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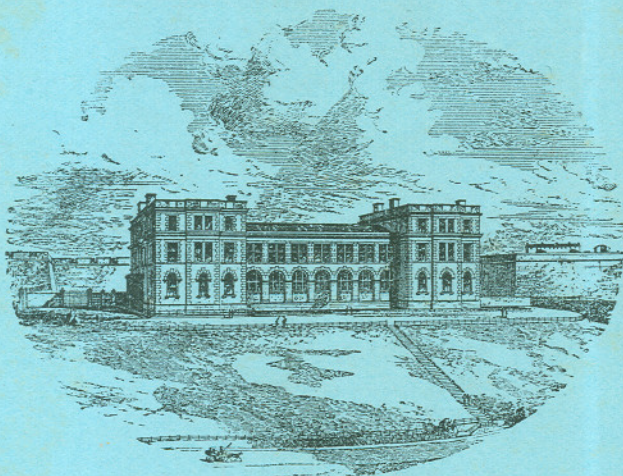
Journal

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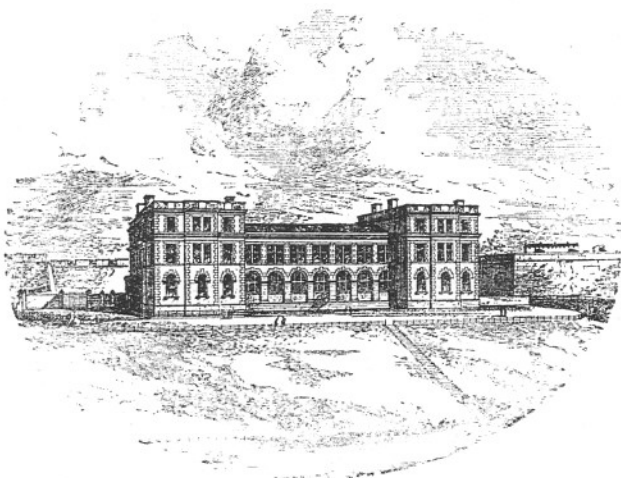
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PLYMOUTH

The Decapoda collected by the "Huxley" from the North Side of the Bay of Biscay in August, 1906.

By

Stanley Kemp, B.A.

THE collection of Decapoda made by the *Huxley* during her short cruise on the north side of the Bay of Biscay is an extensive one; it comprises no less than forty-nine species—a number which speaks well for the efficiency of the gear employed.

Although, as might be expected, the majority of the species obtained are well-known members of the N.E. Atlantic fauna, the material presents many points of interest. Five species not hitherto known to extend south of the British Isles were found by the *Huxley*, and in several cases important additions have been made to our knowledge of the bathymetric range.

A specimen which has been tentatively referred to *Periclimenes Kornii* (Lo Bianco) is of the greatest possible interest, for no deep-water representative of the family Palæmonidæ was hitherto known from the N.E. Atlantic. Unfortunately, the species is represented only by a fragment of a single individual; this is particularly irritating, for the collection, as a whole, is in a remarkably good state of preservation.

No close comparison can be made between the species in the present collection and those found by the *Caudan* in 1895, for the latter expedition worked considerably to the south of the area investigated by the *Huxley*; nevertheless, two species, *Spongicola Koehleri* and *Uroptychus Bouvieri*, which were first described from material obtained by the *Caudan*, have again been found. Until now, both these forms were known only from the type specimens.

My thanks are due to Dr. E. J. Allen for the opportunity of examining this interesting collection.

DECAPODA NATANTIA.

PENÆIDEA.

SERGESTIDÆ.

Sergestes arcticus, Krøyer.

Station VIII.	Surface.	Many, 9-22 mm.*
"	X.	Surface. One, very small.
"	XII.	246 fathoms. Five, 29-35 mm.

The majority of the specimens only measure from 9 to 15 mm. in length, and the largest (35 mm.) is not half grown. The examples from St. XII were probably caught in midwater during the ascent of the net.

STENOPIDEA.

STENOPIDÆ.

Spongicola † *Koehleri*, Caullery.

Station XIII. 412 fathoms. Twenty-three, 25-46 mm., and several very young, about 8 mm.

Prior to the date of the *Huxley's* cruise, this interesting species was known only from five specimens dredged in the Bay of Biscay in 770 fathoms by the *Caudan* expedition. The additional examples, while in the main confirming the accuracy of Caullery's ‡ description, show a very considerable amount of variation in the spinulation of the carapace and certain appendages. This variation is indeed so great that no specimen in the collection exhibits precisely the same armature on both sides of its body. The following notes indicate the numbers of spines and spinules observed in some of the more important positions.

The rostrum bears from 6 to 9 teeth on its dorsal aspect. Ventrally there are two ridges (for the rostrum is triangular in section), each of which is furnished with from 0 to 4 spinules. Occasionally the foremost spinule is median in position owing to the confluence of the two ridges near the apex. The rounded antero-inferior angle of the carapace bears from 1 to 4 short spines, and from 1 to 4 are situated on the lateral face of the carapace a little behind the margin. At the

* The measurements of all the Natantia mentioned in this paper were taken from the tip of the rostrum to the apex of the telson.

† Bouvier (*Mem. Mus. Comp. Zool.*, Harvard, XXVII, 3, 1909, p. 264) gives a useful table for the discrimination of the five species comprised in this genus.

‡ "Schizopodes et Décapodes de la Campagne du Caudan." *Ann. Univ. Lyon*, XXVI, 1895, p. 382.

base of the rostrum on either side there are from 0 to 3 spines, while the posterior margin of the gastric groove may be wholly unarmed or may be provided with as many as twelve spinules. There are from 2 to 5 spinules, often blunt and inconspicuous, on the outer margin of the antennal scale, and from 4 to 14 on either side of the telson. There may also be one or two stout spines on the internal margin of the merus of third pereopod.

The eyes, as Caullery has observed, are devoid of black pigment, except for an annular band at the proximal edge of the cornea. The small and rudimentary exopod which Spence Bate has figured* at the base of the third maxilliped of *Spongicola venusta* is not found in *S. Koehleri*.

The *Huxley*, like the *Caudan*, obtained several very young specimens of this species. Those in the present collection measure about 8 mm. in length and evidently represent the earliest free-living stage, for some remain curled up as though still within the eggshell. The rostrum and all the appendages of the cephalothorax are well developed in these specimens, while the eyes are just as deficient in pigmentation as they are in the adult. The pereopods are fully segmented, and chelæ are present on the first three pairs, those of the third pair being very noticeable owing to their large size. Conspicuous exopods are retained on the first three pairs. The pleopods are well formed, but the uropods are not yet free and the telson is slightly emarginate distally.

An ovigerous female was found to be carrying sixty-two eggs.

As in the case of the type specimens, the examples of *S. Koehleri* collected by the *Huxley* were living in the sponge *Regadrella phœnix*; as a rule a single individual was found inside each sponge.

CARIDEA.

PASIPHÆIDÆ.

Pasiphaë sivado (Risso).

Station VIII.	Surface.	Many, 35-55 mm.
„ IX.	240 fathoms.	Eight, 59-69 mm.
„ X.	Surface.	Many, 8-26 mm.
„ XII.	246 fathoms.	Twenty-nine, 21-40 mm.
„ XIII.	412 fathoms.	Two, 40 and 58 mm.

Only once previously has this species been recorded from depths exceeding 400 fathoms: by Adensamer, from 543 fathoms in the Mediterranean.

* *Challenger Report*, 1888, Pl. XXIX, Fig. i".

Pasiphaë princeps, Smith.

Station XII. 246 fathoms. One, 69 mm.

This solitary individual is specifically identical with a number of specimens found off the west coast of Ireland. These, although differing in certain features from the original description, have been determined as *P. princeps*, Smith, a species closely allied to *P. tarda*, Kröyer, but extending much further south.

P. princeps had not hitherto been found in as little as 246 fathoms.

PANDALIDÆ.

Pandalus leptocerus, Smith, var. **Bonnieri**, Caullery.

Station IX. 240 fathoms. Twenty-six, 35-ca.110 mm.

„ XII. 246 fathoms. Thirty-three, 25-98 mm.

Pandalus propinquus, G. O. Sars.

Station VII. 444 fathoms. Nineteen, 27-70 mm.

„ IX. 240 fathoms. Eight, 27-34 mm.

„ XII. 246 fathoms. Fifteen, 18-35 mm.

„ XIII. 412 fathoms. Thirteen, 31-ca.75 mm.

P. propinquus had not previously been recorded as far south as the Bay of Biscay.

Plesionika martia (A. Milne-Edwards).

Station XII. 246 fathoms. Five; one perfect, 90 mm.

(?) „ XIII. 412 fathoms. One, large, in very bad condition.

The large individual from Station XIII cannot be satisfactorily determined. It appears to have been swallowed by a fish and partially digested.

Pandalina brevirostris (Rathke).

Station IX. 240 fathoms. Three, 21-25 mm.

„ XII. 246 fathoms. Thirty, 12-25 mm.

Several of the female specimens are ovigerous.

HIPPOLYTIDÆ.

Hippolyte varians, Leach.

Station II. 75 fathoms. One, 17 mm.; an ovigerous female.

Caridion Gordoni (Spence Bate).

Station IX. 240 fathoms. Four, 16-21 mm.

„ XII. 246 fathoms. Eight, 14-18.5 mm.

The rostra of these specimens bear from six to nine teeth above and from one to three below.

C. Gordoni was not previously known to the south of the British Isles, and hitherto had not been trawled in depths exceeding 200 fathoms.

PROCESSIDÆ.

Processa canaliculata, Leach.

Station II. 75 fathoms. Two, 26 and 30 mm.

„ V. 109 fathoms. Seventy-six, 21–50 mm.

Two of the specimens have abnormal eyes. The cornea on one side is well developed and of the usual size, whereas that on the other side is much smaller with, in one case, a curious swelling on the inner face of the stalk. The rostrum of the latter specimen is also unusually short, and is not furnished with its full complement of setæ.

PALÆMONIDÆ.

Periclimenes Kornii? Lo Bianco.

Station XIII. 412 fathoms. Fragment.

The rostrum of this specimen is broken off at the base, and the whole of the abdomen is missing. This is particularly unfortunate, for the specimen is, as far as I am aware, the only deep-water Palæmonid which has been found in the North-East Atlantic. It appears to be most closely allied to the imperfectly described *Periclimenes (Anchistia) Kornii* (Lo Bianco),* found near Capri in about 600 fathoms, but is considerably larger and differs from the Italian author's figure in the lengths of various segments of the pereopods.

The carapace measures 7 mm. from the back of the orbit to the hinder margin of the carapace; it is therefore probable that the specimen was originally more than twice as long as the types of *P. Kornii*, which were only 13–15 mm. in total length.

The rostrum is broken, but four dorsal teeth are present on the anterior third of the carapace behind the orbital notch. The dorsal carina is clear and distinct for three-quarters the length of the carapace, fading away further back. Both hepatic and antennal spines are present. The eyes are deeply pigmented and the cornea is wider than the stalk. The outer antennular flagellum is split into two rami, the inner one (which is also the thicker) being slightly longer than the fused basal part. The lamellar portion of the antennal scale is produced acutely at its inner distal angle, and reaches considerably beyond the stout spine which terminates the straight outer margin.

The first pair of pereopods reaches beyond the apex of the antennal scale by the whole length of the propodus; the merus and

* Lo Bianco. *Mitt. Zoo. Stat. Neapel*, 1903, p. 250, tav. 7, fig. 13.

carpus are nearly equal in length, each being about one and a half times as long as the chela. The second pair is characterised by the very long but comparatively slender chela, which is twice the length of the merus. The carpus is very short, about one-third the length of the merus, and the dactylus is half the length of the palm. The dactylus is strongly curved and sharply pointed apically; it bears a prominent longitudinal carina on either side and a sharp tooth internally in the middle of its basal third. The fixed finger is carinate along its internal aspect only, and bears, in its basal third, two teeth, between which the dactylar tooth fits when the claw is closed. In the last three pairs of pereopods the propodus is slightly longer than the merus, the carpus is three-fifths the length of the propodus, and the dactylus is very short, simple, curved, and claw-like.

CRANGONIDÆ.

Crangon Allmanni, Kinahan.

Station II.	75 fathoms.	Nine, 18-26 mm.
„ V.	109 fathoms.	Many, 14-25 mm.
„ XI.	146 fathoms.	Many, 12-27 mm.

The capture of this species on the north side of the Bay of Biscay in 146 fathoms establishes new records both for its horizontal and bathymetric distribution. *C. Allmanni* had not hitherto been found south of the British Isles, and was not previously known from depths exceeding 100 fathoms.

The small size of the specimens seems to indicate that the species is unable to attain its maximum development in deep water.

Philocheras* echinulatus, M. Sars.

Station IX.	240 fathoms.	Forty-eight, 14-34 mm.
„ XII.	246 fathoms.	Many, 14-35 mm.

This species was not previously known as far south as the Bay of Biscay.

Philocheras bispinosus, Hailstone, var. **neglectus**, G. O. Sars.

Station II.	75 fathoms.	Two, 11.5 mm.
-------------	-------------	---------------

These two specimens show no trace of the brown pigment which is sometimes such a prominent feature of the var. *neglectus* when living. The surface of the carapace and abdomen is, however, without trace of tubercles, and is pitted with microscopic punctuations exactly as in the forms with transverse brown bands.

* *Philocheras*, Stebbing, *nom. nov. vice Cheraphilus*.

P. bispinosus var. *neglectus* had not hitherto been recorded from as far south as the Bay of Biscay nor from as much as 75 fathoms. The typical form is, however, known to extend to the Azores and has been found off the west coast of Ireland in as much as 200 fathoms.

Ægeon Lacazei (Gourret).

- Station IX. 240 fathoms. Fourteen, 19.5-28 mm.
 ,, XII. 246 fathoms. Eight, 20-25 mm.

This scarce species is closely allied to the common Mediterranean form *A. cataphractus*. It was originally described by Gourret from specimens found in the vicinity of Marseilles, and since then twelve examples have been trawled off the west coast of Ireland between 160 and 374 fathoms.

Pontophilus spinosus (Leach).

- Station IX. 240 fathoms. Seven, 28-38 mm.
 ,, XII. 246 fathoms. Three, 9-12 mm.

Pontophilus norvegicus, M. Sars.

- Station XII. 246 fathoms. Three, 13-17 mm.

DECAPODA REPTANTIA.

ERYONIDEA.

ERYONIDÆ.

Polycheles typhlops, Heller.

- Station XII. 246 fathoms. Two, 29 mm.

SCYLLARIDEA.

PALINURIDÆ.

"*Phyllosoma*" (larva).

- Station VIII. Surface. Two.

GALATHEIDEA.

UROPTYCHIDÆ.

Uroptychus rubrovittatus (A. Milne-Edwards).

- Station VII. 444 fathoms. Four, 15-30 mm.*
 ,, XII. 412 fathoms. One, 19 mm.

* The measurements of all the Galatheidea mentioned in this paper were taken from the apex of the rostrum to the extremity of the telson, with the abdomen stretched out in macrurous fashion.

Uroptychus nitidus var. **concolor** (A. Milne-Edwards).

Station XIII. 412 fathoms. One, 30 mm.

Uroptychus Bouvieri, Caullery.

Station XIII. 412 fathoms. Three, 14·5–22 mm.

This is the first time this species has been recorded since it was described by Caullery.* The type specimens, two males, were found by the *Caudan* expedition between 218 and 273 fathoms.

Two of the examples collected by the *Huxley* are ovigerous females, and measure 22 and 20·5 mm. from the tip of the rostrum to the apex of the telson; the third is a male, 14·5 mm. in length. The first pereopods measure 26, 24, and 23·5 mm. respectively, thus showing that this limb is much more strongly developed in the male than in the female.

Little can be added to Caullery's careful description. The small median denticle behind the base of the rostrum is absent in all the specimens, the lateral spines on the carapace vary in number from five to six, and the antennal scale reaches to two-thirds the length of the rostrum, and is narrower at its base than in the figure of the type. The notch in the sternal plastron is, in the female, rectangular in shape, and considerably deeper than in the male.

Only four longitudinal rows of spines can be found on the merus and carpus of the first pereopod, and the internal edge of the propodus of the same limb is upturned and denticulate proximally and is separated from the smooth dorsal surface by a well-defined groove.

The eggs, which appear to be on the point of hatching, measure about 1·5 mm in length.

Gastroptychus formosus (A. M.-Edw. and Bouvier).

Station VII. 444 fathoms. One, 18 mm.

„ XIII. 412 fathoms. Two, 21 and 38 mm.

One of the specimens from Station XIII is an ovigerous female.

GALATHEIDÆ.

Galathodes tridentatus (Esmark).

Station VII. 444 fathoms. Twenty-four, 6·5–28 mm.

„ XIII. 412 fathoms. Four, 18–20 mm.

Nine females are ovigerous.

* "Schizopodes et Décapodes de la Campagne du Caudan," *Ann. Univ. Lyon*, XXVI, 1895, p. 394.

Galathea nexa, Embleton.

- Station II. 75 fathoms. Two, 16 and 23 mm.
 ,, V. 109 fathoms. One small; broken.
 ,, XI. 146 fathoms. Six, 10-21 mm.
 ,, XII. 246 fathoms. Two, 15 and 18 mm.

Compared with Bonnier's figures the third maxillipede in these specimens bears a closer resemblance to *G. dispersa* than to *G. nexa*. Recent authors are, however, agreed that these two forms are merely variations of a single species, and although the form known as *dispersa* is far the commoner, yet this name must lapse in favour of *nexa*, which has priority.*

Munida bamffica (Pennant).

- Station IX. 240 fathoms. Two, 24 and 33 mm.
 ,, XII. 246 fathoms. Four, 25-33 mm.
 ,, XIII. 412 fathoms. One, 23 mm.

Although the specimens are small, they all present the scaly appearance on the thoracic sternum which so readily separates this species from its close ally *Munida tenuimana*.*

PAGURIDEA.

PAGURIDÆ.

Eupagurus bernhardus (Linn.).

- Station II. 75 fathoms. One, very small.

Eupagurus Prideauxi (Leach).

- Station II. 75 fathoms. Five.
 ,, V. 109 fathoms. Four.
 ,, VI. 87 fathoms. One.

In the largest specimen, which is an ovigerous female from Station VI, the carapace measures 15 mm. in length.

Eupagurus variabilis, A. M.-Edw. and Bouvier.

- Station IX. 240 fathoms. Twenty-six.
 ,, XI. 146 fathoms. Sixteen.
 ,, XII. 246 fathoms. Twelve.
 ,, XIII. 412 fathoms. One.

The largest example, taken at Station IX, measures 62 mm. from the hinder margin of the cephalothorax to the distal extremity of the large chela. All the specimens are typical in form with the exception of two, in which the propodus of the right chela is slightly excavate.

* v. Hansen, *Danish Ingolf Malacostraca*, 1908, pp. 31 and 32.

Eupagurus carneus, Pocock.

- Station VII. 444 fathoms. One.
 ,, XIII. 412 fathoms. Two.

The largest specimen measures only 28 mm. from the hinder margin of the cephalothorax to the distal extremity of the large chela.

Anapagurus lævis (W. Thompson).

- Station II. 75 fathoms. Seven.
 ,, V. 109 fathoms. Twenty-two.
 ,, IX. 240 fathoms. Two.
 ,, XI. 146 fathoms. Six.
 ,, XII. 246 fathoms. Four.

OXYSTOMATA.

DORIPPIDÆ.

Cymonomus granulatus (Norman).

- Station XII. 246 fathoms. One, 4 mm.*

LEUCOSIIDÆ.

Ebalia tuberosa (Pennant).

- Station V. 109 fathoms. One, 12.5 mm.

This species does not seem to have been recorded hitherto from as much as 109 fathoms.

Ebalia tumefacta (Montagu).

- Station II. 75 fathoms. One, 7 mm.
 ,, V. 109 fathoms. Two, 8 and 8.5 mm.

Ebalia nux, Norman.

- Station V. 109 fathoms. One, 7.5 mm.
 ,, IX. 240 fathoms. Eight, 6.5-8.5 mm.
 ,, XI. 146 fathoms. Three, 7-7.5 mm.
 ,, XII. 412 fathoms. 7-8 mm.

Several of the specimens are ovigerous females.

BRACHYGNATHA.

PORTUNIDÆ.

Portunus holsatus, Fabricius.

- Station II. 75 fathoms. Eighteen, 10.5-18 mm.
 ,, V. 109 fathoms. Three, 6.5-8.5 mm.

* Length of carapace.

The specimens are all very young, but in my opinion they can be referred with safety to this species. Hitherto *P. holsatus* has not been recorded from depths exceeding 70 fathoms.

Portunus pusillus, Leach.

Station V. 109 fathoms. Eighteen, 5-9 mm.

It is with some doubt that these small specimens are referred to *P. pusillus*. The median frontal tooth is, in several instances, not more advanced than the lateral, but it is probable that with growth this feature would become more apparent.

Portunus tuberculatus, Roux.

Station III. 75 fathoms. Two, 27 and 29 mm.

„ V. 109 fathoms. Three, 17-21 mm.

„ VI. 87 fathoms. Three, 21-22 mm.

Polybius Henslowi, Leach.

Station II. 75 fathoms. One, 36 mm.

Bathynectes superba (Costa).

Station VII. 444 fathoms. Two; one 11 mm., one broken.

„ IX. 240 fathoms. Seven, about 5.5 mm.

„ XII. 246 fathoms. Fifty-three; one large and very macerated, the rest 5-6 mm.

„ XIII. 412 fathoms. Four.

In the small specimens the form of the carapace resembles Bouvier's figure* of an individual 4.5 mm. in length; the frontal margin is four-lobed, the second and fourth antero-lateral spines are extremely short, while the fifth is not specially longer than the third. In the two large specimens from Station XIII the hindmost spines of the antero-lateral series are very long; they measure 50 and 46 mm. in breadth without these spines, while, including them, they measure 84 and 80 mm.

Geryon sp. ?

Station XII. 246 fathoms. One, broken, 5 mm.

ATELECYCLIDÆ.

Atelecyclus septemdentatus (Montagu).

Station II. 75 fathoms. Two, 15 and 17 mm.

* Bouvier, *Rés. Camp. Sci. Monaco*, XIII, 1899, Pl. II, fig. 1.

MAIIDÆ.

Inachus dorsettensis (Pennant).

- Station V. 109 fathoms. Four, 13-18 mm.
 „ VI. 87 fathoms. Two, 19 and 20 mm.

Inachus leptochirus, Leach.

- Station II. 75 fathoms. Sixteen, 12-22 mm.
 „ VI. 87 fathoms. Five, 18-22 mm.
 „ XI. 146 fathoms. Two, 9 and 12·5 mm.

Stenorhynchus longirostris (Fabricius).

- Station II. 75 fathoms. Twelve, 10-20 mm.
 „ V. 109 fathoms. Two, 9·5 and 17·5 mm.
 „ IX. 240 fathoms. One, 22 mm.

Lispognathus Thomsoni (Norman).

- Station VII. 444 fathoms. One, 7 mm.
 „ XIII. 412 fathoms. Four, 4-7 mm.

Ergasticus Clouei, A. Milne-Edwards.

- Station V. 109 fathoms. One, 8 mm.
 „ IX. 240 fathoms. One, 11 mm.
 „ XII. 246 fathoms. Four, 9-18 mm.

Hyas coarctatus, Leach.

- Station VI. 87 fathoms. One, 26 mm.

LIST OF SPECIES, AND THE STATIONS AT WHICH THEY OCCURRED.

STATION No.	II	III	V	VI	VII	VIII	IX	X	XI	XII	XIII
Latitude, N.	48° 24'	48° 24'	47° 48'	47° 46'	47° 36'	47° 30'	48° 7'	48° 7'	48° 10'	48° 7'	48° 7'
Longitude, W.	6° 28'	6° 33'	7° 46'	7° 12'	7° 31'	7° 31'	8° 13'	8° 13'	8° 11'	8° 13'	8° 13'
Fathoms	75	75	100	87	444	Surface.	240	Surface.	146	246	412
Natantia.											
<i>Sergestes arcticus</i>	many	...	1	...	5	
<i>Spongiicola Koehleri</i>	23
<i>Pasiphaë sivado</i>	many	8	many	...	29	2
<i>Pasiphaë princeps</i>	1	
<i>Pandalus leptocercus</i> v. <i>Bonnieri</i>	26	33	
<i>Pandalus propinquus</i>	19	...	8	15	13
<i>Plesionika martia</i>	5	?
<i>Pandalina brevirostris</i>	3	30	
<i>Hippolyte varians</i>	1	
<i>Caridion Gordoni</i>	4	8	
<i>Processa canaliculata</i>	2	...	76	
<i>Periclimenes Korni</i> ?	1
<i>Crangon Allmanni</i>	9	...	many	many
<i>Philocheras echinulatus</i>	48	many	
<i>Philocheras bispinosus</i> v. <i>neglectus</i>	2	
<i>Ægeon Lacazei</i>	14	8	
<i>Pontophilus spinosus</i>	7	3	
<i>Pontophilus norvegicus</i>	3	
Reptantia.											
<i>Polycheles typhlops</i>	2	
' <i>Phyllosoma larva</i>	2	
<i>Uroptychus rubrovittatus</i>	4	1
<i>Uroptychus nitidus</i> v. <i>concolor</i>	1
<i>Uroptychus Bouvieri</i>	3
<i>Gastroptychus formosus</i>	1	2

On the Artificial Culture of Marine Plankton Organisms.

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Introduction. The observations to be recorded in this Paper were commenced in March, 1905. They originated in an attempt to find a general method for rearing marine larval forms. Several investigators had previously succeeded in rearing Echinoderms, Molluscs, and Polychætes from artificially fertilized eggs under laboratory conditions, but the process was generally difficult and the results more or less uncertain. The most promising method seemed to be that adopted by Caswell Grave (26), who was able to rear his larvæ by feeding them on diatoms. Grave obtained his diatoms by placing sand, collected from the sea bottom, in aquaria and using such diatoms as developed from this material. All the methods, however, suffered from the uncertainty of not knowing what organisms were introduced into the aquaria in which the larvæ were to be reared, either in the original sea-water or along with the food-supply.

It appeared, therefore, at an early stage of the work, worth while to make an attempt to carry out rearing experiments on a more definite and precise plan, to endeavour, in fact, to introduce the larvæ to be reared into sterile sea-water, and to feed them with pure cultures of a suitable food. This was the ideal to be aimed at. As a matter of fact, it has seldom, if ever, been attained in practice; nevertheless a considerable measure of success has been achieved by working upon these lines, and during the course of the work innumerable problems relating to the physical conditions under which plankton organisms can best flourish have presented themselves. Some account of the experiments made may be of interest to other workers, although many of the problems raised are not yet solved, notwithstanding the fact that some 1500 cultural experiments have been under observation. It is rather with a view of stimulating other work upon similar lines, than of bringing forward conclusive results, that this paper is being published.

In the summer of 1907, Mr. E. W. Nelson became associated with the investigation, and since that date the experimental work has been carried out by him. The discussions in this paper of a more chemical character, particularly the section on alkalinity, are almost entirely the work of Mr. Nelson, and we have both had throughout the advantage of the constant advice and help of Mr. D. J. Matthews on all such matters.

I. CULTURE OF PLANKTON DIATOMS.

A. PRACTICAL CULTURE METHODS.

1. *Miquel's Method.* Attention was first directed to the culture of Plankton diatoms; and the methods, which had been elaborated by Miquel (11) for fresh-water diatoms and had been found by him to succeed with marine-bottom diatoms, were tried.

The essential features of Miquel's method, as applied to marine diatoms, are as follows:—

Two solutions are prepared:—

SOLUTION A.

Magnesium sulphate	10 gm.
Sodium chloride	10 „
Sodium sulphate	5 „
Ammonium nitrate	1 „
Potassium nitrate	2 „
Sodium nitrate	2 „
Potassium bromide	0.2 „
Potassium iodide	0.1 „
Water	100 „

SOLUTION B.*

Sodium phosphate	4 gm.
Calcium chloride (dry)	4 „
Hydrochloric acid	† 2 cc.
Ferric chloride	‡ 2 „
Water	80 „

Forty drops of Solution A and 10 to 20 drops of Solution B are added to each 1000 cc. of sea-water, and the sea-water is sterilized by keeping it at 70° C. for about 20 minutes.

According to Miquel it is also necessary to add "organic nutritive material in the form of bran, straw, or filaments of weed such as *Zostera*. Macerations of these should be made up separately, some time before they are required for use, and should be carefully filtered and sterilized. Organic matter must, however, be used very sparingly,

* "The preparation of Solution A presents no difficulty; Solution B should be made up as follows: To the Sodium phosphate dissolved in 40 cc. of water are added first the 2 cc. of Hydrochloric acid, then the 2 cc. of hydrous Ferric chloride and then the 4 gm. of Calcium chloride dissolved in 40 cc. of water, taking care to shake the mixture, which I call Phospho-ferro-calcic solution. The addition of this last solution to the maceration throws down a slight brownish flocculent precipitate, formed for the most part of Ferric oxide, which should be carefully separated from the liquid used for cultivations."

† "Acid chlorhydrique pur à 22°." Presumably meaning degrees Baumé=sp. gr. 1.169.

‡ "Perchlorure de fer liquide à 45°." As above=sp. gr. 1.421.

or else putrefaction will set in and the cultures will be irrevocably lost." As a matter of fact, we have found that such organic infusions are unnecessary, when dealing with plankton diatoms, and it has not been our practice to employ them (cf., however, p. 445).

Miquel obtained cultures of single species of diatoms either by picking out individual diatoms under the microscope and introducing them into the prepared water, or by adding a small quantity of water containing a mixture of diatoms and other organisms to some prepared water, and subdividing this into a number of tubes. If the subdivision has been carried out sufficiently some of the tubes may contain one kind of diatom only, from which fresh cultures can be made. In this way, by repeated subdivision, cultures can be obtained which, by inoculating fresh quantities of prepared water from time to time, may, with care, be maintained indefinitely. Such cultures, however, must practically always contain bacteria, and Miquel distinguishes them from bacteria-free cultures, which he terms "Cultures des Diatomées à l'état de pureté absolue." The latter he found very difficult to obtain, but, through repeated washing in sterile water, followed by fractional subdivision, he succeeded in getting some in which he could find no trace of bacteria by ordinary bacteriological methods (cf. Miquel 11, p. 155; cf. also Richter, 16-18).

We propose to call any diatom culture, which can be carried on practically indefinitely by inoculating fresh supplies of prepared water, a "*persistent*" culture, the term "*pure*" culture being reserved for cultures which can be proved to contain not more than one organism. We are not satisfied that we have yet succeeded in obtaining cultures of the latter kind. For the most part our persistent cultures contain one species of diatom only, and are free from all organisms larger than small flagellates.

In our earlier experiments with plankton diatoms, we obtained persistent cultures, containing a single species of diatom, by both of the methods recommended by Miquel. We, however, have rarely succeeded by picking out single diatoms or chains of diatoms, for although we have passed the selected diatom through several changes of sterilized sea-water, the resulting cultures, even when the diatoms have multiplied to some extent, have generally shown evidence of contamination by harmful organisms, and have soon died down. Only in one of the earliest experiments, and in one more recent, has complete success resulted. In the first case a small chain of six or eight frustules of *Skeletonema costatum*, picked out in April, 1905, gave rise to a culture which still persists (Nov., 1909). Subcultures can still be obtained even from the original flask inoculated in April, 1905. In the

second case a chain of 8 or 9 cells of *Chaetoceras densum*, picked out from a Petri dish culture, has given a particularly good growth.

The method of dilution and subdivision has been more successful and persistent cultures of a number of species have been obtained in this way.

A more ready method of obtaining the cultures is, we have found, to add one or two drops of plankton to, say, 250 cc. of a suitable sterile culture medium, and to pour this into shallow glass dishes (Petri dishes). The dishes should be placed in a position as free as possible from vibration, and where they can be easily examined with a lens *in situ*. The temperature should be kept as constant as possible and the dishes exposed to light of moderate intensity, direct sunlight being avoided. In the course of a few days, colonies of diatoms of different species will be seen at different spots on the bottom of the Petri dishes. These can be picked out with a fine pipette and transferred to flasks containing fresh culture medium. The colonies should be picked out from the Petri dishes at as early a stage as possible, because if left too long some one organism, a diatom or a flagellate, may have multiplied so rapidly that the whole of the water in the dish becomes infected with it. In this case persistent cultures of a single species would not be obtained. The above method is similar to one described by Miquel, excepting that he placed gelatinous silica at the bottom of the vessel. Some very successful persistent cultures were obtained from the following experiment, which will serve to illustrate the method:—A sample of plankton, from a very fine-mesh bolting-silk tow-net, was diluted down with sterile sea-water, until a single drop examined under a two-thirds-inch objective contained on an average ten organisms, chiefly diatoms of various species. Petri dishes (4 in.), containing 60 cc. each of Miquel sea-water, were then inoculated with various numbers of drops of the diluted plankton. The two dishes, to which two and three drops respectively were added, gave the best results; and from these persistent cultures of several species of diatoms were obtained. Hence we may conclude that the most advantageous number of single cells or short chains of cells to be added to a 4 in. Petri dish, containing 60 cc. culture medium, is about 20 to 30.

We have succeeded in obtaining the following species of Plankton diatoms in persistent cultures:—

- Asterionella japonica*, Cleve.
- Biddulphia mobiliensis* (Bail.), Grun.
- Biddulphia regia* (M. Schultze).*

* See pp. 461.

- Chaetoceras densum*, Cleve.
Chaetoceras decipiens, Cleve.
Chaetoceras constrictum, Gran.
Cocconeis scutellum, Ehr. var. *minutissima*, Grun.
Coscinodiscus eccentricus, Ehr.*
Coscinodiscus Granii, Gough.
Ditylium Brightwellii (West), Grun.
Lauderia borealis, Gran.
Nitzschia closterium, W. Sm.
Nitzschia closterium, W. Sm., *forma minutissima*.
Nitzschia seriata, Cleve.
Rhizosolenia stouterfothii, H. Perag.
Skeletonema costatum (Grev.)
Streptotheca thamensis, Shrubs.
Thalassiosira decipiens, Grun.*

It is hardly necessary to add that in dealing with these cultures, similar precautions to those used in bacteriological work must be taken, all vessels and instruments being carefully sterilized before they are brought into contact with the prepared sea-water. The cultures are best made in small, wide-mouthed flasks, which may be plugged with cotton wool, or simply covered with watch-glasses. The flasks should be kept at as uniform a temperature as possible (from 12°–17° C.) and should be exposed to strong daylight, direct sunlight being avoided. A flask should not be more than half filled with culture fluid, so that the surface exposed to the air may be large in proportion to the volume of fluid.

Other Methods. The addition of the solutions devised by Miquel to sea-water has in all cases given us good cultures of diatoms, and the method is certain in its action. We have, however, made numerous experiments by treating sea-water in other ways, with a view to finding out what are the best conditions under which plankton diatoms will grow, and of arriving at some explanation of the action of the different salts contained in Miquel's solutions.

2. *Houghton Gill's Method.* H. Houghton Gill (5), a contemporary of Miquel, made use of a culture medium not essentially different from that employed by the latter. Unfortunately he died before publishing his work, but an account of his principal results is given by Van Heurck. In his final method Houghton Gill made use of four distinct solutions, as follows:—

* See p. 460.

SOLUTION 1.

Crystallized sodium phosphate	2	gram.
Calcium chloride	4	„
Syrup of iron chloride	0.5	„
Strong hydrochloric acid	1	„
Water	100	„

SOLUTION 2.

Crystallized magnesium sulphate	4	„
„ sodium sulphate	4	„
„ potassium nitrate	4	„
Common salt (sodium chloride)	8	„
Potassium bromide	0.2	„
Potassium iodide	0.2	„
Water	100	„

SOLUTION 3.

Crystallized sodium carbonate	4	„
Water	100	„

SOLUTION 4.

Well-washed, precipitated calcium silicate	25	„
Water	75	„

All the salts employed must be chemically pure. Three cc. of each of these liquids are added to 1000 cc. of fresh water or sea-water (according to circumstances), and the whole sterilized. In his earlier work Houghton Gill added a sterilized infusion of grass or of diatoms, but it is not clear from the accounts whether this was still employed with the above solutions. We have obtained very good cultures with the above solutions, to which we did not add any organic infusion.

3 (a). *Modification of Miquel's method. "Miquel Sea-water."*

Since several of the components in Miquel's formula for solution A (p. 423) are obviously unnecessary, when sea-water is being used as the basis of the culture-medium, we adopted for our own work the following modifications:—After some preliminary experiments it was found, as would be expected from the composition of sea-water, that the only salts of value to the medium are the three nitrates, KNO_3 , NaNO_3 , NH_4NO_3 , and possibly KBr and KI . The omission of the two latter was soon found to make no difference. Experiments also showed that the formula for solution A could, without any appreciable detriment to results, be further simplified to the one salt, KNO_3 , or NaNO_3 , but not NH_4NO_3 . At first the amount of KNO_3 , dissolved in 100 cc. distilled water, used to make the modified solution A, was the same as the sum of the weights of the nitrates in

Miquel's own formula, viz. 5 gm. But later experiments showed that a considerably greater concentration of KNO_3 than this gave more lasting cultures; the strength of solution, and amount to be added to a litre of sea-water, in order to obtain the best results, being 2 cc., 2M, KNO_3 .

In the case of solution B no modification has been adopted, but it has been found that small variations in the amounts of the ingredients used do not affect the results. A convenient method for measuring the right amount of FeCl_3 is to warm the salt until it just melts in its own water of crystallization, and to pipette out 2 cc. with a previously warmed pipette. No temperature corrections need be considered. Also 2 cc. of the ordinary pure concentrated hydrochloric acid at room temperature will suffice.

Our own formula for preparing Miquel sea-water is now:—

SOLUTION A.*

Potassium nitrate	20.2 gm.	} = 2M KNO_3
Distilled water	100 „	

SOLUTION B.†

Sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$)	4 „
Calcium chloride ($\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$)	4 „
Ferric chloride (melted)	2 cc.
Hydrochloric acid (pure, concentrated)	2 cc.
Distilled water	80 cc.

To each 1000 cc. of sea-water‡ add 2 cc. solution A and 1 cc. solution B, and sterilize by heating to 70°C . When cool, decant off the clear liquids from the precipitate, which will have formed when solution B is added to the sea-water.

As a rule our cultures were made in 60 cc. of this medium, contained in short-necked, wide-mouthed flasks of 125 cc. capacity, so that the proportion of air-surface to volume of liquid was large.

The medium was found to give constantly satisfactory results. On inoculation from a persistent culture of such diatoms as *Thalassiosira*, *Skeletonema*, *Chaetoceras*, etc., a growth visible to the eye is obtained in about ten days, and then multiplication takes place very rapidly. In from three weeks to a month's time a very considerable growth will be seen making a brown, flocculent mass at the bottom and back of the vessel containing the culture.

* This strength has only been used in the most recent experiments; and solution A in this paper, unless otherwise stated, means the five per cent solution of KNO_3 .

† For preparing this solution see p. 423.

‡ "Miquel water" seems to succeed equally well, whether it is made by adding Miquel's solutions to "outside water" or to "tank water" (cf. p. 437).

In from three to four months the culture begins to show signs of exhaustion and the frustules lose colour, but they do not, as in the case of sterilized outside and tank water, completely die off. A great number certainly do die, but some remain in a resting condition, and often, after a period of six months or so, these begin to multiply again and the culture regains its former vigour. This is probably due to the food-stuffs contained in the dead frustules going into solution again, possibly by means of bacterial action. This periodicity in cultures is interesting in that it resembles what takes place in the ocean. Cultures in this medium will persist indefinitely, so far as our experience goes. The oldest culture in our possession is one of *Skeletonema costatum*, made at the very commencement of this work, dated April, 1905. Although the frustules in this culture are quite unrecognizable as any diatom now, on making a subculture in fresh Miquel a normal and healthy growth can always be obtained.

In old cultures the diatoms are nearly always found to be very much deformed, and often appear to be only a mass of broken-down chromatophores. Whether regeneration can be successfully obtained from a single chromatophore, which must presumably be contained within a cell-wall of some kind, has not been definitely decided, but results seem to point in this direction.

At the start of a culture a tendency to teratological forms is often exhibited, but when the growth is well advanced, the shape of the frustules is usually quite normal.

(b) *English Channel Water* ("Outside Water").—In a large number of our experiments sea-water brought in from outside the Plymouth breakwater, and therefore taken at some distance from the shore, has been used. This is referred to as "outside water." It has an average salinity of about 35.0 ‰, and the temperature range for the year is from 8° C. to 16° C.

If a sample of "outside water" is inoculated from a persistent culture of a plankton diatom, a small growth is obtained in from five to fifteen days. But soon minute bottom forms of diatoms, other algæ, flagellates, infusoria, etc., appear, and the inoculated species is lost. The total growth of any form is never large. If the growth of these foreign forms is prevented by sterilizing the water before inoculation, a considerably better growth of the plankton form is obtained. The water was, as a rule, sterilized by simply heating to 70° C., which temperature was found to be quite adequate. Boiling gave equally good results, but the former was preferred, as less concentration due to evaporation took place. Even under these conditions

no permanent culture can be obtained, the diatoms soon beginning to lose colour and getting into an exhausted condition. Death takes place in from two to three months after the culture has been started, and in many cases considerably sooner. Long before inability to start new cultures, the test of death, has been established, the valves appear on examination quite colourless and practically empty.

Samples of outside water, taken at times when the quantity of plankton was widely different, gave no appreciable variation in the results obtained by culture methods. It is, however, doubtful whether differences in the amounts of growth in cultures, proportional to the seasonal variation in the quantity of phytoplankton, would be sufficiently marked to be appreciable.

The total growth under cultural conditions, although small for a culture, is very much greater than any natural plankton that has come within our experience.

(c) *Tank-water*.—"Tank-water" or water taken from the supply of sea-water circulating through the tanks of the Aquarium at Plymouth, shows some striking and interesting differences from "outside water." This water is pumped up from the sea, just below the Laboratory, into two large, covered-in, settling reservoirs, with a capacity of 50,000 gallons each. Pumping is only done at high water, spring tides, so as to get the least contaminated water, and no water is pumped that does not show a specific gravity, measured with a hydrometer, of $\rho^{17.5} = 26.00$ ($S = 34.00$) or over. The water is allowed to settle for about a fortnight before being used for the general circulation.

The tanks themselves are made of slate and glass, and the pipes which convey the sea-water to them are of vulcanite, so that the water does not come in contact with metal, excepting in the pumps, which are of cast-iron. The two settling reservoirs are used alternately, for about a week each. From time to time, tide and water allowing, waste is replenished, and about twice a year each reservoir is emptied, cleaned out and refilled. The aquarium takes about 20,000 gallons, and this is in circulation with one of the two 50,000-gallon reservoirs. An estimate of the amount of life in the tanks of the aquarium must be exceedingly rough, but the intensity of the larger forms of life is far greater than anything met with in natural waters. About 500 fish and 2000 invertebrates, including all forms as large as an *Actinia equina*, might be somewhere near the mark. So it will be seen that the accumulation of excretory products must be a by no means negligible factor. The flora of the tanks is very restricted, and is chiefly composed of minute forms of algæ. Minute naviculoid diatoms,

Ectocarpus, *Cladophora*, *Enteromorpha*, *Vaucheria*, and unicellular algae are the commonest forms. The large seaweeds, such as *Fucus* and *Laminaria*, do not live long if introduced. Plankton diatoms, although a great number must be pumped up when the reservoirs are being filled, are not represented.

As in the case of outside water, a sample of "tank-water," inoculated from a persistent culture, will only give a very small growth, minute forms, etc., soon multiplying and choking out the plankton form. The ultimate growth of minute unicellular algae other than diatoms is often considerable, and many quite unknown and unidentified forms have been obtained. The total growth of vegetable forms is always found to be greater than in the case of outside water.

In cultures of plankton diatoms made with sterilized tank water, a very great improvement on outside sterilized water was always noted. The culture of the diatom used to inoculate this medium persists for a considerable period, and the colour of the frustules remains normal for two to three months.

(d) *Animal-Charcoal Water*.—The use of animal charcoal, as a means of purifying the water in small aquaria, has for a long time been known and practised by those who have kept such aquaria in inland places. At an early stage in our experiments, water from a tank, which was not in a satisfactory condition, was treated with some powdered animal charcoal and filtered. It was noticed that a good growth of diatoms took place in this water. Systematic experiments with the use of animal charcoal were then commenced, and these have resulted in a method of great value, both for the culture of diatoms and for the rearing of pelagic larvæ.

Animal charcoal is made by the carbonization of bones,* and is sold in two grades known as "pure" and "commercial." Our earlier experiments were all made with "pure" animal charcoal, but subsequently the "commercial" animal charcoal was largely used and appears to give equally good, if not better results. In both cases the animal charcoal is used in the powdered form. Animal-charcoal water is prepared as follows:—

1. A quantity of sea-water is sterilized by heating it in a flask to

* Analysis of Animal Charcoal, from Thorpe's *Dictionary of Applied Chemistry*:—

Carbon	10.51
Ca., Mg. phosphates, Ca. fluoride, etc.	80.21
Calcium carbonate	8.30
Other mineral matter	0.98
	<hr/>
	100.00
	<hr/>

70° C., at which temperature it should be kept for about twenty minutes. At the same time some animal charcoal is heated sufficiently to sterilize but not to burn it, covered over and allowed to cool. When both are quite cold, the charcoal is added to the water (ca. 15 gm. to 1000 cc.) and well shaken up in it several times. After an interval of half an hour or more the water is filtered through fine filter cloth,* the whole filter having been first sterilized with boiling sea-water, and is received in a sterile flask. It is then ready for use.

2. For many experiments, where larger quantities of water were required, the sea-water was not sterilized before being treated with animal charcoal. In this case, if the first part of the filtrate be rejected, the subsequent water will generally be practically sterile, and few, if any, extraneous organisms will develop in it.

3. At a later date an automatic apparatus was set up in the Plymouth Laboratory, by which very considerable quantities of sea-water could be treated with animal charcoal, and subsequently filtered through a "Berkefeld" filter; water treated in this manner we call "Berkefeld water." *Tank-water was always used in this apparatus, and was mixed with animal charcoal,† in a clean sulphuric acid carboy, by blowing air through with a pair of bellows. The mixture was allowed to settle for at least twenty-four hours and then syphoned over into an inverted bell-jar, with a tubulure at the bottom, into which the Berkefeld candle was fitted. Filtration under these conditions was found to be rather slow; so, in order to increase its rate, an apparatus was devised by which the pressure on the filter was considerably augmented.

This apparatus (see Fig. 1) consists of a glazed earthenware "tobacco jar" with two tubulures, one at the side, the other at the bottom, and a lid which can be screwed down tightly on to a rubber washer, by means of a triangular metal arrangement fitting into grooves above the lid.‡ The internal dimensions of our jars are 11 × 6 inches, and the diameter of the opening at the top is 3¼ inches. The tubulures are coned, with the smaller diameter external, and make a good fit for a No. 8 rubber bung. When setting up this apparatus, a bung, through which a short glass tube bent at right angles is passed, is fitted into the

* The filter cloth used for this purpose is the same as is made for use in filter presses, and is known as Extra-Super Swansdown. To prevent this becoming clogged another cloth, known as Hydraulic Twill, was, as a rule, used over it.

† Ca. 300 gm. to 20 litres of water.

‡ These jars were made, to our specification, by Messrs. Price, Powell, and Co., Bristol. The clamps usually supplied with such jars are not strong enough to obtain a tight joint, but these are easily replaced by stronger ones.

side tubulure. This tube is connected, by means of rubber pressure tubing, to another glass tube leading down from the bottom of a small inverted bell-jar, placed some height above (in our case 14 feet, which gives a pressure of ca. 6 lbs. to the square inch inside the jar). A screw pinch-cock on this connection serves as a tap. The carboy containing the treated water stands just above the bell-jar, and is fitted with a tightly fitting rubber bung, through which two tubes pass. One is an ordinary syphon, the other the only air inlet into the carboy. This latter automatically keeps the level of the water in the bell-jar

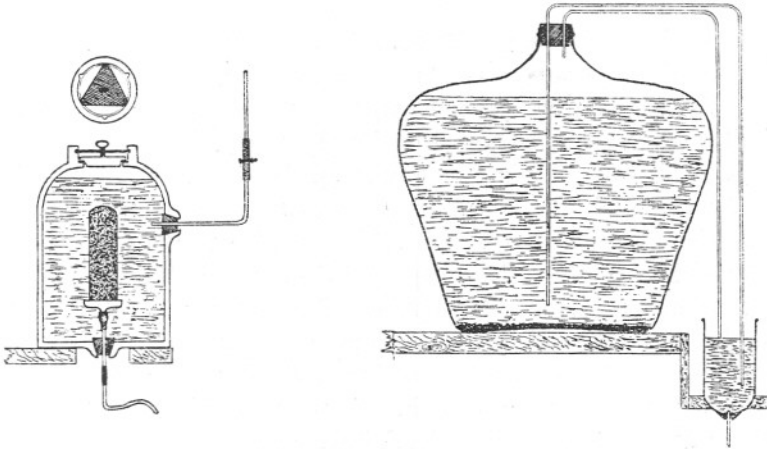


FIG. 1.—Diagram of apparatus for preparing sterile sea-water by filtration, without contact with metal.

constant, by closing the air-inlet as soon as the water covers the end of the tube. When filtering water, the *modus operandi* is as follows:—

The carboy is filled with tank-water, treated, and allowed to settle as before. The Berkefeld candle,* bung, delivery tube, and connections (see Fig. 1) are sterilized by boiling for half an hour and fitted into place from within. (The delivery tube is shaped so that any drops of water, accidentally running down outside it, do not enter the vessel receiving the filtrate; and the jar should be large enough to allow the hand to fit the filter into place without much trouble.) The pinch-cock is closed and the syphon from the carboy started, which will automatically stop if the bung has been properly fitted. This should be watched to avoid accidents. The pinch-cock is then opened until the water rises in the jar well above the top of the candle, but still leaving some air space. The lid can now be fitted into place and screwed down. The tightness of this joint can be tested by pouring a little water into

* No. 5. Porcelain mount; length 8 ins., diameter 2 ins.

the crack round the lid, and observing if any bubbles are formed when the pinch-cock is opened. If all is right, no bubbles will be seen, and a good stream of water will flow out from the delivery tube. Our apparatus will filter about twenty litres an hour, and the filtrate is exceptionally bright and clear. The candle should be sterilized every three or four days that the apparatus is in use, to avoid indirect contamination by growths of organisms through the substance of the filter.* The water while passing through this apparatus only comes into contact with glass, earthenware, and rubber, the use of metal having been purposely avoided.

(e) *Peroxide of Hydrogen Water.* As it seemed probable that the action of animal charcoal was due to contact oxidation with the oxygen occluded in the charcoal, experiments were made to determine whether a similar effect could be produced by the use of hydrogen peroxide (H_2O_2). This was used in two ways. In the first method a sufficient quantity of H_2O_2 was added to the sea-water to ensure complete sterilization (1 cc. of H_2O_2 of 20 vols. strength per 1000 cc. of tank-water was found to be satisfactory), and the excess of H_2O_2 was decomposed by adding manganese dioxide. The water was then filtered through filter cloth, and the filtrate appeared to remain quite sterile. Good cultures of *Chaetoceras constrictum*, *Biddulphia mobiliensis*, and *Skeletonema costatum* were made in this water, which seemed to be as good as water treated by the animal-charcoal method.

The second way of using the peroxide of hydrogen was to start with water sterilized by heating to $70^\circ C.$, and to add to this H_2O_2 , in small quantities at a time, until its presence could just be detected on testing the sea-water with permanganate of potash. In these circumstances, the first amounts of H_2O_2 are decomposed in the oxidation of organic substances in the water, and a very slight excess of H_2O_2 persists. For tank-water 1 cc. of 1 vol. H_2O_2 per 1000 cc. was found to give the best general effect. Cultures grown in water prepared in this way developed satisfactorily, being practically equal to those made in animal-charcoal water, but they became exhausted rather quickly.

The treatment of aquarium water with ozone was also tried, as this seems to offer a possibility of treating large quantities of water,† such as the whole bulk of water in an aquarium circulation, without very considerable expense. Experiments on a small scale, which we were able to make, unfortunately only with imperfect apparatus, showed

* See Bulloch and Craw., *Jour. of Hygiene*, VI, No. 3 (1906) p. 409.

† The use of ozonized air for the purification of fresh water for town-water supplies has been adopted in some localities. See Bridge, J. H. Paper read before Franklin Institute, reprinted in *English Mechanic* (1907), pp. 369 and 392.

that water treated with ozonized oxygen gave distinctly better cultures than untreated water. Although the sea-water was not absolutely sterilized by the treatment to which we actually subjected it, a sample of water which was visibly clouded with bacteria became quite clear and bright.

(f) *Cultures in these Media.* In order to make clear the different results, which are obtained by using these different waters, we will describe the probable result which would be got from a series of flasks set up with the following media, and each inoculated with a persistent culture of a true plankton diatom, such as *Thalassiosira*, *Skeletonema*, or *Chaetoceras*.

A. "Outside water" untreated.

Small growth in from five to fifteen days, almost immediately swamped by growths of foreign forms; the latter, however, will never be large.

B. "Outside water" sterilized.

Slightly larger growth, very soon becoming exhausted.

C. "Tank-water" untreated.

Same result as in A, but growths will be much larger, healthier, and will last longer.

D. "Tank-water" sterilized.

A fair growth of the inoculated species, but the total growth will not be as great as in C; the diatoms will retain their normal appearance for some time.

E. "Outside water" + Miquel's solutions A and B, sterilized.

Best culture in series, both in quantity and quality. The diatoms will remain normal and healthy for a very long period.

F. "Outside water" sterilized and treated with Animal Charcoal.

Fair growth, especially at first; diatoms will soon grow pale and become exhausted; better than D.

G. "Tank-water" sterilized and treated with Animal Charcoal.

As F, only growth will be slightly greater and will last considerably longer. Third best in series.

H. "Tank-water" treated with Animal Charcoal and filtered through Berkefeld filter.

This will usually be the second-best culture in the series, but the difference between this and G will only be slight.

K. "Outside water" treated with H_2O_2 .

This will most resemble F, but will not be quite so good.

L. "Tank-water" treated with H_2O_2 .

A distinct improvement over K. This medium is rather variable, and in some cases the growth obtained has been quite equal to F, if not better.

B. EXPERIMENTS WITH A VIEW TO DETERMINING THE CONDITIONS WHICH UNDERLIE THE SUCCESSFUL CULTURE OF DIATOMS.

The attempt to make cultures of diatoms for use as food, when rearing pelagic larvæ, led naturally to an effort to determine the best culture medium and the most favourable conditions for the rapid and continuous growth of diatoms. Before success can be attained in this direction exact knowledge as to the nature of the essential food-stuffs—and in fact as to the general physiology of the Diatomaceæ—is necessary.* Numerous experiments, extending over the last three years, have been carried out, with a view to obtaining such knowledge, and the results, though still by no means complete or conclusive, are perhaps worth recording.

A great difficulty which has to be met in carrying out such investigations on marine diatoms is caused by the fact that, when sea-water is used as a basis for the culture media, we are dealing with a solution of a very complex and very variable character, the exact nature of which it is extremely difficult to determine. The most direct method of research, namely, chemical analysis, has not proved of much service, owing to the uncertainty and in many cases impossibility of accurate determinations, in sea-water, of such minute quantities of substances as those upon which the growth of plankton diatoms has been found to depend.

We have had, therefore, to rely, for the most part, on the lengthy and tedious process of analysis by "trial and error," the experiments being largely conducted on lines suggested by Liebig's well-known "law of minimums" (Pfeffer, vol. i., p. 413). The ideal at which we aim is to find a culture medium, with artificially prepared sea-water as its basis, such that the absence, or diminution in quantity, of any one of its constituents would have a profound effect upon the growth of diatoms in it. Whether the conditions regulating growth in such a medium would be at all comparable to the natural conditions of life in the sea is a question that would have to be decided by experiment, but in any case this could be made a starting point for much more definite research than has yet been attempted. Up to the present time we have not, unfortunately, succeeded in finding such a culture medium. Throughout the work we have had very great difficulty, in spite of much care and many precautions, in obtaining consistent results. It may even happen that, in two flasks containing the same culture medium, inoculated with the same culture of diatom and standing side

* For general references to literature see Bibliography, especially Miquel (12), Richter (18).

by side, under exactly identical conditions, as far as can be recognized, quite different degrees of growth will be observed. All experiments must therefore be frequently repeated before entire confidence can be felt in any conclusions which they seem to indicate.

It must be remembered, also, that in all the persistent cultures of diatoms that we have used, bacteria have probably been present, and this fact has probably had some influence on the result. Unfortunately our attempts to obtain absolutely pure cultures have not met with success.

Methods. In carrying out the experiments to be described in this section the procedure has been as follows:—All media have been prepared from sterile sea-water, and sterile vessels and instruments have always been used. The cultures have usually been made in 60 cc. of liquid, in short-necked, wide-mouthed flasks of 125 cc. capacity. When a number of cultures were to be compared, the flasks were kept standing in a row together in such a way as to keep the physical conditions as similar as possible. Control cultures in standard media were included in each series, so that results from different series could be compared by reference to the controls. The various media were inoculated from a persistent culture of a species of plankton diatom, which in the great majority of cases was *Thalassiosira decipiens* (p. 460). When preparing the different media the methods used were, as far as possible, identical, and although only about 60 cc. was needed for a culture, a litre was made up, so that errors due to measuring very minute quantities might be avoided. The media were all freshly prepared for each comparative series of cultures, the same sample of sea-water being used, when the basis of any two or more was the same. Comparative estimates of the amount of growth in the different cultures were made by eye alone. Any difference between amounts of growth that has been described here as appreciable has always been accompanied by a marked difference in appearance to the eye on holding the cultures up to the light. A few drops from each culture were also, from time to time, examined microscopically as a test of the quality and purity of the growth.

The sea-water employed. The sea-water employed as a basis for the culture media has been either (1) "outside water" or (2) "tank-water." A general description of these will be found on pp. 429-431. An accurate chemical analysis of both types of water would probably make clear many difficult points, but, as already pointed out, no chemical methods of sufficient delicacy have yet been devised.

We have seen that if we compare "tank-water," i.e. water from the closed circulation of the Plymouth Aquarium, with off-shore sea-water *in situ*, a most obvious difference is the much increased density of the larger forms of animal life in the former, combined with the almost complete absence of plant life. Hence the concentration of excretory products in the tank-water must be very much higher than in outside water. Other factors, such as increased bacterial action, artificial aeration, etc., in tank-water, must also be taken into account (cf. Vernon, 58; Smith, 56). There seems to be direct evidence to show that the concentration of nitrates, possibly due to the action of nitrifying bacteria on the products of excretion, such as urea, ammonia, etc., is considerably higher in the tank-water, and the presence of soluble organic matter, in concentrations never met with in the sea, can almost certainly be assumed. It is probably due to the presence of these nitrates and soluble organic substances that sterilized tank-water is a much better medium in which to grow diatoms than sterilized outside water (see p. 435).

The constituents of Miquel's solutions. It has already been stated that no better medium for the culture of plankton diatoms has been found by us than the solutions recommended by Miquel, although these solutions may be modified and simplified in various ways with equally good results. The formulæ recommended by Houghton Gill give very similar cultures. The essential features of Miquel's and Houghton Gill's methods, when adapted to sea-water, are the same. Miquel's solution A, and Gill's solution 2, can both be replaced by a solution of potassium nitrate (p. 427). Again, Miquel's solution B and Gill's solution 1 only differ in the proportionate amounts in which the various constituents are prescribed. The formulæ are:—

	<i>Miquel's sol. B.</i>	<i>H. Gill's sol. 1.</i>
Na ₂ HPO ₄ , 12H ₂ O	4 grm.	2 grm.
Ca Cl ₂	4 ,,	4 ,,
Fe Cl ₃ (syrupus)	2 cc.	0.5 ,,
HCl (concentrated)	2 cc.	1 ,,
Water	80 cc.	100 ,,
	Use 1 cc. per 1000.	Use 3 cc. per 1000.

The proportionate amounts added to equal volumes of sea-water are:—

	<i>Miquel's sol. B.</i>	<i>H. Gill's sol. 1.</i>
Na ₂ HPO ₄	10	12
Ca Cl ₂	10	24
Fe Cl ₃	5	3
HCl	5	6

Since cultures can be obtained with no appreciable difference by using media prepared by adding either of these solutions, together with Miquel's solution A, to sea-water, a considerable latitude in the proportions of the salts present is tolerated.

We must now consider what is the *rôle* of the various constituents in Miquel sea-water. The part played by any salt of a culture medium may be considered as being either, firstly, "nutritive," or secondly, "protective."* Under the first heading, any direct addition of food material must be included; under the second, any removal or neutralization of harmful substances, such as toxins and possibly bacteria, and any more remote effects, which, although influencing growth, do not directly enter into the metabolism of the plant.

Our experiments have proved that solution A can be reduced to a simple solution of potassium nitrate† without detriment (cf. p. 427), and that the amount of growth is, within limits, roughly proportional to the amount of KNO_3 added, as the following experiment shows:—

Inoculated from persistent culture of *Thalassiosira decipiens*.

- A. Normal Miquel sea-water.
Growth as usual.
- B. Ditto, but only one-half amount of sol. A.
Good growth at first, but exhausted sooner than A.
- C. Ditto, but $2\frac{1}{2}$ times amount of sol. A.
Was slower than either A or B at start, but afterwards was better than A or B and lasted longer.
- D. Ditto, but five times amount of sol. A.
As C, but in greater degree.

Considering the nature of the substance added, and its already well-known action in plant metabolism, these results, coupled with the fact that exhausted cultures can often be regenerated by the simple addition of nitrates (see below, p. 444), are quite consistent with the assumption that sol. A is simply nutritive in action. The concentration of nitrates in natural sea-water is so low (Brandt, 47) that the amount available in a culture of untreated water very soon becomes completely exhausted, and it is this deficiency that sol. A probably corrects.

Considering now the action of sol. B, it must first be observed that increased concentration of nitrates alone will not explain the whole

* Loeb, *The Dynamics of Living Matter* (New York, 1906), p. 77.

† For the sake of convenience, the expression sol. A will be used throughout the rest of this paper to indicate a simple solution of potassium nitrate (5 per cent) and sol. B to indicate Miquel's phospho-ferricalcic solution (p. 423). Unless otherwise stated, the amounts of each added to 1000 cc. sea-water will be normal, i.e. 2 cc. sol. A and 1 cc. sol. B.

action of Miquel's solutions, for no increase in growth is obtained when nitrates or sol. A only are added to sea-water. To illustrate this point an account of an actual experiment may be given:—

Inoculated with *Thalassiosira decipiens*.

- A. Normal Miquel sea-water.
Good strong culture, in every way normal.
- B. Outside water sterilized.
Small growth at first; very soon exhausted.
- C. Ditto + sol. A.
No improvement over B.
- D. Ditto + sol. B.
Fair growth. Great improvement on B and C, but exhausted considerably before A.
- E. Tank-water sterilized.
Appreciably better than B, but growth not large.
- F. Ditto + sol. A.
Not even as good as E.
- G. Ditto + sol. B.
Next best in series to A; lasted longer than D, and had better colour.

To generalize, no improved culture is obtained with sol. A alone, but a fair, though not very lasting, growth can result from using sol. B only.

The action of sol. B is to some extent obscured by the fact that, when this solution is added to the alkaline sea-water, a precipitate is formed. This precipitate is at first white, but, on heating or standing for some time, it becomes greenish yellow. We are indebted to Mr. D. J. Matthews for the following analyses.

Ten litres of normal Miquel sea-water were prepared, and the precipitate was collected on a filter paper washed and dried at 100° C.

Weight of dry precipitate from 10 litres = 0.2949 grm.

Analysis of Dry Precipitate.

	Per cent.
P ₂ O ₅	26.36
Fe ₂ O ₃	41.31
CaO	7.63
H ₂ O	24.86
	100.16

Or, the precipitate from 1 litre of normal Miquel sea-water contains:—

P ₂ O ₅00777 grm.
Fe ₂ O ₃01218 „
CaO00225 „

An analysis of 1 cc. Miquel sol. B, the amount added to 1 litre Miquel sea-water, gave:—

P_2O_500825	gram.
Fe_2O_30105	„
CaO0145	„

Comparing these figures, it seems probable that, when added to sea-water, all the iron in sol. B is precipitated, and a certain amount also of the phosphate and calcium. The additive effect on the sea-water is therefore a slightly increased concentration of phosphate and calcium.

An analysis of a sample of tank-water for phosphorus, before and after treatment with sol. B (1 cc. per thousand), gave the following figures:—

Tank-water5 mgrm. P per litre = .00163	gram. P_2O_5
Tank-water + sol. B						
(without precipitate)	.	1.5	„	„	„	= .00488 „ „

It will be noticed that the figures from the different analyses do not agree very well. This is probably due to the fact that different samples were used for analyses in each case, and also to the fact that the solutions were made up in the ordinary way, without any special precautions, volumes, for instance, being measured in cylindrical glasses, pipettes, etc.

Cultures were tried in sea-water containing the normal amount of sol. A, plus the normal constituents of sol. B, less all the iron and less the amount of phosphate that would combine with the iron to form basic ferric phosphate ($P_2O_5 \cdot 2Fe_2O_3 \cdot 12H_2O$). This solution should have very nearly the same chemical composition as normal Miquel sea-water from which the precipitate has been removed. Successful cultures could not, however, be obtained in it. Neither could cultures be grown in sea-water to which had been added the normal amount of sol. A and 1 mgrm. P (as sodium phosphate) per litre.

To ascertain the effects of the different constituents of sol. B, experiments were carried out with separate solutions of these constituents, each of the same strength as in Miquel's formula. Different combinations of these solutions were added, together with sol. A, to sterilized sea-water, and the resulting media were inoculated in the usual way. It was found necessary to repeat these experiments a great number of times, as the results obtained were rather contradictory. To illustrate the methods used, a list of the different media, and notes on the cultures obtained in them, are given below. These media were inoculated from cultures of *Thalassiosira decipiens*, and the

cultures were kept under observation for at least four months. Series were made as uniformly as possible, and controls in standard media were included in each. The strength of the various solutions used in these experiments was the same as in Miquel's formula.

- A. Outside water + sol. A + sol. B (normal Miquel sea-water).
First control.
- B. Outside water + sol. A + Na_2HPO_4 sol. + FeCl_3 sol. + CaCl_2 sol.
Second control.
Good normal cultures were always obtained in these two controls.
- C. Outside water + sol. A + Na_2HPO_4 sol.
A very uncertain medium. Sometimes no growth has been recorded and at other times a fair growth results, but these cultures are never equal to normal Miquel.
- D. Outside water + sol. A + FeCl_3 sol.
Occasionally a very small growth has been obtained, but at the best it is very poor.
- E. Outside water + sol. A + CaCl_2 sol.
About equal to D.
- F. Outside water + sol. A + Na_2HPO_4 sol. + FeCl_3 sol.
Uncertain as C; no cultures have been obtained equal to the best in C.
- G. Outside water + sol. A + Na_2HPO_4 sol. + CaCl_2 sol.
Some cultures very nearly equal to the controls have been obtained in this medium.
- H. Outside water + sol. A + FeCl_3 sol. + CaCl_2 sol.
Poor, about equal to D.

Analysing the above results, we see that—

- (1) None of these modifications of sol. B give results equal to sol. B itself.
- (2) The best result is obtained from the combination of the phosphate and calcium chloride solutions.
- (3) Of the solutions used singly the phosphate is the best, the iron and calcium chloride being about equal.
- (4) The addition of FeCl_3 to Na_2HPO_4 , or the addition to CaCl_2 to FeCl_3 , does not improve the medium to any extent.

Experiments were also made to determine whether the precipitate thrown down in sea-water by Miquel's sol. B, itself had any influence on culture media. A quantity of this precipitate was prepared, filtered off, and then added to outside sea-water + sol. A (nitrates). A small growth was obtained, which was a distinct improvement on the control without the precipitate, but exhaustion soon set in.

Further discussion of the mode of action of sol. B, and as to whether that action is purely nutritive, or partly nutritive and partly

protective, is better postponed until a later section, after the action of animal charcoal and other substances has been considered (see p. 455).

Animal Charcoal and Peroxide of Hydrogen. The most successful culture medium for plankton diatoms, next to Miquel sea-water, is that prepared from animal charcoal (cf. p. 435). Animal-charcoal water gives at first almost as good cultures of plankton diatoms as Miquel sea-water, but the tendency to paleness and exhaustion appears much sooner. The best cultures were obtained in "Berkefeld water," that is, tank-water from the Plymouth Aquarium treated with powdered commercial animal charcoal and filtered through a Berkefeld filter. Tank-water as a basis for animal-charcoal water is very much better than outside water, probably on account of the higher concentration of nitrates, etc.

There is a very striking resemblance between the effect of animal charcoal and of Miquel's sol. B upon sea-water used for diatom cultures, and the growths obtained by using tank-water + sol. B and tank animal-charcoal water are very similar in character. If Miquel's sol. A is added to animal-charcoal water, there is a great improvement, both in the colour and quantity of diatom growth, and in the case of *Thalassiosira decipiens* the chains are long and well formed. With animal-charcoal water + sol. B, on the other hand, practically no growth was obtained.

It is possible that a certain amount of phosphate, and perhaps of calcium, from the animal charcoal, goes into solution and serves as a "nutritive" material for the diatoms. But we are inclined to think that its chief action is "protective," and due to its power of occluding gases, such gases being in a state of higher chemical activity than under normal conditions.*

As was explained in a previous section (p. 434), the possibility that the action of animal charcoal might have some sort of effect comparable to oxidation, led us to experiment with hydrogen peroxide. Fair growths of diatom could be obtained in sea-water prepared in the manner described, but they always showed a tendency to rather rapid exhaustion. As in the case of animal-charcoal water, tank-water proved a much better basis for treatment with H_2O_2 than outside water.

Reviving Exhausted Cultures. Several experiments were carried out with water from old, exhausted cultures. The sediment was filtered

* Against this view would seem to be the fact, that when powdered cocoanut charcoal, which has a still higher power of occluding gases, was used in the place of animal charcoal, very poor cultures were obtained.

the final result was, if anything, slightly better. As Miquel points out, these infusions must be made very dilute, otherwise growths of bacteria, moulds, etc., will completely swamp the diatoms. Karsten (7), in some interesting experiments, showed that *Nitzschia palea* (Kutz), W.Sm., could be made to alter completely its mode of nutrition. On placing this diatom in organic nutrient solutions, it lost all chloro-

off, the filtrate was sterilized by heat, and then treated by various methods.

In one typical experiment the following was the result:—

Water from an exhausted culture of *Skeletonema costatum* in Miquel sea-water, reinoculated with the same diatom:—

- A. Filtered and sterilized.
No growth obtained.
- B. Ditto + sol. A (nitrates only).
Good culture, but did not last very long; further addition of nitrates made no improvement.
- C. Ditto + sol. B.
No growth.
- D. Ditto + sol. A + sol. B.
Very good growth, lasting considerably longer than B.
- E. Ditto + an. char.
No growth.

Exhausted cultures in animal-charcoal water gave the same general results on treatment and reinoculation. In an old culture of *Biddulphia mobiliensis* in outside water + sol. B only, which was in a very exhausted condition (nine months old), the addition of KNO_3 gave a very rapid regeneration, and the diatoms became of normal colour and form. This renewed growth, however, did not last very long, and a further addition of KNO_3 did not give any result. The addition of sodium phosphate also failed to stimulate growth. The same rapid regeneration, on the addition of potassium nitrate, has been obtained with almost every medium, but a second attempt has always failed.

Silica. A very noticeable character of the true plankton species of marine diatom is, that their skeletons are very markedly less silicious than the great majority of other forms. Their valves are only feebly marked, if at all, and they will not stand the vigorous treatment of cleaning with acids and heat that is commonly used in the case of fresh-water diatoms. In cultural forms, this absence of silica is still more obvious, and no marking can usually be seen on even those forms which, under natural conditions, are the most silicious, e.g. *Coscinodiscus eccentricus*. Deformed and distorted frustules are the rule in certain stages of growth in our cultures, and it is often very hard to make out more than the thinnest coating of silica. It is quite probable that this deformity can be accounted for simply by the absence of a strong silicious skeleton. As a rule, the more rapid the growth, the more teratological forms will be found. In untreated outside water little deformity will take place, but in normal Miquel, where very rapid growth takes place, the diatoms may assume almost

any conceivable shape. The form of the frustules tends to come back to the normal again, when the culture is well started, and in old stages the majority will be perfectly formed, although small and pale. It was found that the addition of silica (in early experiments as fragments of potassium silicate) was, as far as could be judged, immaterial, which fact led to the conclusion that a sufficiency dissolved out from the glass flasks in which the cultures were kept. During rapid growth, it is possible that the silica does not dissolve out fast enough to supply the demand, although it is also possible that diatoms, during rapid division, cannot absorb silica and form a perfect skeleton, even when the supply is abundant. Richter (18) has proved the necessity of either CaSi_2O_5 or $\text{K}_2\text{Si}_2\text{O}_5$ for the growth of *Nitzschia palea*, grown in pure cultures. We tried the addition of silica in various forms, and in one instance, in a culture of *Coscinodiscus eccentricus*, to which a little precipitated calcium silicate had been added, the uniformity and markings of the valves were much more regular than in the control. The presence of a trace of pure, dialysed silica, also, in one experiment, gave an improved regularity of form, but the quantity or rapidity of growth did not seem to be affected. No sign of regeneration could be obtained in exhausted cultures by the addition of silica.

Organic Infusions.—Miquel recommends the use in culture media of infusions of organic substances such as bran, straw, diatom broth, etc., in addition to the saline solution. He does not make it quite clear if he ever dispensed with them at all. In his general directions, he certainly states that the addition of both saline and organic nutrient material is necessary. As would be expected from the general metabolism of plants, the saline constituents are sufficient for growth. At the same time, excellent cultures have been obtained from dilute organic infusions, both with and without the addition of Miquel's sols. A and B. About a square inch of *Ulva* was boiled in 600 cc. sea-water for half an hour, cooled and filtered. In this medium an excellent growth of *Coscinodiscus eccentricus* in one case, and *Biddulphia mobiliensis* in another, was obtained, the growth lasting for some considerable time.

Infusions, made in the same way from a small piece of fresh fish, gave the same results, and although growth was rather slower at first, the final result was, if anything, slightly better. As Miquel points out, these infusions must be made very dilute, otherwise growths of bacteria, moulds, etc., will completely swamp the diatoms. Karsten (7), in some interesting experiments, showed that *Nitzschia palea* (Kütz), W.Sm., could be made to alter completely its mode of nutrition. On placing this diatom in organic nutrient solutions, it lost all chloro-

phyll and became colourless, but in saline media the chlorophyll would not regenerate, and the nutrition change back from heterotrophic to autotrophic.*

Of course, with our infusions, it cannot be said that the diatoms were necessarily feeding on dissolved organic material, as some necessary, saline, nutritive materials could have dissolved out from the weed or fish. If the former is the case, it might explain the superiority of tank-water over outside water, since the tank-water must contain a much higher percentage of organic substances in solution. If an alternative mode of nutrition autotrophic or mixotrophic could be proved, especially in the case of the "bottom" forms of diatoms, a great many phenomena could be explained, but the evidence is as yet far too slight to warrant any such assumption.

Artificial Sea-water.—As we have explained in a previous section, the ideal aimed at, in this part of our work, has been to obtain strong growths of diatomaceae in purely artificially prepared solutions of simple salts. If this end could be satisfactorily attained, the difficulties due to the unknown and variable composition of natural sea-water at once disappear. According to van 't Hoff (35) sea-water is a solution containing salts in the following molecular concentrations:—

NaCl 100.0, KCl 2.2, MgCl₂ 7.8, MgSO₄ 3.8, CaCl₂ 1.0 (varies).

Using these molecular concentrations, a sea-water of any desired salinity can be prepared. The chlorine content of average Atlantic water is about Cl=19.4, and samples of artificial sea-water were prepared with the same chlorine value, thus:—

NaCl	26.75
KCl75
MgCl ₂	3.42
CaCl ₂51
MgSO ₄	2.10
Double-distilled water	966.47
		<hr/>
		1000.00
		<hr/>

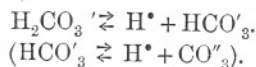
To make this solution comparable to natural sea-water, the "alkalinity" must be raised by the addition of an alkali such as Na₂CO₃. After the importance of "alkalinity" as a factor had come before our notice, 2.4 cc. M/2 Na₂CO₃ was always added to the above solution in order to make the amount of base in equilibrium with CO₂ equivalent to the usual 40 mgrm. OH°/∞ (p. 450).

* Cf. Zumstein, *Zur Morphologie u. Physiologie d. Euglena gracilis*. Leipzig. 1899.

The only success we attained with artificial sea-water as a basis for culture media was with four isolated cultures in one of our earlier experiments. Two of these were cultures of *Coscinodiscus excentricus* in artificial sea-water + Miquel's solutions A and B. The two cultures were identical except that one was in an ordinary Bohemian glass flask and the other in a "resistance glass" flask. No difference between these two could be seen. The growth obtained in both was in every way equal to normal Miquel sea-water, and is still fair, although over two years old. The other two successful cultures were growths of the same diatom in the same media, plus a small quantity of weed infusion, made by boiling up a small piece of *Ulva* in artificial sea-water. These gave just as good results, but the addition of unknown factors from the weed detracts from their general interest. In spite of frequent attempts, over fifty in number, we have not been able to repeat this experiment, which may possibly be due to some accidental impurity in the salts or distilled water from which the successful media were prepared.

Alkalinity. Tornöe (43) and Dittmar (33) were the first to investigate the fact that sea-water showed on analysis an apparent excess of base over acid, which excess they termed "the alkalinity of sea-water." Dittmar defines the alkalinity of sea-water as "a measure of its potential carbonate of lime," but this definition, and his supposition that this excess of base combines directly with dissolved CO_2 to form carbonates and, further, but only in very small proportion, bicarbonates, is liable to give a quite erroneous idea of the state of equilibrium actually occurring in the ocean. For, as Fox (34) has shown, "sea-water reacts *in situ* very nearly neutral, and actually just slightly more *acid* than distilled water." This is due to the fact that sea-water always contains a considerable quantity of dissolved CO_2 .

If a salt solution with *neutral* reaction, that is containing H^\bullet and OH' ions in concentrations equal to one another and the same as for pure water, be exposed to an atmosphere containing CO_2 , a definite amount, depending on pressure, temperature, and salinity, would go into solution. This CO_2 would combine with water and form the very weak acid H_2CO_3 , which would ionize with the formation of the free H^\bullet ions thus:—



The second stage of dissociation is so small as to be negligible. The concentration of H^\bullet being now increased, and OH' decreased, the

solution would have an acid reaction. The actual amount of CO_2 thus dissolved would always be small; for instance, a salt solution of strength $\text{Cl}=20.00$ (average Atlantic water $\text{Cl}=19.4$) will at 10°C . dissolve about .3 cc. CO_2 per litre from an atmosphere containing 3‰ CO_2 (about normal). But the ocean is found to contain very much greater quantities than this, 60 cc., or two hundred times this amount, being a not unusual figure for the total CO_2 . The difference between this amount and the .3 cc. or so dissolved by the neutral salt solution, as above, is kept in equilibrium with the 3‰ CO_2 of the atmosphere, by the amount of "excess" base equivalent to the amount of acid neutralized when an acid such as HCl is added to sea-water in excess. If a solution identical with sea-water but absolutely free from CO_2 (a practical chemical impossibility) could be obtained, then there would be present an excess of base over acid, and consequently an excess of OH' ions over H' ions, and an alkaline reaction. On exposing such a solution to the atmosphere, CO_2 would go into solution, ionize, and the H' ions thus set free would react with the OH' ions, due to the excess base, to form water. And this reaction would continue to take place, on more CO_2 dissolving, until all the excess OH' ions were neutralized, at which point the solution would react neutral. Now, as before with the neutral salt solution, a further small amount of CO_2 would go into solution, bringing the solution into equilibrium with the atmosphere, and the excess H' ions thus formed would give an acid reaction. The final result would be a solution exactly identical with natural sea-water. The total CO_2 found in sea-water can be considered as existing in two parts: the larger part in equilibrium with free base, its amount depending on temperature, pressure, and alkalinity; the smaller in equilibrium with the partial pressure of CO_2 in the atmosphere, its amount depending on temperature, pressure, and salinity. Although sea-water *in situ* has an acid reaction, it still maintains the property of being able to neutralize a certain amount of any acid stronger than H_2CO_3 , that is any acid which, on dissociation, forms a stronger concentration of H' ions; for the stronger acid will turn out the H_2CO_3 in equilibrium with the "excess base" and CO_2 will be evolved.

In consideration of these points a less confusing definition of the "alkalinity of sea-water" would perhaps be a *measure of its potential capability of neutralizing a strong acid* with the evolution of CO_2* . This can be conveniently expressed, as is usual, in mgrm. $\text{OH}'\text{‰}$.

Some of our earlier experiments seemed to show that "alkalinity" was a factor of considerable importance for the successful growth

* Such as HCl , with a high degree of ionization.

of cultures of plankton diatoms; so an attempt was made to analyse the various samples of water both before and after treatment as culture media. The method adopted was a modification of that used by Tornøe and Dittmar. Solutions of NaOH and H₂SO₄ of strength N/50, by intention, were made up and stored in 5-litre "aspirator" bottles. Two accurately graduated burettes standing side by side were connected to these by tubes, so that they could be readily filled by gravity. All air inlets to burettes and stock bottles were fitted with tubes of soda lime. A standard solution of Na₂CO₃ of exactly known alkalinity, approximately that of average sea-water (40.00 mgrm. OH %), was prepared by diluting down from a N/10 solution, all operations being performed by weighing. These standards were stored in stoppered bottles of the fairly insoluble dark green glass, but those that had been kept for any length of time were not trusted, fresh standards being prepared. On analysis these standards agreed with one another to well within .1 mgrm. OH % . The water used for diluting the standards was distilled water from the laboratory still, redistilled in all-glass apparatus with potassium bichromate and sulphuric acid.

When carrying out an analysis, equal volumes (about 100 cc.) of sample and standard were measured out into Jena glass Erlenmeyer flasks with a Knudsen automatic pipette. The specific gravity of each was determined by weighing in a 25 cc. pycnometer. Sample and standard were then titrated by running in acid from the burette and back titrating with alkali, using a 1% alcoholic solution of aurine as an indicator and keeping the liquid boiling. The acid to alkali equivalent was determined by titrating a pipetteful of double-distilled water in the same manner. The mean of at least four readings was always used. Let N and n be number of burette divisions of alkali equivalent to standard and sample respectively, and D and d their density at the time of pipetting out. Then if A is the alkalinity of the standard and X the required alkalinity of sample:—

$$X = A \frac{Dn}{Nd}$$

Since all operations were carried out at the same room temperature, no corrections for temperature are necessary.

In spite of the greatest care consistent results could not be obtained by this method of analysis. A sample analysed against the same standard would sometimes give results varying as much as 0.5 mgrm. and occasionally 1.0 mgrm. OH %. The work on indicators by Salm (42) and its application to this question has only recently come to our notice, and it is our intention to experiment on this in future research.

The figures quoted below as the results of analyses have been rounded off as whole numbers, since their interest lies in their comparative rather than their absolute value, for convenience they are quoted as "alkalinity," although we are fully conscious that the methods used do not warrant this assumption, and that their actual chemical significance is still obscure.

The mean value for "outside water" was found to be fairly constant at 40.0 mgrm. OH %₁₀₀, which figure agrees with results obtained by others for average ocean water. Samples from the aquarium tanks never gave as high figures as this, the average being approximately 37.5 mgrm. OH %₁₀₀. From this it seems that the amount of base in equilibrium with CO₂ in tank-water is appreciably less than in outside water. A series of thirteen samples taken from seven miles beyond the Eddystone to well inside the Cattewater (an inner tidal harbour near Plymouth) showed a gradual lowering of the alkalinity from the normal 40, to 38 mgrm. OH %₁₀₀ as the water became more estuarine and polluted.

The addition of Miquel's sol. B to sea-water was found, on analysis, to reduce the "alkalinity" by an amount equivalent to 10 mgrm. OH %₁₀₀ or more. The 1 cc. sol. B added to a litre of sea-water, in itself contains a certain amount of free acid, equivalent to less than 4 mgrm. OH %₁₀₀. But this reduction of alkalinity cannot be accounted for by the addition of free acid alone, because if only a quarter of the amount of sol. B is added, the alkalinity of the sample will be found to be, if anything, only very slightly higher. Also, if the various constituents of sol. B are added as separate solutions, thus obviating any addition of free acid, a reduction equivalent to about 6 mgrm. OH %₁₀₀ is still obtained. The presence of ferric chloride in sol. B gives a possible explanation of this phenomenon. If a solution of ferric chloride is added to a solution of a soluble carbonate, a reaction, which can be expressed by the following equation, takes place:—



When the ferric chloride is added to sea-water, the final result will be that a certain amount of the "excess base" which was in equilibrium with CO₂, will then be in equilibrium with the chlorine, available on the precipitation of hydrated ferric oxide, with a consequent liberation of CO₂, and a reduction in "alkalinity" will, therefore, take place.

An analogy between the actions of Miquel's sol. B and animal charcoal can be seen in the fact that water treated with animal charcoal also shows a reduced "alkalinity," the amount being very variable in different samples.

Sea-water treated with H_2O_2 also showed a lowering of the alkalinity, but in a much less degree when, as usual, minimal quantities were used.

Control experiments on double-distilled water, which had been treated with these substances, were tried, but great difficulty was found in obtaining an end point with the indicator. As far as could be judged, distilled water treated with sol. B (quantities as with sea-water) showed a negative "alkalinity," equivalent to about 8 mgrm. $OH^{\circ}/_{\infty}$, and in the case of animal charcoal a positive alkalinity equivalent to 6 mgrm. $OH^{\circ}/_{\infty}$, but the colour change was so slow that these results are only the roughest estimates. The possibility that the above results are due to some effect on the indicator, which entirely cloaks the true alkalinity, must always be taken into consideration.

Before any attempts at analysis had been made, the probability that considerable differences might be found in the alkalinity of the various media had presented itself. Improvement in the growth of diatom cultures was found to result from the purely empirical addition of $NaHCO_3$, this result being most marked in normal Miquel sea-water, outside water + sol. B only, and Berkefeld water. No growth could be obtained in either tank-water or Miquel sea-water to which had been added 1 cc. HCl (pure, concentrated) per litre, but on again raising the alkalinity of the latter by the addition of $NaHCO_3$ or KOH good normal growths resulted. Richter (18) and H. Gill (5), also, both state that a weak alkaline reaction is necessary for the growth of diatoms.

In our most recent experiments, all the media have been analysed for alkalinity, and those given in detail below illustrate the importance of determining this factor. Cultures of *Thalassiosira decipiens* were made in the following media:—

- A. Tank-water. Control.
Poor growth, hardly normal. Later, good growth of minute forms, etc.
- B. Tank-water treated with cold commercial animal charcoal and filtered.
Very good growth indeed.
- C. Tank-water treated with cold *pure* an. char. and filtered.
Very poor growth, comparable to A without minute forms.
- D. Tank-water treated with pure an. char. as in C, but the an. char. was added red hot.
Fair growth, much superior to C, but not up to B.

The sample of pure an. char. used here had been previously found to give very poor results, and it was also quite contrary to our experience that any improvement in growth should be obtained by adding it hot.

But if we examine the results of analyses of these media for alkalinity a probable explanation presents itself. The following figures are only comparative:—

A—38 mgrm.	OH ‰	(used as standard).
B—37	„	„ (higher than usual).
C—16	„	„ (very low indeed).
D—34	„	„

It will be seen that the amount of growth in each treated sample follows the alkalinity very closely.

Solutions of Na_2CO_3 , NaHCO_3 and HCl were made up, so that 4 cc. of any one contained an amount of acid or alkali equivalent to 10 mgrm. OH. From these a series of normal Miquel sea-waters of different alkalinities were prepared. Cultures of *Thalassiosira decipiens* were grown in these media.

- A. Normal Miquel sea-water. Control. A = 32.7 mgrm. OH ‰.
Perfectly normal growth.
- B. Ditto + 4 cc. Na_2CO_3 per litre. A = 41.7 mgrm. OH ‰ (= +9.0).*
No difference between this culture and A.
- C. Ditto + 8 cc. Na_2CO_3 per litre. A = 50.2 mgrm. OH ‰ (= +17.5).
Best culture in series in quality and quantity.
- D. Ditto + 4 cc. NaHCO_3 per litre. A = 42.4 mgrm. OH ‰ (= +9.7).
Slightly better than control.
- E. Ditto + 8 cc. NaHCO_3 per litre. A = 51.5 mgrm. OH ‰ (= +18.8).
As D.
- F. Ditto + 4 cc. HCl per litre. A = 22.2 mgrm. OH ‰ (= -10.5).
Fair growth but never up to control, exhausted much sooner.
- G. Ditto + 8 cc. HCl per litre. A = 11.1 mgrm. OH ‰ (= -21.6).
Poorest in series.

Except in the cases where the alkalinity was lowered by the addition of HCl , the results obtained from this series were not up to expectation. Nevertheless the majority showed a distinct improvement from increased "alkalinity" and in C, where the alkalinity had been raised 17.5 mgrm. OH ‰, this improvement was very marked.

Another point illustrated by cultural experiment is that in two samples of an. char. water, one with "outside" and the other with "tank-water" as a basis, the amount of growth in the latter considerably exceeded that in the former, and at the same time it was found that, with the tank-water, the alkalinity had not been reduced to the same extent as in the case of the outside water.

How far apparently anomalous results, which have so frequently

* Figures in parentheses are difference in alkalinity from control, in mgrm. OH ‰.

occurred in our experimental work, could be explained by unforeseen changes in "alkalinity," can only be answered by future research.

Salinity.—The salinity (or amount of salts dissolved in a litre of sea-water) of the outside water used in these experiments only varied between small limits, $S=34.5-35.5\text{‰}$. The salinity of "tank-water" is also fairly constant, the average being about $S=34.9\text{‰}$; water is only pumped up into the reservoirs at high water, spring tides, and unless the salinity on analysis is well above $S=34.5\text{‰}$ no water is taken. Experiments to show what effect salinity pure and simple had on the growth of diatoms were undertaken. Samples of sea-water of various salinities were prepared by diluting down "outside water" with double-distilled water, and by concentrating "outside water" by slow evaporation. Two litres of "outside water" $S=34.9$ were evaporated down to the bulk of one litre, giving a 50%* concentration. Miquel solutions 4 cc. A, 2 cc. B, were now added, and the solution was divided into ten culture vessels, 20 cc. in each. Double-distilled water was added, 2 cc. to the first, 4 cc. to the second, 20 cc. to the last, so that a series of media were obtained, varying in salinity from normal to nearly 50% concentration, each containing the same amount of Miquel's nutrient solutions. These were inoculated from a mixed culture of *Skeletonema costatum*, *Biddulphia mobiliensis*, and *Coscinodiscus eccentricus*. A good growth took place in all except the two with highest concentration. Of these two, the last remained practically sterile and the growth in the other was very poor. The limit of concentration, therefore, seems to lie between 35 and 40%. In the same way series of lowered salinities were prepared, and cultures of the same diatoms were grown in these. Dilution up to 100% did not seem to make any difference at all in the quantity or quality of growth. In a series extending the dilution to 200% even in the cultures of lowest salinity, a fair quantity of growth took place. The range of salinities covered by the various series was $S=12\text{‰}$ to $S=60\text{‰}$ and within these limits no effect on growth could be observed, except in the very highest, where a distinct deterioration was noted.

An attempt to grow *Coscinodiscus eccentricus* in tap water + Miquel's solutions was tried, and it was thought that some slight multiplication took place, although it was certainly not at all considerable. Inoculating a culture of normal Miquel sea-water from this after six weeks gave no growth.

Light. Of all the factors controlling the rate of growth of a culture,

* i.e. from every 100 cc. sea-water 50 cc. H_2O had been subtracted.

light seems to be by far the most important. Without light a culture soon dies off completely, showing marked signs of malnutrition very soon after having been placed in the dark, the brown pigment being the first to go, and later the chlorophyll. A culture (*Thalassiosira*) placed in the dark for five months was found to be completely killed, the diatoms being quite colourless. In cultures kept in bulbous flasks or any spherical vessel, the strongest and earliest growth always takes place at the side of the vessel away from the source of light, where the light will be found to be concentrated owing to the lens effect of a sphere of water. By painting a flask black on the outside up to the water-line of the medium, a very marked diminution in the *rate* of growth was obtained. The total growth was not affected, but depends on the available quantity of food-stuffs present.

Experiments on the reaction of cultures to different rays of the spectrum, obtained by coloured glass, were tried, but no results obtained. Miquel obtained marked results with yellow light, but in our experience, with plankton diatoms, satisfactory cultures could not be obtained under these conditions.

Temperature. The highest temperature which diatoms and allied forms can stand was about uniform for all the species tested, and lay between 35°–40° C. Cultures of the following species, viz. *Asterionella japonica*, *Nitzschia closterium*, minute naviculoid diatom, *Pleurococcus mucosus*, *Chilomonas* sp., were slowly heated in a water bath, and at every rise of 5° C. from 15° C. to 45° C., a few drops of the culture were pipetted out and a fresh flask inoculated. In all the flasks cultures were obtained where the heating process had not been carried above 35° C., but none in those where the temperature had exceeded this.

In the earlier stages of experimentation the cultures of diatoms were kept in various places about the Laboratory, and so were under quite different temperature conditions. Those placed in the warmer situations, i.e. near hot-water pipes, as a rule gave the most satisfactory results. In all the later work the cultures have been kept in one room, and an attempt has been made to keep the temperature of this room as nearly as possible constant at 15° C. A continuous record of its temperature has been kept by means of a recording thermograph, and no very great change of temperature has been noted. In a few isolated cases the temperature has dropped as low as 9° C., and in hot weather has risen just above 20° C., but these have only been for very short periods, the average temperature having kept remarkably constant. An apparatus in which flasks could be kept at

different uniform temperatures from 10° to 25° C., by means of hot air, was used, but no really satisfactory result could be obtained. About 17° C. seemed to give the maximum growth, and the cultures below this temperature were usually superior to those above.

General Conclusions. The general conclusions to be drawn from the experiments described in this section, which were made with a view to determining the conditions that underlie the successful culture of diatoms, may now be discussed. Although the experiments have involved the making of some 750 different cultures, our conclusions on many of the questions raised are still indefinite, and much further work will be necessary before a satisfactory answer can be given to them.

If we wish to obtain the maximum quantity of healthy growth of a plankton diatom, the diatom must first be obtained as free as possible from all other organisms, if not in a "pure" culture, at least in a "persistent" culture. All culture media should be sterilized either by heat or filtration, and the experiments should be conducted under sterile conditions. Starting with normal sea-water as the basis for the culture medium, it seems to be first necessary to raise the concentration of the nitrates, and possibly also of the phosphates, in solution. But this simple addition of nutrient materials will not in itself suffice. Some other action, such as that exerted by Miquel's sol. B, by animal charcoal, or by peroxide of hydrogen, seems to be imperative in nearly every case. The exact nature of this action we have not been able conclusively to determine. If the substance contained in sol. B were purely nutritive in character, we should expect that, when alterations in the amounts of the different ingredients were made, or when any one of the ingredients was omitted altogether, the differences in the quantity of growth would show a direct relation to the kind of modification introduced. But our usual experience has been that sol. B can be modified within certain limits, without producing any appreciable effect upon the resulting cultures, whilst if these limits are exceeded, there is an almost complete inhibition of growth. In supplying a necessary increase of phosphates, both Miquel's sol. B and animal charcoal may and probably do act as "nutritive" substances, but, since the addition of phosphates alone does not yield cultures comparable with those produced by either of these, and since, excepting phosphates, there is no possible common nutritive substance in their composition, we are led to conclude that, in addition to any nutritive effect, they must exert some other action. This view is supported by the results obtained by using H_2O_2 . This substance cannot be directly "nutritive," although it may be so indirectly, by oxidizing into useful

food-material substances which the diatoms are incapable of using in their metabolism, e.g. nitrites into nitrates. The absence of any increase in phosphates, when using H_2O_2 , may possibly be the reason why better results were not obtained with this medium. The action which, in addition to any nutritive value, we must assume that sol. B, animal charcoal, and H_2O_2 can all effect, would appear to fall into the class of "protective" actions (p. 439). It is quite conceivable that, with different samples of sea-water, this "protective" action is not necessary in every case, and this would account for the anomalous results met with when using sea-water + nitrates + phosphates only, in which medium sometimes good cultures, but more often the reverse, are obtained. The effect of Miquel's sol. B, animal charcoal, and H_2O_2 on the "alkalinity" of the sea-water, also points to some chemical change, which does not directly enter into the metabolism of the plants.

It may be pointed out that the action of such substances as finely powdered carbon, and ferric oxide precipitates, has been shown to produce a favourable effect on nutrient solutions used for the culture of certain higher plants, and it has been suggested that the beneficial action of these substances is the removal of toxic elements from the media (Breazeale, 3). Such removal of toxins would fall under our definition of "protective" action.

Of nutritive substances, other than those already mentioned, we have still to consider (1) silica and (2) dissolved oxygen and carbonic acid. Having regard to the conditions under which our cultures have been grown, i.e. in glass flasks, the question of silica does not seem to enter into the problems which we have discussed. A few words must, however, be said as to the dissolved gases. Whipple (62) and Baldwin (44) have drawn attention to the observed relations, which are found in natural waters, between algal growths and the amounts of dissolved oxygen and carbonic acid. That these factors are of great importance cannot be doubted, but in our cultures it seems reasonable to suppose that the conditions of saturation of these gases are the same in all, since series of cultures in standard media, such as Miquel sea-water or Berkefeld water, can be set up with the certainty that, if not every one, at least a very high percentage, will give normal results.

Of the purely physical factors, light is by far the most important. Within limits, the rate of growth in a suitable medium seems to depend directly on the intensity of the light (Whipple, 60). Absence of light, as would be expected, soon completely kills the diatoms.

Temperature also seems to affect the rate of growth to a certain extent, but for those temperatures at which we have experimented it does not appear to alter the quantity of growth.

Salinity, apart from the quantities of available nutrient materials, can be varied within large limits without appreciable effect on the diatoms.

II. MIXED CULTURES.

In what has been said up to the present, we have been dealing with persistent cultures containing a single species of diatom, which are comparatively, if not entirely, free from admixture of other organisms. The study of cultures which contain a considerable mixture of organisms is not without interest.

A number of experiments have been made on the following lines. About 10,000 cc. of water, taken at some distance from shore, was placed in a tall bell-jar fitted with a "plunger," which keeps the water in constant movement. (*Journ. Mar. Biol. Assoc.*, Vol. 5, p. 176). The water was treated with Miquel's solutions in normal proportions, and a considerable quantity of plankton taken with a fine-meshed net (150 meshes to the inch) was added, say, 10 or 20 cc. of a moderately rich sample of tow-netting. The experiments were made during the spring and summer months, and the general course of events has been the same, with a certain amount of difference in detail according to the nature of the plankton present at the time.

During the first two days the water often became cloudy, owing to the rapid multiplication of small flagellate infusoria, though this was not always the case. Plankton Copepods and other animals gradually died off, though some survived for as long as a week or ten days. The plankton diatoms, on the other hand, generally multiplied rapidly during the early days of the experiments, the first to become abundant in the body of the water being usually *Skeletonema costatum*, which at the end of a week might be so thick, that a number of chains could be seen in every drop of water examined with the microscope. Along with the *Skeletonema* were found other plankton diatoms, such as *Lauderia borealis*, *Chaetoceras* (two or three species), *Biddulphia mobilensis*, *Ditylium Brightwellii*, and in nearly every case *Thalassiosira decipiens*. These latter diatoms were present in moderate numbers only, when the *Skeletonema* was at its height; but as the *Skeletonema* died down they increased in quantity. At the same time *Nitzschia closterium* commenced to appear, both amongst the precipitate on the bottom of the jar, and in the general body of the water. Small green flagellates often began to get numerous also at this stage. The true plankton diatoms were at their height about a fortnight after the experiments were started. At this time a great many diatoms of all

kinds were to be found amongst the precipitate at the bottom of the jar, *Asterionella japonica* and *Coscinodiscus excentricus* being often numerous here. During the course of the next week, however, *Nitzschia closterium* rapidly increased in quantity until, not only the sides of the jar were coated with it, but the whole mass of the water became thick and opaque. By this time the plankton diatoms had all disappeared, with the exception of those which may survive for a considerable period amongst the precipitate at the bottom of the jar. Bottom diatoms (*Navicula*, etc.) had begun to grow on the sides of the jar, and small green and brown algæ (*Pleurococcus mucosus*, *Ectocarpus*, etc.) also appeared. Infusoria (*Euplotes* and other smaller forms) then became numerous, and as the *Nitzschia* and bottom diatoms increased on the glass, large numbers of *Amoebæ* made their appearance among them. The jars continued in this condition for many months, the algæ becoming more and more predominant.

From these experiments, as well as from instances of mixed cultures obtained in the course of our attempts to secure persistent cultures of single species of diatoms, it seems usual that, in a culture obtained by inoculating Miquel sea-water with plankton taken freshly from the sea, the true plankton diatoms are the first to develop in considerable numbers. Subsequently bottom diatoms and algæ of various kinds become abundant, and the true plankton forms die out.

A complete explanation of this sequence of events would probably be of a very complicated character, and we have practically no evidence from our experiments which bears very directly on the question. It would seem, however, that the early predominance of the plankton forms in the cultures would naturally follow from the fact that, in the plankton material used for inoculation, these plankton forms are numerous, whilst bottom diatoms and spores of algæ are rare. The subsequent very great predominance of such a species as *Nitzschia closterium* may be due simply to a very much more rapid growth rate, though it is difficult to avoid the impression that the organisms which finally take possession of the cultures, are in some way directly inimical to those which they supersede, not merely by robbing them of their food-supply, but perhaps, also, by the production of toxic substances. This suggestion does not, however, give an adequate explanation of the essential facts concerning these organisms. We have to consider two sets of species: (1) the true Plankton forms, which flourish in the open sea and can be grown quite easily in the laboratory, provided the cultures remain pure; and (2) what we may call "aquarium" or "bottom forms," which under experimental conditions invariably take possession, when present in mixed cultures, whilst the plankton forms

are killed off. Why is it that, although species of the second class are always present in small numbers in plankton taken from the sea, they are there altogether outnumbered by the true Plankton forms, whereas under conditions such as those of our experiments they invariably succeed in gaining the upper hand? What are the factors which determine the difference in behaviour of these two sets of organisms in the sea and in the culture vessels? The whole question offers a very fruitful field for further experiment. The evidence at present available is so slight that further discussion of it here is not likely to be of much service.

The details of two experiments which we have made, bearing on the subject of mixed cultures, may, however, be recorded.

A flask, containing about 1000 cc. of sea-water treated with Miquel's solutions, was inoculated with approximately equal amounts of certain persistent cultures of diatoms, which we possessed at the time. The following diatoms were in this way introduced:—*Chaetoceras constrictum*, *Biddulphia mobiliensis*, *Skeletonema costatum*, *Coscinodiscus excentricus*, *Streptotheca thamensis*. The flagellate (*Chilomonas* sp.) was also introduced, since it was present in the culture of *Coscinodiscus*. The experiment was started on August 26th, 1907. On September 6th (11 days) *Biddulphia*, *Coscinodiscus* and *Chaetoceras* were increasing rapidly and were very healthy. *Skeletonema* was not so good, and no *Streptotheca* was found.

On October 2nd (37 days) *Biddulphia* was numerous and healthy, *Coscinodiscus* was healthy but not so numerous. *Skeletonema* was poor, and *Chaetoceras* was not seen. Flagellates (*Chilomonas*) had become very numerous.

On October 31st (66 days) all the diatoms were in very poor condition, *Coscinodiscus* being slightly better than the others. The flagellates (*Chilomonas*) were extremely thick, giving the water a deep red colour.

Subsequently a small green alga (*Pleurococcus mucosus*) appeared, having probably been derived from the *Coscinodiscus* culture. This increased very greatly in quantity, whilst the flagellates became inconspicuous.

On July 28th, 1909 (1 year 11 months), some *Coscinodiscus*, which were still in a healthy condition, were seen in a sample examined from the flask. A great quantity of *Pleurococcus*, in a healthy condition, was also present, but no other organisms were noted. On this date a subculture was made from the flask in normal outside Miquel. The subculture gave a considerable growth of *Skeletonema*, the cells being, however, of a very abnormal character, and a good many normal and

healthy *Coscinodiscus* were found in each sample examined. The whole culture was crowded with *Chilomonas* in a very active state, which gave the whole contents of the flask a deep red-brown colour. Up to August 24th, the green alga (*Pleurococcus*) had not become sufficiently abundant to be detected by the naked-eye appearance of the flask, though it could be seen in samples examined with the microscope.

In another experiment, a flask of Miquel sea-water was inoculated (May 4th, 1908) from two cultures, one containing the green alga (*Pleurococcus mucosus*) and the other *Thalassiosira decipiens*. At first both did well, and on May 20th (16 days) there was a very good crop both of the diatom and the alga. Gradually, however, the alga became predominant, and on October 14th (163 days) only quite empty frustules of *Thalassiosira* could be found, whilst the growth of *Pleurococcus* was abundant and healthy. The only case where a diatom was observed to flourish in the presence of this green alga was in a culture of *Nitzschia*, a bottom form. In this case a very abundant growth of the diatom was obtained, but the *Pleurococcus* did not multiply to any extent, although it could always be found on microscopic examination.

III. NOTES ON PARTICULAR SPECIES OF DIATOMS, ON THEIR METHODS OF REPRODUCTION, AND ON OTHER ALGÆ OCCURRING IN CULTURES.

A list has been already given (p. 425) of those species of diatoms which we have obtained in "persistent" cultures. Of these, a species belonging to the genus *Thalassiosira* has been used for experimental work in the great majority of cases. We are not quite certain as to the identity of the species, but since it most resembles *T. decipiens*, Grun., we have called it by that name, although it does not exactly conform to the published descriptions of that form. The most characteristic feature of this particular species is the eccentric markings on the valves, which are also seen on the valves of the diatom *Coscinodiscus excentricus*, Ehr., and, as is typical of the genus, the frustules are united into chains by a delicate filament. Jørgensen (50, p. 96) describes the valves as "decidedly convex," Gran (49) as "flat," and both agree that there are marginal spines and a single asymmetrical spine. Our cultural forms are united together by a filament into chains, some of which are made up of five hundred cells and more, but the distance between each is considerably smaller than that figured by Gran. The valves are quite flat and the marginal spines are often

present, although this is not always the case. The odd asymmetrical apiculus can nearly always be seen. The eccentric markings have only been observed in a few isolated cases, and are then usually very indistinct. In one culture these markings on the valves were very distinct, and were also easily seen on the megafrustules (cf. below) which developed in it, but in none of the several generations of cultures started from this one have we been able to find any traces of marking at all. The genus seems to be in considerable confusion, and it is probable that the conflicting descriptions given by different observers are due to variations in what is really one species.

Persistent cultures of *Coscinodiscus excentricus*, Ehr., have also been obtained, and it is interesting to note that this diatom sometimes forms chains, but they are rather exceptional. These chains are never as long as those commonly found with *Thalassiosira*, two or four cells only being the rule. The filament joining the valves is also finer and more easily broken. The two species are quite distinct, and cultures of them can be discriminated by a practised eye.

Two species of the genus *Biddulphia* are commonly met with in our cultures, namely *Biddulphia mobiliensis* (Bail.), Grun., and *Biddulphia regia*, M. Schultze. These two forms are generally regarded as one species, but Ostenfeld (54) has recently shown that they are really distinct. We have obtained persistent cultures of both forms from several different samples of plankton, and the two species are easily recognizable, never merging into one another. When Petri dishes, inoculated from plankton (see p. 425), contain both species, the colonies can be easily distinguished with a small hand lens.

The most generally accepted theory of the reproduction of the diatomaceæ is briefly that the cells divide by simple fission, but on account of the rigid character of the cell walls each division necessitates a decrease in size of the new valve, since this must always be formed inside the old valve. So the frustules gradually get smaller and smaller as multiplication proceeds, thus necessitating some process by which the original size can be re-established. This takes place by the formation of what are known as auxospores, which ultimately form megafrustules, and these in turn multiply by division until the minimum limit of size has again been reached. There are also several special processes of reproduction, but no occurrence of any of these has been noted in our work (cf. Miquel, 14).

The diatoms in our cultures multiply by simple fission, and although there is, in nearly every case, a considerable diminution in size when compared with specimens from the plankton, this diminution soon

seems to reach a limit, where further decrease does not take place. In chains of *Thalassiosira*, several hundred cells in length, no difference in size between individuals could be made out. Auxospores are commonly formed with every species, but only in the cultures of *Coscinodiscus* and *Thalassiosira* have megafrustules been found, and in these they are very exceptional. These megafrustules seem to divide once or twice and then die or form new auxospores. What exactly is the fate of these auxospores, which are often exceedingly numerous, we have not been able to make out. It seems that cultural conditions are not favourable to this mode of reproduction, and that the auxospores do not further the multiplication of the diatom at all. If this were not the case, stages of the formation of auxospores into frustules must have been seen in some at least of the very numerous samples examined. As it is, what has been said to take place is, that the cell contents expand and force apart the valves of the diatom and emerge as a spherical body about three or four times the diameter of the parent cell. The chromatophores and diatomin then collect to one side, forming a compact cap against the cell wall. Beyond this point no stages have been found, except in the case of the few cultures where megafrustules were formed. In these the chromatophores, etc., gradually formed into the shape of the diatom (*Coscinodiscus*), the silicious coat with plain eccentric markings was easily seen inside the spore, and lastly, the cell wall of the spore burst, leaving the megafrustule free. The megafrustule was measured and found to have a diameter three times that of the parent cell.

In the case of the diatom we have very largely used for feeding larvæ, etc., namely *Nitzschia closterium*, *forma minutissima*, a great number of cultures have been made, all originating from the single drop from which the first persistent culture was obtained. The total amount of growth in all the various cultures has been enormous, and the number of generations must be quite inconceivable. No diminution in size has, however, been appreciable, and no sign of any method of re-establishment of size has been seen, although these cultures have been under constant observation for over two years. This seems to prove that the theory of gradual decrease in size with successive generations cannot be generally applied.

The following experiment on the rate of multiplication of *Thalassiosira* in normal Miquel sea-water was carried out. A single drop from a fresh and vigorous culture was kept under a microscope as a hanging-drop preparation in a moist chamber. The number of diatoms in this drop was counted from time to time, and the results are given in the following table:—

Day.	Number of frustules.	Geometric progression.
11th	59	63
14th	62	68
19th	85	85
27th	140	120
34th	170	160
41st	190	220

The curve obtained by plotting the number of diatoms against the number of days approximates the curve of an ordinary geometric progression, where the ratio is two and the periods are equal to sixteen days. To show this the figures read off from the curve at the same intervals as the diatoms are appended in the table. From this it will be seen that, after a start had been made and before exhaustion set in, the numbers obtained agree fairly closely with the assumption that every diatom divided once in a period of sixteen days. Probably in normal cultural conditions the rate of multiplication greatly exceeds this figure on account of better lighting, etc. (cp. Miquel, 12).

Besides diatoms, many other organisms appear in these cultures. We are indebted to Prof. G. S. West for the identification of a form of unicellular alga, which is very common and difficult to avoid when attempting to obtain persistent cultures of the *Diatomaceæ*, namely *Pleurococcus mucosus* (Kutz.), Rabenh. This small green alga, if once introduced into a culture of a plankton diatom, will soon multiply at the expense of the latter with its ultimate extinction. It is very hardy and cultures of it in almost every medium seem to last indefinitely. Multiplication beyond a certain point probably does not occur, but the cells retain their colour and normal shape and will start active reproduction if suitable nutrient material is provided.

In cultures inoculated from plankton many other forms of unicellular and filamentous algæ thrive. Several species belonging to the classes *Rhodophyceæ* and *Myxophyceæ* commonly occur, but we have not been able to identify them. The most usual filamentous forms of *Chlorophyceæ* are *Enteromorpha*, *Vaucheria*, *Rhizoclonium*, etc. It is interesting to note that it was the unintentional appearance of young plants of *Laminaria digitata* in some of our Petri dishes that led Mr. Drew (4) to cultivate this alga in Miquel sea-water and so discover its early life history. Cultivations of marine algæ by these methods would without doubt yield many new species, and would also provide rich material for the study of their modes of reproduction.

Many forms of flagellates live either together with diatoms or alone. Among these is an unidentified species of *Chilomonas* which we have obtained in persistent culture. It multiplies very rapidly, colouring

the whole medium a deep red-brown. It flourishes in Miquel sea-water, and its nutrition is evidently autotrophic. In one culture in Miquel sea-water inoculated with plankton a number of Coccospheres developed, probably *Coccosphæra atlantica*, Ostenf. Other flagellates and ciliated infusoria are very commonly met with, such as *Bodo*, *Euplotes*, *Euglena*, etc., which all seem to depend on the diatoms or other vegetable organisms for their food material.

IV. THE REARING OF MARINE LARVÆ.

In the rearing of pelagic larval forms of marine animals,* the principle which we have followed has been to introduce into pure, sterile sea-water the larvæ to be reared, together with a pure culture of a suitable food. As far as practicable all other organisms have been excluded from the rearing vessels. It should be added that the food used in all successful experiments has been of a vegetable nature, and has continued to grow actively in the vessels. This is important from the point of view of oxygen supply. Under the above conditions, or rather under the nearest approach to them at which we have been able to arrive, no change of water has been found necessary.

Methods.—It will, perhaps, best make the matter plain if we first of all describe the actual procedure which we now follow in the case of such an animal as *Echinus esculentus* or *E. acutus*. The water to be used is first of all prepared by treating water from the aquarium tanks with powdered animal charcoal, filtering it through a Berkefeld filter (p. 431), and collecting it in sterilized glass vessels. All instruments and pipettes are sterilized by baking in an oven, and a fresh sterile pipette is used for each operation during the progress of the work. Specimens of *Echinus* are then opened until a perfectly ripe female has been found, that is to say, one in which the eggs separate quite freely, when a portion of the ovary is shaken in sea-water.

Pieces of ovary taken from a little below the exposed surface are then placed in sterile sea-water in a shallow glass dish and shaken with forceps in order to get the eggs well separated, or a number of eggs from the centre of the ovary are drawn up with a pipette and placed in the water. A very small quantity of active sperm from a ripe male is then added, very little being sufficient to fertilize a large number of eggs. Excess of sperm should be avoided owing to its liability to putrefy. After an interval of ten or fifteen minutes the water containing the eggs is filtered through bolting silk of 100 meshes per inch, which just allows single eggs to pass through, whilst keeping

* See *Bibliography*; especially Grave (26), MacBride (28-30), Doncaster (25), etc.

back clusters of eggs or other large material. The filtrate is divided amongst a number of tall narrow beakers containing sterile sea-water, and the beakers, after being covered with a glass plate, are placed where the temperature will be uniform and not rise much above 15° C. In the course of twenty-four hours the healthy larvæ will swim up to the surface and can be easily seen and removed from vessels of this shape. They are transferred by means of sterile pipettes to jars* of sterile sea-water, about fifty to seventy larvæ being put in each jar of 2000 c.c. sea-water. At the same time, a good pipetteful of a pure culture of diatom is added to each jar. The small diatom *Nitzschia closterium, forma minutissima*, we have found most useful, as its size is suitable, and it grows well in animal-charcoal tank-water, floating throughout the body of the water, and so being in intimate admixture with the larvæ. The jars are placed in a moderate light and at as even a temperature as possible.† No further attention is necessary until the larvæ have metamorphosed. The metamorphosis takes place in from six to nine weeks after fertilization. Larvæ may be taken out from time to time and examined to see if they are feeding well. If the diatoms do not grow sufficiently rapidly in the jar, more should be added from the culture flasks. We are more often troubled, however, towards the end of an experiment by an excessive abundance of diatoms. In this case the jar may either be put in a darker place or some of the water may be drawn off and replaced by a fresh supply of sterile sea-water. Care should be taken to have a sufficient supply of food at the beginning of the experiment, so that the larvæ may be able to feed as soon as they are ready for food.

The method just described can be modified in various ways without detriment to the result. Sufficient sterilization of the water may be effected by heating to 70° C. for fifteen minutes, after which it should be aerated by violent shaking; "outside" water may be used instead of "tank-water," and may be treated with Miquel's solutions in the ordinary way, to ensure a satisfactory growth of the food-diatom.

With regard to the food organisms, we have tried to obtain as large a variety of these in pure culture as possible, and then to make trial of a number of them with each batch of larvæ on which we have experimented. If no suitable pure cultures are available, success can sometimes be obtained by adding a few drops of tow-netting, collected with a fine-meshed net (180 meshes per inch), directly to the treated

* The vessels we use are ordinary green glass sweet-jars, having a capacity of about 2000 c.c., which are kept covered with the glass stoppers provided with such jars, from which the cork band has been removed.

† In hot weather we often stand the jars in one of the tanks of circulating aquarium water, which maintains them at a very uniform temperature.

sterile water containing the larvæ. In this case one depends on the chance of a suitable food organism growing in the vessel unaccompanied by any destructive organism. On several occasions a satisfactory result has been reached by proceeding in this way, and the method is generally worth a trial, seeing that the number of larvæ obtainable from an ordinary fertilization is very large, and many different experiments are easily made with them.

We will now give details of some of the results obtained by making use of the methods described, or of their modifications.

Echinus acutus.—The first successful experiment was made with this species. Eggs fertilized on June 13th, 1905, produced healthy larvæ, 50 to 75 of which were placed, three days later, in a glass jar containing 2000 c.c. of outside sea-water, filtered through animal charcoal, to which modified Miquel solutions were added. They were fed on a diatom culture, containing a small species of *Chaetoceras*, which did not form chains, a small diatom probably belonging to the genus *Melosira*, a small Naviculoid diatom, two minute flagellates, and a small green organism, probably one of the *Pleurococcaceæ*. The vessel stood in a shallow tank, through which a stream of aquarium water was flowing, and the temperature was fairly constant at 15° or 16° C., though there is one record of 19° C. at the end of July. The first two young *Echinus* were seen on July 25th, 42 days after fertilization, and on August 1st 20 were counted. On August 5th (the 53rd day) a careful search through the jar gave 21 young *Echinus* of normal size attached to the glass, 6 minute but fully formed *Echinus*, about 23 still in the Pluteus stage, roughly half of which were well advanced. On August 16th some of the water, which had not been changed since the beginning of the experiment, was replaced by "outside" water. On October 5th (16 weeks after fertilization) 12 *Echinus* were still alive. Some pieces of red seaweed were placed in the jar, upon which the *Echinus* fixed themselves and fed. Several of these specimens lived for over a year, but sufficient attention was not given to finding suitable food for them after the metamorphosis, so that they did not grow very large.

Echinus esculentus.—Three successful experiments have been made with *E. esculentus*. In the first (eggs fertilized April 5th, 1907) "outside" water treated with animal charcoal and filtered through filter-cloth, but not otherwise sterilized, was used. A number of jars of 2000 c.c. capacity containing larvæ were set up, and, to the most of these, various diatom cultures then in our possession were added, none of which, however, gave a satisfactory result. In two jars, on the

other hand, to which no culture was added, there was considerable growth of diatoms and of a flagellate, upon which the Plutei fed. The first young *Echinus* were recorded in both jars on June 8th (64 days), but may have been present a few days earlier. Eventually from 30 to 40 metamorphosed in one jar and about 12 in the other. The temperature varied from 10.5° C. to 12.5° C.

In the second experiment (eggs fertilized June 8th, 1908), made with similar water, the larvæ were fed on a pure culture of *Nitzschia closterium*, var., and six had completely metamorphosed on July 26th (48 days after fertilization), two more subsequently coming through. The temperature was generally 15° to 16° or 17° C.

In the third experiment (eggs fertilized March 29th, 1909) aquarium tank-water treated with animal charcoal and then filtered through a Berkefeld filter was used. Plutei, fed with a pure culture of a small flagellate (probably *Chilomonas* sp.) grew satisfactorily, and eight young *Echinus* were found on June 5th (68 days after fertilization), which had probably metamorphosed some days earlier. Two other jars in which *Nitzschia closterium*, var., was used as food, were not successful, probably because the growth of diatoms became too thick towards the end of the experiment.

Echinus miliaris. In the first experiment with this species animal-charcoal Berkefeld water was used, each jar containing as usual 2000 c.c. In one jar the Plutei, from eggs fertilized on August 27th, 1907, were fed on a pure culture of *Nitzschia closterium*, var. On October 4th, i.e. thirty-eight days after fertilization, one *Echinus* had just metamorphosed. On October 29th about a dozen healthy-looking *Echini* were climbing about the jar, and many were still in a healthy condition on January 8th, 1908. Temperatures: September, 15° to 19° C., October, 16° dropping to 13° towards end, November, 12° to 11° C., December, 15° to 10° C.

To another jar containing larvæ from the same batch a few drops of fresh Plankton were added as food. The Plutei in this case fed on flagellates and *Nitzschia* which grew in the jar, and several metamorphosed.

In a second experiment with eggs fertilized on September 13th, 1907, the larvæ were fed with *Nitzschia closterium*, but although there were a few well-advanced Plutei still living on January 8th, 1908, none completed the metamorphosis.

Cucumaria saxicola. A female *Cucumaria*, one of a number in a dish containing "outside" water, laid eggs, which were fertilized, and segmented on May 12th, 1906. A number of these were placed in a

flask in 800 c.c. of "outside" water, which had been sterilized by heating and then treated with animal charcoal and filtered. About 1 c.c. of fine plankton, containing diatoms, was added to the flask on May 12th. On May 25th some of the water was poured off and a new supply added. As the amount of food seemed small, some culture of a green alga (*Pleurococcus mucosus* (Kutz.), Rabenh.) was added, and this continued to grow well in the flask. The larvæ continued healthy and formed young *Cucumaria*, of which many were still alive on July 25th, 1907, i.e. fourteen months after fertilization. Some of the water was changed in this flask on May 30th, 1906, June 18th, 1906, September 15th, 1906, and July 25th, 1907. Although many of these *Cucumaria* remained quite healthy they did not grow to any great size. Probably the food which was suitable to the larvæ and early stages, ought to have been changed as the animals grew older.

Pomatoceros triqueter. The larvæ of *Pomatoceros* are perhaps the easiest to rear, and give the most certain results of any with which we have experimented. They do well on the minute variety of *Nitzschia closterium*, but will feed upon almost any small diatom. Since the adults live in calcareous tubes attached to stones, and the tubes have to be broken open before the eggs can be obtained, it is not easy to get the latter free from infection of other organisms. If, therefore, the eggs are fertilized and placed in sterilized animal-charcoal water with only moderate precautions, sufficient growth of diatoms or other organisms will generally take place in the jar to feed the larvæ and bring them to the adult state. When once fixed to the glass the worms are very hardy and healthy, and a stream of ordinary aquarium water can be run through the jar. They then grow rapidly and attain a size equal to any found on the shore. The following experiment may be given in detail to illustrate the time occupied in development. On August 29th, 1907, eggs of *Pomatoceros triqueter* were fertilized in animal-charcoal Berkefeld water, and some pure culture of *Nitzschia closterium*, var., added. The larvæ fed well, and on October 1st (i.e. thirty-three days after fertilization) a great number had fixed on the sides of the jar and made quite normal tubes. A constant stream of the ordinary aquarium water was then allowed to run through the jar, and the worms continued to grow and flourish, reaching a large size, and are still alive and healthy (November, 1909). A similar result was obtained from the same batch of eggs by feeding on a pure culture of a flagellate infusorian. Temperatures during these two experiments were between 15° C. and 19° C.

Chaetopterus variopedatus. Four experiments were made with this

species. The food which gave most promise of success was the diatom *Nitzschia closterium*, var. Larvæ from eggs fertilized on July 20th, 1908, fed on this material lived until October 30th, and reached an advanced stage. They did not, however, adopt the adult habit and form tubes. Two larvæ were also reared to an advanced stage by using flagellates and, in later stages, the diatom *Skeletonema costatum* as food.

Sabellaria alveolata. One experiment only was made with this species on eggs fertilized on July 19th, 1908. The eggs were fertilized in "outside" water and the larvæ subsequently transferred to jars containing animal-charcoal Berkefeld aquarium water. They were fed on a pure culture of *Nitzschia closterium*, var., and kept healthy and active and developed well until nearly the end of October, when, simultaneously with a sudden drop in the temperature from 15° and 16° C. to 12° and 9° C., they sank to the bottom of the vessel and in about three days were all dead. Temperatures:—During July and August, the temperature kept fairly constant at about 17° C., with a range from 15° to 19° C. During September it was generally about 15° C., and continued at about this level until the fall in the middle of October.

Archidoris tuberculata. A good many trials have been made to rear the larvæ of nudibranchiate molluscs, but up to the present not much success has been achieved. The best experiment was one made with larvæ of *Archidoris tuberculata*. A number of veligers of this species hatched out on May 8th, 1908, from some spawn, which had just been collected from the shore. Some of these were put in a flask containing 1000 c.c. of sterilized animal-charcoal water and about 1 c.c. of fine plankton was added. On May 14th a few veligers were transferred to another flask of sterilized animal-charcoal water and some pure culture of the green alga, *Pleurococcus mucosus*, was added. Whereas the larvæ in the original flask did not live long, those provided with the green alga fed well and developed for some time. A number of them were active and vigorous on July 4th, i.e. 51 days after hatching, and several were still swimming at the end of July. On August 15th none could be seen moving, but two of those which lay on the bottom, when examined with the microscope, showed no sign of decomposition. The animal was retracted in the shell, but the tissue looked healthy, and the eye-spots and otoliths could be seen. The growth in the flask seemed to be a quite pure culture of *Pleurococcus*. Larvæ were examined again on September 14th, and appeared much as in August, the tissue still showing no sign of dis-

integration. The flask was not again examined microscopically until July 25th of the following year (1907). No sign of the larvæ could then be seen, but the culture of *Pleurococcus* remained pure and healthy.

Subsequent experiments were made with spawn, which was deposited by the females in confinement. Although the spawn hatched and gave apparently healthy larvæ, these did not live for more than a few days.

Calanus finmarchicus. A single experiment is perhaps worth recording, as showing that it ought to be possible to rear this species without great difficulty. On August 8th, 1905, to a flask containing 1000 c.c. of outside water (unsterilized) there was added $\frac{1}{2}$ c.c. of Miquel's solution B and $\frac{1}{2}$ c.c. of a 1.5 per cent solution of anhydrous sodium carbonate. A few *Calanus finmarchicus* and some decapod Zoeas were put in, together with a quantity of a culture containing mixed diatoms. On September 8th all the Zoeas were dead, but three *Calanus* were alive, and *Nitzschia* and a number of bottom diatoms were very plentiful. On September 17th the three large *Calanus* were alive and vigorous, and a considerable number of *Nauplii* were seen in the flask. By September 22nd two of the *Nauplii* had developed into young *Calanus*. These, however, did not live for more than a week or ten days, and the adults also died. The flask was abandoned on November 13th, the water in it not having been changed since the commencement of the experiment.

Echinus hybrid. A successful experiment on crossing *E. esculentus* and *E. acutus* was carried out by Mr. W. De Morgan, who was working at the Plymouth Laboratory. We provided him with treated water and diatom cultures for food, and he followed our methods. We are indebted to him for allowing us to publish these results. Some eggs from a ripe *E. esculentus* were fertilized by active sperm from *E. acutus* in sterilized water on March 29th, 1909. Healthy larvæ were obtained and were transferred two days later to tank-water, which had been treated with animal charcoal and filtered through a Berkefeld filter. A culture of *Nitzschia closterium*, var., was added as food, and the larvæ developed rapidly, feeding well. Several were completely metamorphosed on May 7th, or thirty-nine days after fertilization. In all thirty young hybrids were obtained, and a number of these are still alive and feeding on red weeds.

Sacculina carcini. Mr. Geoffrey Smith has recorded the fact (*Quart. Journ. Micr. Sci.*, LI, 1907, p. 625) that he was able to rear the larvæ of *Sacculina* up to the Cypris stage, when they attached themselves to

their host, *Carcinus maenas*. These larvæ were kept in aquarium tank-water treated with animal charcoal and filtered through a Berkefeld filter. In this case the question of food did not arise, as the larvæ do not feed after hatching. It must be noted, however, that these larvæ had previously been reared by Müller and by Delage.

Summary of Method for Rearing Larvæ. We have found that the best results in rearing marine larvæ have been attained by taking the following precautions:—

1. The eggs of the female selected must be really ripe, and the spermatozoa of the male active.
2. The smallest quantity of sperm necessary to fertilize the eggs should be used.
3. Sterile sea-water, treated in such a way that diatoms, etc., will grow well in it, should be used. No frequent change of water is then necessary.
4. All dishes, jars, instruments, and pipettes should be carefully sterilized before use. Every possible effort should be made to prevent the introduction into the rearing-jars of any organisms other than the larvæ to be reared, and organisms on which they feed. The jars should be covered with loosely fitting glass covers.
5. The eggs after fertilization must be separated from all foreign matter, pieces of ovary, or testis, etc. As soon as the larvæ swim up they should be pipetted off into fresh vessels of treated water, so as to leave behind any unsegmented eggs, etc.
6. The food organisms should be small in size, so that the larvæ can draw them into the mouth by ciliary currents. The food should distribute itself through the body of the liquid and not settle too readily on the bottom of the vessel. (This is one of the great advantages of the diatom *Nitzschia closterium*, *forma minutissima*.)
7. The food should be abundant early, so that the larvæ may commence feeding as soon as they are able to do so. The food, however, must not be allowed to get excessively thick in the water. It can be kept down by diminishing the light or by changing some of the water.
8. The temperature should be kept as constant as possible. Within limits, the actual degree of temperature is not so important as the avoidance of rapid change of temperature.
9. A good north light, not exposed to direct sunlight, is most suitable for the rearing-jars.

10. In determining the amount of water to be used in any particular vessel, regard must be had to the amount of water surface exposed to the air, which should be large in proportion to the volume of the water.
11. A change of food is generally required after the metamorphosis of the larvæ.

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On the Species *Upogebia stellata* and *Gebia deltura*.

By

W. De Morgan, F.Z.S.

As remarked by Stebbing (*A History of Recent Crustacea*, p. 185): "Upogebia, Leach, 1814, was founded to receive another species discovered by the industrious Montagu, and described by him in 1805 (1808) as *Cancer astacus stellatus*. . . . It seems to have escaped the notice of writers subsequent to Leach that the earliest name of this genus was Upogebia, which therefore must be retained in preference to Leach's own alteration of it into Gebia, or Risso's Gebios."

Some doubt still appears to exist whether *Upogebia stellata* (Leach) and *Gebia deltura* (Leach) are distinct, or merely sexual forms of the same species.

Leach gives excellent figures of *Gebia stellata* (*Malac. Podolph. Brit.*, table xxi, figs. 1-9) and *Gebia deltura* (figs. 9-10). He regarded them as distinct species, and remarks of *Gebia deltura*: "This species lives with *Gebia stellata*, with which it was confounded, until the distinctions were discovered by Mr. J. D. C. Sowerby." I am unable to find out whether Mr. Sowerby recorded his description.

Bell (*British Stalk-eyed Crustacea*, pp. 223-5) describes *Gebia stellata* and *Gebia deltura* under the genus Gebia (Leach), of the Thalassinidæ, and gives good figures of both. He appears to doubt, however, whether they are distinct species, and of *G. deltura* writes: "This species, if it be indeed distinct, differs from the former, *G. stellata*, in the following particulars: the whole animal is very much larger, sometimes not less than twice the length, and more than proportionately wider. The carapace is much broader and more spreading at the sides. The legs are more robust; the arm of the first pair is not more than twice as long as it is broad, the wrist even shorter than broad, the hand thicker, and the fingers more nearly of equal length. The setæ of the external antennæ are shorter in proportion, being, according to Leach's figure, not more than half the length of the body. The abdomen is broader, more spread, and much less firm in its texture, the sides being almost membranaceous, and the abdominal false feet larger and more voluminous than in the other species. The different lamellæ of the

tail differ also in some particulars, the exterior being rather broader than it is long, and the middle one, or terminal segment, of the abdomen nearly quadrate. In all other respects the two species very greatly resemble each other."

In a note Bell remarks: "The term 'deltoid' appears to be very much misplaced in describing this part."

If Leach referred to the central lamella of the tail, the term is misleading, as that plate is certainly subquadrate in form. But, as pointed out by Stebbing (*History of Crustacea*, p. 186), Leach was no doubt referring to the minor branch of the Uropods, which may reasonably be described as "deltoid."

Bell further remarks: "I confess I am very doubtful if it will not prove on further investigation that the two British forms, and perhaps also *G. littoralis* of Risso, constitute but one species. The form and development of the abdomen, and the great development of the abdominal false feet in *G. deltura*, are certainly very much like peculiarities belonging to the female sex, and calculated for the support and protection of the ova."

Norman appears to consider that there is only one species. In his *Crustacea of Devon and Cornwall*, p. 12, he has:—

"*Upogebia stellata* (Montagu) = *Gebia deltura* (Leach)."

At the Marine Biological Laboratory, Plymouth, I have had the opportunity of examining a good many specimens of both forms, both alive and in spirit. The two forms are always found together at Salcombe, and a day's hunting may produce a dozen specimens. *Stellata* is rather more common than *deltura*.

I have kept several of the *stellata* form in berry under circulation, and the zoæas have hatched out, and one specimen of the *deltura* form, in berry, which also hatched. In neither case, however, was I able to rear the larvæ. There are thus males and females of both forms. The genital opening of both forms is situated in the females on the basipodite of the 3rd thoracic appendage, and is covered by a diaphragm. It is very easy to see. In the males, the opening is on the basipodite of the 5th thoracic segment. Close to it, there is a small tuft of setæ. It is not so easy to distinguish as in the female.

The females of both forms possess modified copulatory appendages, and may be recognised by them, as they are absent in the males.

Among the Thalassinidæ, *Upogebia* forms a rare exception to the general rule on this point (*vide* Calman, in *Treatise of Zoology*, ed. by Ray Lankester, part vii, p. 274).

In large specimens it is easy to distinguish between *deltura* and *stellata*. The width of the abdominal plates in *deltura* is very noticeable, and the rostrum is blunter. It is altogether a more massive animal, and the spotted appearance, whence the name *stellata*,

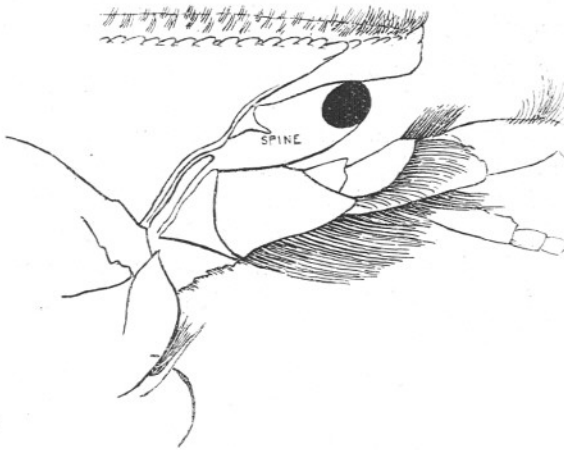


FIG. 1.—*Upogebia stellata*, showing spine.
Cam. luc. × 27.

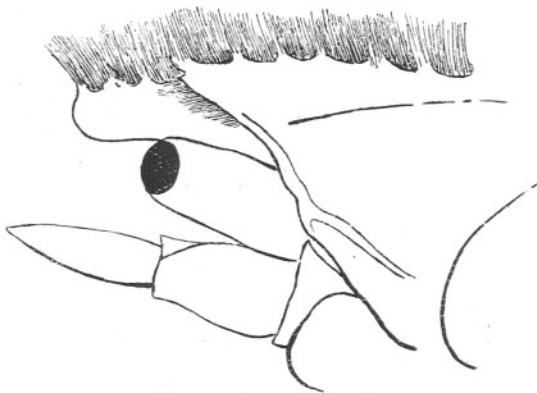


FIG. 2.—*Gebia deltura*.
Cam. luc. × 27.

is wanting. In *deltura* the dactylopod is stouter and blunter, and more nearly equals the process of the propodite in length. On the inner side, where the dactylopod hinges, there are two blunt spines. In *stellata* the "fingers" are much slighter, the dactylopod longer and slenderer, and the opposite process smaller, than in *deltura*.

The hairs on the rostrum and carapace, and also on the edges of the abdominal plates, are longer and thicker in *deltura* than in *stellata*, and give it a more shaggy appearance.

In small specimens, however, these differences are not so marked. But *stellata* has one mark which always distinguishes it from *deltura*, namely, a small spine on the curved edge of the frontal margin of the carapace behind the eye-stalks. In ordinary specimens it is easily seen; in very small ones a lens may be required to detect it, but its presence in *stellata* is constant. In *deltura* it is absent, and the margin of the carapace forms an unbroken curve. The spine is shown in the figure (*cf.* Figs. 1 and 2).

This spine is not shown in the figures of either Leach or Bell. It would hardly be visible on so small a scale; also, it would hardly be seen in the position in which the animal is drawn. From the above considerations it appears that *Upogebia stellata* and *Gebia deltura* are clearly distinct species.

Marine Biological Association of the United Kingdom.

Report of the Council, 1908-9.

The Council and Officers.

Four ordinary meetings and one special meeting of the Council have been held during the year, at which the average attendance has been ten.

The Laboratories at Plymouth and Lowestoft have both been visited by Committees of the Council.

The thanks of the Council are due to the Councils of the Royal Society and of the Linnean Society for the use of their rooms for the meetings.

The Council have been requested by H.M. Government to continue the work which they have been doing in connection with the International Fishery Investigations for a further year.

The Laboratories.

No large repairs have been necessary to the buildings and machinery at Plymouth. The new centrifugal pump has continued to give satisfactory results, and the self-sown invertebrate fauna in the tanks of the aquarium has been larger than usual. At Lowestoft, arrangements have been made with the landlord of the house occupied by the Association to continue the tenancy for a further year.

The Boats.

The steam-trawler *Huxley* has carried out the international work in the North Sea and English Channel. She was laid up at Plymouth for three months during the winter.

The *Oithona* has worked at Plymouth during the summer months, the collecting in the winter being done, as in previous years, by the sailing-boat *Anton Dohrn*.

The Staff.

Mr. R. A. Todd and Dr. W. Wallace have been promoted to the rank of Naturalist, whilst Mr. G. T. Atkinson and Mr. H. J. B.

Wollaston have been appointed Assistant Naturalists. The staff is now composed as follows:—

Director—E. J. ALLEN, D.S.C.

PLYMOUTH LABORATORY.

Assistant Director—L. R. CRAWSHAY, M.A.

Hydrographer (International Investigations)—D. J. MATTHEWS.

Assistant Naturalists—A. E. HEFFORD, B.S.C., E. W. NELSON.

Assistant Naturalist (International Investigations)—A. J. MASON-JONES, M.S.C.

LOWESTOFT LABORATORY (*International Investigations*).

Assistant Director—J. O. BORLEY, M.A.

Naturalists—W. WALLACE, D.S.C. R. A. TODD, B.S.C.

Statistical Assistant—Miss R. M. LEE, M.A.

Assistant Naturalists—G. T. ATKINSON. H. J. BUCHANAN WOLLASTON.

Occupation of Tables.

The following Naturalists have occupied tables at the Plymouth Laboratory during the year:—

- Miss BAINBRIDGE, London (Fish Parasites).
- Miss BAMFORD, Cambridge (General Zoology).
- F. J. BRIDGMAN, London (Sponges).
- A. F. COVENTRY, Oxford (Cell Lineage).
- W. C. DE MORGAN, Plymouth (Crustacea and Echinoderms).
- E. R. DOWNING, PH.D., Marquette, Michigan (Arenicola).
- G. H. DREW, Plymouth (Pathology of Fishes).
- Sir CHARLES ELIOT, K.C.M.G., Sheffield (Nudibranchs).
- T. J. EVANS, M.A., Sheffield (General Zoology).
- F. W. GAMBLE, F.R.S., Manchester (Colour Physiology of Crustacea and Fishes).
- T. GOODEY, B.S.C., Birmingham (Aurelia).
- F. H. GRAVELY, M.S.C., Manchester (Polychæte Larvæ).
- G. H. GROSVENOR, M.A., Oxford (Actinia).
- Miss A. ISGROVE, M.S.C., Manchester (Mollusca).
- J. W. JENKINSON, M.A., Oxford (Embryology).
- W. O. R. KING, Cambridge (Polychætes).
- C. H. MARTIN, B.A., Glasgow (Protozoa).
- J. PEARSON, D.S.C., Liverpool (Cancer).
- F. A. POTTS, B.A., Cambridge (Maldanidae).
- C. SHEARER, M.A., Cambridge (Histiobdella).
- Rev. J. H. SCALES, London (General Zoology).
- Rev. G. WADDINGTON, London (General Zoology).
- R. WHITEHOUSE, B.S.C., Birmingham (Fishes).
- H. J. B. WOLLASTON, Lowestoft (Fishes).
- W. WOODLAND, D.S.C., London (Gobius).

In addition to the above, twenty-four students attended the Laboratory during the Easter vacation, when Mr. G. H. Grosvenor conducted the usual course of instruction in Marine Biology.

The Library.

The thanks of the Association are due for the following books and current numbers of periodicals presented to the Library during the past year:—

- Académie Imp. des Sciences de St. Pétersbourg. Bulletin.
 American Museum of Natural History. Bulletin.
 — Report.
 American Microscopical Society. Transactions.
 American Philosophical Society. Proceedings.
 Armstrong College. Calendar.
 Australian Museum. Records.
 — Report.
 Bergens Museum. Aarbog.
 — An Account of the Crustacea of Norway, etc. ; by G. O. Sars.
 Bernice Pauahi Bishop Museum, Honolulu. Occasional Papers.
 — Fauna Hawaiiensis.
 — Memoirs.
 Board of Agriculture and Fisheries. Annual Report of Proceedings under the Salmon and Freshwater Fisheries Acts.
 — Annual Report of Proceedings under Acts relating to Sea Fisheries.
 — Report of Proceedings of Annual Meeting.
 — Return of the Number of Steam Trawlers Registered at Ports in the States of Western Europe in 1907.
 — Report on the Research Work of the Board in relation to the Plaice Fisheries of the North Sea.
 Boston Society of Natural History. Proceedings.
 Bristol Naturalists Society. Proceedings.
 British Association for the Advancement of Science. Report.
 British Museum. National Antarctic Expedition, 1901-4. Natural History.
 — Guide to the Gallery of Fishes in the Department of Zoology.
 Brooklyn Institute of Arts and Sciences. Cold Spring Harbor Monographs.
 — Science Bulletin.
 Bryn Mawr College. Monographs, Reprint Series.
 Bulletin Scientifique de la France et de la Belgique.
 Cairo Zoological Gardens. Report.
 — Special Report.
 California Academy of Sciences. Proceedings.
 College of Science, Tokyo. Journal.
 College voor de Zeevisscherijen. Verslag van den Staat der Nederlandsche Zeevisscherijen.
 Colombo Museum. Spolia Zeylanica.
 Conchological Society of Great Britain and Ireland. Journal of Conchology.
 Conseil perm. internat. pour l'Exploration de la Mer. Bulletin Trimestriel des Résultats acquis pendant les Croisières Périodiques.
 — Bulletin Statistique.
 — Publications de Circonstance.
 — Rapports et Procès-Verbaux des Réunions.
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 Danish Biological Station. Report to the Board of Agriculture.

- Kgl. Danske Videnskabernes Selskab. Oversigt.
 — Skrifter.
- Dept. of Agriculture, etc., Ireland. Reports.
 — Scientific Investigations.
 — The Department's Fishery Cruiser *Helga*.
- Dept. of Commerce and Labor, U.S.A. Pamphlets.
 — Report of the Commissioner of Fisheries.
- Dept. of Fisheries, New South Wales. Annual Report.
 — Edible Fishes of New South Wales. By D. G. Stead.
 — New Fishes from New South Wales. By D. G. Stead.
 — The Beaked Salmon (*Gonorhynchus gonorhynchus*, Linnaeus). By D. G. Stead.
- Dept. of Marine and Fisheries, Canada. Annual Report.
 — Supplement to the 32nd Annual Report.
 — Georgian Bay Fisheries Commission, 1905-8. Report and Recommendations.
 — Dominion British Columbia Fisheries Commission, 1905-7. Report and Recommendations.
- Deutsche Zoologische Gesellschaft. Verhandlungen.
 Deutscher Fischerei Verein. Zeitschrift für Fischerei.
 Deutscher Seefischerei Verein. Mitteilungen.
- Dominion Museum, New Zealand. Bulletin.
- Falmouth Observatory. Meteorological and Magnetic Reports.
- La Feuille des Jeunes Naturalistes.
- Field Museum of Natural History. Annual Report.
 — Publications.
- Finnländische Hydrographisch-Biologische Untersuchungen.
- Fisheries Society of Japan. Journal.
- The Fisherman's Nautical Almanac; by O. T. Olsen.
- Fishery Board of Scotland. Annual Report.
- Fiskeri-Beretning, 1907-8.
- Government Museum, Madras. Report.
- Illinois State Laboratory of Natural History. Bulletin.
- Imperial University, Tokyo. Calendar.
- Indian Museum. Illustrations of the Zoology of R.I.M.S. Ship *Investigator*.
- Institut de Zoologie, Montpellier. Travaux.
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- R. Irish Academy. Proceedings.
- Kansas University. Science Bulletin.
- Kommission zur wissenschaftlichen Untersuchung der Deutschen Meere, etc. Wissenschaftliche Meeresuntersuchungen.
- Kommissionen for Havundersøgelser, Copenhagen. Meddelelser, series Fiskeri, Hydrografi, Plankton.
 — Skrifter.
- Kgl. Norske Videnskabernes Selskab. Skrifter.
- Laboratoire Biologique de St. Pétersbourg. Bulletin.
- Lancashire Sea Fisheries Laboratory. Report.
- Lancashire and Western Sea Fisheries. Superintendent's Report.
- Leland Stanford Junior University. Publications.
- Linnean Society, N. S. Wales. Proceedings.
- Liverpool Biological Society. Proceedings and Transactions.

- Liverpool Marine Biology Committee. Marine Biological Station at Port Erin. Report.
- Manchester Microscopical Society. Annual Report and Transactions.
- Marine Biological Association of the West of Scotland. Report.
- Marine Biological Laboratory, Woods Holl. Biological Bulletin.
- Mededeelingen over Visscherij.
- Meteorological Office. Monthly Pilot Charts, North Atlantic and Mediterranean.
- Monthly Pilot Charts, Indian Ocean and Red Sea.
- Annual Report of the Committee.
- R. Microscopical Society. Journal.
- Ministère de l'Instruction Publique, France. Nouvelles Archives des Missions Scientifiques.
- Ministère de la Marine, France. Travaux du Service Scientifique des Pêches Maritimes en 1906.
- Musée Océanographique de Monaco. Bulletin.
- Museo de La Plata. Anales.
- Revista.
- Museo Nacional, Buenos Aires. Anales.
- Museum of Comparative Zoology, Harvard College. Bulletin.
- Memoirs.
- Report.
- Muséum National d'Histoire Naturelle, Paris. Bulletin.
- Nouvelles Archives.
- The Museums Journal.
- Naturforschende Gessellschaft in Basel. Verhandlungen.
- Naturhistorischen Museum, Hamburg. Mitteilungen.
- Neapel. Mitteilungen aus der Zoologischen Station.
- Nederlandsche Dierkundige Vereeniging. Verslag.
- Tijdschrift.
- New York Academy of Sciences. Annals.
- New York Zoological Society. Bulletin.
- Report.
- New Zealand Institute. Transactions and Proceedings.
- Nikolsk : l'Etablissement de Pisciculture.
- Iz Nikol'skagho R'ibovodnagho Zavoda.
- Norges Fiskeristyrelse. Aarsberetning vedkommende Norges Fiskerier.
- Northumberland Sea Fisheries Committee. Report on Scientific Investigations.
- La Nuova Notarisia.
- Oberlin College. The Wilson Bulletin.
- Otago Acclimatisation Society. Report.
- Work of Acclimatisation ; by R. Chisholm.
- Owens College, Manchester. On a new Phytophagous Mite, *Lohmannia insignis*, Berl. var. *dissimilis* n. var., with notes on other species of economic importance ; by C. G. Hewitt.
- The Physical Basis of Hereditary Characters ; by S. J. Hickson.
- On the systematic position of *Eunephthya maldivensis*, Hickson ; by S. J. Hickson.
- The Percy Sladen Trust Expedition to the Indian Ocean in 1905, XIX. The Stylasterina of the Indian Ocean ; by S. J. Hickson and H. M. England.
- On the Structure of *Dendrosoma radians* ; by S. J. Hickson and J. T. Wadsworth.

- Oxford University Museum. Catalogue of Books added to the Radcliffe Library.
- Physiographiske Forening. Christiania. Nyt Magazin for Naturvidenskaberne.
- Plymouth Museum and Art Gallery. Annual Report.
- Quarterly Journal of Microscopical Science. (Presented by Sir E. Ray Lankester, K.C.B., F.R.S.)
- Queensland Museum. Annals.
- Rijksinstituut voor het Onderzoek der Zee. Helder. Jaarboek.
- Verhandelingen.
- Vangstatistieken van Hollandsche Stoomtrawlers.
- Royal Society of Edinburgh. Proceedings.
- Transactions.
- Royal Society of London. Philosophical Transactions.
- Proceedings.
- National Antarctic Expedition, 1901-04. Physical Observations. Meteorology. Album of Photographs and Sketches.
- Reports of the Evolution Committee.
- Year-Book.
- Royal Society of Victoria. Proceedings.
- Selskabet for de Norske Fiskeriers Fremme. Norsk Fiskeritidende.
- Senckenbergische naturforschende Gesellschaft, Frankfurt. Bericht.
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General Work at the Plymouth Laboratory.

A report by Mr. L. R. Crawshay has been published in the Journal of the Association (Vol. VIII, Pt. 3) on an experiment in the keeping of Salmon in sea-water at the Plymouth Laboratory, which was carried out for the Duke of Bedford. Salmon smolts, which were two years old when first transferred to sea-water in February, 1906, showed signs of maturity in November of the same year. They were then transferred to fresh water, and produced fertile ova. In March, 1907, the fish were returned to sea-water, and they were again returned to fresh water, and spawned in the autumn of that year.

The smolts in the above experiment had been reared artificially in the hatchery at Endsleigh. A similar experiment is now being carried on with wild smolts.

Two reports on the Western Mackerel Fishery have been published

in the Journal. One, by Mr. G. E. Bullen, deals with the food of the mackerel, and suggests a correlation between the abundance of mackerel on the fishing grounds off the Cornish coast in May, and the amount of Copepod plankton, upon which the fish feed, present in the water at the time. The second paper, by Dr. Allen, attempts to carry the question a step further, and shows some evidence for thinking that the abundance of mackerel in May varies with the amount of sunshine in the earlier months of the year (February and March). It is suggested that the amount of sunshine influences the growth of diatoms and other plant life, which in its turn influences the Copepod plankton upon which the mackerel feed.

Mr. E. W. Nelson has again been engaged, in association with Dr. Allen, in experiments on the cultivation of marine plankton diatoms and the rearing of pelagic larvæ. A report on this work is now in preparation.

Mr. A. E. Hefford has been occupied in studying the reproduction of teleostean fishes in the neighbourhood of Plymouth by means of observations of the gonads of mature fishes, and of the eggs and larvæ taken in tow-nettings, and in a modified form of the Petersen young-fish trawl.

Records have been kept of the pelagic eggs collected at regular intervals during the early months of the present year. The eggs, with few exceptions, were kept alive in the Laboratory, and observations were made on the developing embryos and the early larval stages. An outstanding feature of the investigation is the preponderance in abundance, though not in number of species, of the eggs of unmarketable fishes over those of marketable forms, those of *Motella* and of *Callionymus lyra* being particularly abundant and of continuous occurrence.

The commencement of spawning for most of the species observed appears to have been earlier this year than usual.

The International Fishery Investigations.

The following is a summary of the work done, and of the conclusions arrived at by the scientific staff working under the direction of the Council.

SECTION I—NORTH SEA WORK.

A. WORK OF THE S.S. "HUXLEY."

From June 1st, 1908, to the end of May, 1909, the *Huxley* made nine voyages, in the course of which 193 hauls of the commercial trawl were made, together with 116 hauls of various smaller nets and gear. The total number of voyages made by the *Huxley* from the commence-

ment of the investigations to the present time is 108; the total number of hauls made with commercial trawls is 1447, that with smaller gear, 1269.

TRAWLING INVESTIGATIONS.—The investigation of fixed stations and fixed lines, which was carried out in the spring of 1908, was repeated in June and August of that year, the gear used being the same as on the first occasion. Trawling also took place along the East Anglian coast, and in The Wash, for the collection of soles; and on the Dutch, Danish, and English coasts, in order to obtain plaice for Vitality Experiments and for Transplantation. The Association is indebted to the Eastern Sea Fisheries District Committee, and to Mr. H. Donnison, their Inspector, for assistance rendered by the *Protector* in connection with the first of these operations. The *Huxley* also obtained eight boxes of plaice from Teignmouth Bay, for the purpose of otolith investigation.

DREDGING INVESTIGATIONS.—Various descriptions of small gear were used both at the fixed stations and elsewhere. During the year 39 samples of the sea bottom were added to the collection already made.

FISH MEASURED.—Over 107,000 fish were measured at sea during the year. As in past years, the entire catch was measured on nearly all occasions. The details as to the number of plaice, haddock, and other species dealt with are as follows:—

YEAR.		PLAICE.	HADDOCK.	OTHERS.		TOTALS.
1902-8*	...	139,964	48,513	295,247	...	483,724
1908-9	...	34,821	1,810	71,153	...	107,784
	Totals	174,785	50,323	366,400	...	591,508

During the past winter the measurements and maturity examination of plaice have been continued on the smacks and fish-market at Lowestoft. From the end of October, 1908, to the close of March, 1909, over 17,000 plaice from the south part of the North Sea have been dealt with.

MARKING EXPERIMENTS.—From the commencement of the investigations 15,887 plaice, together with 713 soles and 552 other fish, have now been marked and liberated by the Association. Of these, 3515 plaice, 51 soles, and 110 other fish have been recovered.

During the year 1908-9, 1385 marked plaice were liberated approximately at the position at which they were captured. The majority of these were marked in the southern extremity of the North Sea, in March, with a view to casting further light on the movements.

* Excluding certain small fish caught in small gear in 1907.

of the large spent fish which are then leaving this portion of the sea. It is intended to conduct similar experiments in January, 1910, as the plaice whose movements it is desired to study can usually be obtained in greater numbers earlier in the year.

Over three thousand fish also were taken in the North Sea and transplanted to other grounds. Of these, 2550 were transplanted to the Dogger Bank, having been procured in about equal numbers on the English, Dutch, and Danish coasts. Plaice taken to the Dogger Bank from the Dutch and Danish coasts in May, 1908, were found, on recapture in December last, to have differed somewhat in their rate of growth. It was accordingly considered advisable to secure comparable data as to the growth rate, on the Dogger Bank, of plaice brought from different localities. During the year 473 plaice were taken to the Devon bays. Twenty-three plaice were brought from the Barents Sea by a member of the staff on the steam trawler *Princess Louise*, and notwithstanding the great change in temperature experienced on the voyage, were liberated in apparently good condition in the North Sea. Twelve of these fish have been recovered, and were found to have grown at a rapid rate.

The following table gives the particulars as to the numbers of plaice recaptured during the year, with the exception of these twelve fish.

Year of Liberation.				Recovered from Ordinary Marking Experiments.	Recovered after Trans- plantation to Dogger Bank. Devon Bays.	
Prior to June 1, 1906	4	13	—
June 1, 1906, to May 31, 1907	25	96	—
„ 1907	„	1908	...	140	211	—
„ 1908	„	1909	...	293	49	30
Total	<u>462</u>	<u>369</u>	<u>30</u>

There have thus been recaptured during the year 861 plaice.

VITALITY EXPERIMENTS.—The plaice caught in ten hauls of the trawl, 3407 in all, were subjected to experiments designed to determine the length of time it is necessary to keep trawled plaice in circulating sea-water, in order satisfactorily to test their condition. While no period was found in all cases sufficient for such tests, the retention of the fish for twenty hours in the tanks, with periodic removal of dead fish, appears greatly to reduce the errors of experiment. A discussion of these experiments has been added to the report on the previous Vitality Experiments carried out by the Association, which has now been published.

B. LABORATORY WORK.

AGE AND GROWTH OF PLAICE.—During the last year a report has been issued dealing with the size and age of plaice at maturity in the North Sea and English Channel. A report has been in preparation dealing with observations on the age and size of over 19,500 plaice collected at different seasons and in different years over a wide area of the North Sea and in the western part of the English Channel. The ages of these fishes have been determined by examination of their otoliths or ear-stones. The investigation of this material has enabled the average size of plaice of given age on many different fishing grounds to be determined, and has brought to light some interesting differences.

Considerable pains have been taken to determine the true average growth of plaice in the region between the English and Dutch coasts, a task which is complicated by the circumstance that the size of plaice of the same age varies according to the distance from land. This difficulty has been overcome by determining the ages and sizes of all plaice caught in continuous lines of trawlings extending from the Dutch coasts into the open sea. The results of several series of observations along these lines agree very closely. They show, among other things, that the average growth of plaice in this region during the first three years is at the rate of 6-7 cm. a year; plaice of three years old averaging 20-21 cm. (about 8 inches) in length. In the western part of the Channel growth of young plaice is more rapid, the average length at three years old being 28 cm. (11 inches).

Plaice do not arrive on the Dogger Bank in any considerable numbers until they are about four years old. In the following year, judging from the average size of five-year-old plaice in this region, they grow faster than plaice of the same age in the southern and eastern parts of the North Sea. This observation is in harmony with the results of the transplantation experiments.

A somewhat sudden diminution in the average rate of growth takes place at the age at which the majority spawn for the first time. In the western part of the Channel these phenomena occur about two years earlier than in the central part of the North Sea.

An investigation of the proportions of the sexes at different ages in collections from the North Sea and English Channel has also brought to light interesting differences, which also appear to be associated with the age at which maturity first occurs in the two sexes in the two regions.

A comparison of the number of plaice of different ages caught per

hour with the trawl (1) in May and (2) in September at various distances along a line between Texel and the Leman Banks, has shown that the mass of each age group is situated further from the Dutch coast in the latter than in the former month. These observations distinctly indicate an off-shore movement of shoals of successive ages in the course of the summer, as has been shown by the results of marking experiments. These results thus confirm conclusions arrived at by Garstang (Internat. Investigations, Mar. Biol. Assoc. Report I, p. 93) from a study of the trawling investigations carried out by the s.s. *Huxley* concerning the source of the plaice found on the Leman Banks and Ground, and those of Redeke (Procès Verbaux, III, Ap. H) concerning the distribution of plaice off the Dutch coast.

TRAWLING INVESTIGATIONS.—The particulars of the trawling stations of the s.s. *Huxley* in 1904–5, illustrated by charts of the trawling courses, have been published, together with detailed measurements of the plaice caught in these years, and summaries, in 10 cm. groups, of these and other species of fish taken in each haul.

An examination of the catch per hour of plaice and dabs taken at the fixed trawling stations during 1908 with the otter and beam trawls, both partially covered by small-meshed net and uncovered, has been made, with a view to gaining information as to the constancy of action of each description of gear, and of the relative powers of capture of the otter and beam trawls. Catches from comparable hauls made in earlier years have also been examined, and the catches from day and night hauls made under similar conditions compared.

From this examination it appears that the day and night catches of plaice made by the s.s. *Huxley* in the North Sea as far north as the Dogger Bank do not differ appreciably. The comparative catching powers of the otter and beam trawls agreed closely with those arrived at by Garstang (Report on the Trawling Investigations, 1902–3, Internat. Investigations, Mar. Biol. Assoc. Report I, 1902–3, p. 74) for the action of these nets in the same region on a similar bottom.

EXPERIMENTS WITH SMALL-MESHED NETS COVERING THE COMMERCIAL TRAWLS.—The measurements of fish taken in trawls covered in whole or in part by small-meshed net have been tabulated. Eliminating twenty experiments in which the net was torn, a total of over 112,700 fish, taken in 118 hauls, have been treated.

In all these experiments the cod-end was covered, sometimes alone, sometimes with part of the batings, with all the batings, with the batings and square, or with all the trawl except the belly. The

following table shows the numbers of fish of various species dealt with:—

	NUMBER		TOTALS.
	Measured.	Computed.	
Plaice	12,565 ...	— ...	12,565
Dabs	47,321 ...	9,638 ...	56,959
Haddock	21,070 ...	— ...	21,070
Cod	1,136 ...	— ...	1,136
Whiting	14,556 ...	6,447 ..	21,003
Totals	96,648 ...	16,085 ...	112,733

From a first examination of the results the following table has been drawn up, showing approximately for the various species the sizes at which 50 per cent of the fish are retained in the cod-end:—

	OTTER TRAWL.	BEAM TRAWL.
Plaice	—	10·5 cm.
Dabs	14 cm.	12 cm.
Haddock	19 cm.	—
Whiting	19–20 cm.	14·5 cm.
Cod	ca. 20 cm.	ca. 14 cm.

It will be seen that the beam trawl, which has a smaller mesh than has the otter, retains a larger percentage of the smaller fish.

MARKING EXPERIMENTS.—The plaice-marking experiments of the years 1906–8 are under examination. The results of these experiments confirm conclusions drawn from previous experiments made in the Southern Bight and on the Eastern Grounds, and add considerably to the knowledge of the movements of plaice on the Flamborough Off and adjacent grounds.

INVERTEBRATE FAUNA.—The report on the Invertebrate Fauna is approaching completion. The records have been classified in grounds whose delimitation has been carried out with reference primarily to the texture of the bottom, with, in the case of the larger grounds, subdivisions based on depth or average salinity, or made by arbitrary lines.

A study has also been made of the frequency of capture of the various species in the grounds chosen, and of the comparative importance of depth and texture in determining distribution in the North Sea.

BOTTOM DEPOSITS.—A report on the bottom deposits is approaching completion. It is based on the examination of 568 samples, together with records obtained from material brought up in trawling and dredging. The distribution of various grades of deposit has been

studied, and a provisional division of the southern part of the North Sea into grounds on the basis of the textures of the bottom carried out.

BOTTOM TRAILER EXPERIMENTS.—The particulars obtained from the cards returned from Mr. Bidder's Bottom Trailer experiments have been arranged and analysed. Three new series of experiments conducted in 1906 have been examined, and three old series of 1904 and 1905 revised. The direction of the bottom currents and the approximate velocities have been ascertained. The series are mutually confirmatory in their indications. A very large number of the bottles have been recovered, 81 per cent of the cards from the first series having been returned. The percentage returned within twelve months of their being put out is between 50 and 60 per cent.

C. FISHERMEN'S RECORDS.

A report on the Lowestoft Trawling Records, dealing with plaice and soles, has been completed and published.

A report on the catches of plaice, soles, turbot, and brill by the Grimsby trawlers is approaching completion. The monthly average catches in different areas have been calculated and analysed. The report deals with 13,246 hauls, made from 1904 to 1907, during nearly 50,000 hours' fishing.

All these species are found to be relatively very numerous on the Eastern Grounds, off the Danish coasts, and to decrease rapidly from east to west, and all, with the exception of brill, show fairly regular seasonal variations in several areas. Plaice show an off-shore movement from the Eastern Ground in the summer, large plaice appear to migrate southwards in the winter, while small plaice disappear almost entirely from the catches at this time.

Soles show a very definite distribution. They are limited to the grounds south of a line drawn from the Horn Reef North Grounds to the neighbourhood of Flamborough Head. North of this line in the region investigated they are very scarce.

Turbot and brill have also been examined.

The records are now being examined with regard to the catches of cod, haddock, and whiting, and the monthly averages for the period 1904 to 1907 have been calculated for each area.

The records have yielded material for determining the relation between various statistical units, and factors connecting the rate of fishing per voyage, per day, per haul, and per hour have been calculated.

SECTION II.—HYDROGRAPHIC AND PLANKTON WORK
IN THE ENGLISH CHANNEL.

In August, 1908, the southerly flow of comparatively fresh water from the Irish Channel was well marked, and the salinity at Station 4, near Parson's Bank, was much lower than in mid channel on the line from Plymouth to Ushant. Any division into layers of varying salinity was less than might have been expected during this month.

By November salinities had increased everywhere in the English Channel, and were nearly the same from surface to bottom. In the Irish Channel, however, the saltier water normally found under the north coast of Cornwall had spread some distance seawards, under the influence of strong easterly winds, as a thin surface film.

Hydrographic investigations had, at the end of 1908, been carried out in the English Channel for six years, and had shown that the water eastward of Start Point is nearly always of the same composition from surface to bottom. In view of the fact that surface water samples are collected every fortnight on four cross-Channel steamers, it was decided to confine the work eastwards of a line drawn from Start Point to the Channel Islands to surface observations only, and to add seven other stations to the westward of the area usually investigated. Five of these new stations lie on the eighth meridian, and No. 37, the most southerly, is a short distance beyond the edge of the continental plateau. The depths here vary very irregularly, but soundings of from 400 to 500 fathoms are to be expected.

The February cruise of 1909 was the first made under the new programme. The water was everywhere nearly homosaline: at Station 37 the salinity was 35.53 ‰ at all depths down to 450 m. (246 fthms.). Unfortunately the wire was not long enough to allow of observations below this depth, and no bottom was found.

The observations in the Irish Channel in May, 1909, show rather complicated conditions, a thick layer of salt water being here superimposed on one of lower salinity. It is probable that this distribution is due, as in November, to strong easterly winds.

At Station 37 the conditions were the same as in February, with the exception that the temperatures were slightly lower, and the surface layer had risen to 35.61 ‰ salinity. On the bottom, however, which was not reached in February, the water had a salinity of 35.62 ‰ and a temperature of 13°. The high bottom salinity has been noticed by several observers, and is generally attributed to a current from the Mediterranean. Until further confirmation is forthcoming, however, the high temperature must be considered doubtful, as it was measured

with a single reversing thermometer, and these instruments ought to be used in pairs, owing to their liability occasionally to give incorrect readings.

Samples of Plankton have been taken as usual on the quarterly cruises, and also at fortnightly periods on light-vessels on the southern and western coasts, and by the s.s. *Devonia* midway between Plymouth and the Channel Islands. Weekly samples have been taken at Plymouth.

The records of species caught on the quarterly cruises are published in the Bulletins of the International Council.

Zooplankton was very abundant in May, August, and November, in the Bristol Channel and around the Scillies. During May, *Pseudo-calanus* preponderated in the samples from this region. In August and November, *Calanus* was present in greater quantity at the western stations.

A new species of *Tintinnus*, which first appeared at E. 20 (off Start Point) in November, 1907, was found occasionally during May, August, and November, 1908, at isolated points from S.W. of Milford, through the English Channel, to the eastern stations, while a few specimens were taken in a netting from Longsands, in the North Sea, during August.

Noctiluca appeared off Milford in May, 1908, and was abundant in the Bristol Channel in August. It gradually spread eastward during November, and has been found very thickly distributed in the mouth of the Channel and at Plymouth during February and May, 1909.

During the year no less than five species of *Ceratium*, characteristic of warm seas, have been taken as far east as the Casquets area. Four were taken in February, 1908, west of Ushant. In May, one of these species dropped out and another appeared in its place. Two were taken in August and four in November. One species appeared off Milford in November.

Observations have been made on the food of *Noctiluca*, and records kept of the diatoms found in them at various times.

Published Memoirs.

The following papers, either wholly or in part the outcome of work done at the Laboratory, have been published elsewhere than in the official publications of the Association:—

DOWNING, E. R.—*The Connections of the Gonadial Blood Vessels and the Form of the Nephridia in the Arenicolidae*. Biological Bulletin, vol. 16, 1909, pp. 246-258.

GAMBLE, F. W.—*The Influence of Light on the Coloration of Certain Marine Animals (Hippolyte, Wrasses)*. Transactions Manchester Literary and Philosophical Society. January 12, 1909.

GOODEY, T.—*A Further Note on the Gonadial Grooves of a Medusa, Aurelia Aurita*. Proceed. Zool. Soc., 1909, pp. 78-81.

MARTIN, C. H.—*Some Observations on Acinetaria*. Quart. Journ. Micr. Sci., vol. 53, 1909, pp. 629-664.

Donations and Receipts.

The receipts for the year for the ordinary work of the Association include the grants from His Majesty's Treasury (£1000) and the Worshipful Company of Fishmongers (£400), Special Donations (£273), Annual Subscriptions (£114), Rent of Tables in the Laboratory (£55), Sale of Specimens (£441), Admission to Tank Room (£120).

The following is a list of the Special Donations:—

	£	s.	d.
Colonel W. Harding	100	0	0
G. H. Fowler, Esq., PH.D.	25	0	0
E. T. Browne, Esq.	25	0	0
John F. P. Rawlinson, Esq., K.C., M.P.	21	0	0
A. E. Shipley, Esq., D.SC., F.R.S.	20	0	0
The Duke of Bedford, K.G.	15	15	0
W. Ambrose Harding, Esq.	15	15	0
E. Waterhouse, Esq.	10	10	0
Lord Avebury, F.R.S.	10	0	0
The Earl of St. Germans	5	0	0
The Right Hon. A. J. Balfour, M.P.	5	0	0
Professor A. Bevan	5	0	0
R. Gurney, Esq.	5	0	0
E. H. Parker, Esq.	5	0	0
F. G. Sinclair, Esq.	5	0	0
	<u>£273</u>	<u>0</u>	<u>0</u>

Vice-Presidents, Officers, and Council.

The following is the list of gentlemen proposed by the Council for election for the year 1909-10:—

President.

Sir RAY LANKESTER, K.C.B., LL.D., F.R.S.

Vice-Presidents.

The Duke of ABERCORN, K.G., C.B.
The Duke of BEDFORD, K.G.
The Earl of ST. GERMANS.
The Earl of DUCIE, F.R.S.
Lord AVEBURY, F.R.S.
Lord TWEEDMOUTH, K.T.
Lord WALSINGHAM, F.R.S.
The Right Hon. A. J. BALFOUR, M.P.,
F.R.S.

The Right Hon. JOSEPH CHAMBERLAIN, M.P.
The Right Hon. AUSTEN CHAMBERLAIN, M.P.
A. C. L. GÜNTHER, Esq., F.R.S.
Sir JOHN MURRAY, K.C.B., F.R.S.
Rev. Canon NORMAN, D.C.L., F.R.S.
EDWIN WATERHOUSE, Esq.

Members of Council.

G. L. ALWARD, Esq.
W. T. CALMAN, Esq., D.Sc.
Prof. A. DENDY, D.Sc., F.R.S.
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S. F. HARMER, Esq., Sc.D., F.R.S.

Commander M. W. CAMPBELL HEPPERT, C.B., R.N.R.
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J. J. LISTER, Esq., F.R.S.
P. CHALMERS MITCHELL, Esq., D.Sc.,
F.R.S.
EDGAR SCHUSTER, Esq., D.Sc.
Prof. D'ARCY W. THOMPSON, C.B.

Chairman of Council.

A. E. SHIPLEY, Esq., D.Sc., F.R.S.

Hon. Treasurer.

J. A. TRAVERS, Esq.

Hon. Secretary.

E. J. ALLEN, Esq., D.Sc.

The following Governors are also members of the Council:—

G. P. BIDDER, Esq., M.A.
E. S. HANBURY, Esq. (Prime Warden
of the Fishmongers' Company).
E. L. BECKWITH, Esq. (Fishmongers'
Company).
Sir RICHARD MARTIN, Bart. (Fish-
mongers' Company).

Prof. G. C. BOURNE, D.Sc. (Oxford
University).
A. E. SHIPLEY, Esq., D.Sc., F.R.S. (Cam-
bridge University).
Prof. W. A. HERDMAN, D.Sc., F.R.S.
(British Association).

Dr.

Statement of Receipts and Payments for

	£	s.	d.	£	s.	d.
To Current Income :—						
H.M. Treasury	1,000	0	0			
Fishmongers' Company.....	400	0	0			
Annual Subscriptions.....	115	9	0			
Rent of Tables	55	5	0	1,570	14	0
„ Extraordinary Receipts :—						
Donations as per Report				273	0	0
„ Charter of Steamboats :—						
S.S. <i>Huxley</i> , for half year	300	0	0			
S.S. <i>Oithona</i> , for special voyage	25	0	0	325	0	0
„ Special Grant from the Fishmongers' Company made in advance to qualify Sir Richard B. Martin as life member of the Council				500	0	0

£2,668 14 0

Examined and found correct.

(Signed) N. E. WATERHOUSE, A.C.A.
W. T. CALMAN.

ARTHUR DENDY.
L. W. BYRNE.

30th June, 1909.

the Year ending 31st May, 1909.

Cr.

	£	s.	d.	£	s.	d.
By Balance from last year, viz. :—						
Loan from Bank	700	0	0			
<i>Less</i> Cash at Bank	£437	5	7			
Cash in hand	18	19	11	456	5	6
						243 14 6
„ Current Expenditure :—						
Salaries and Wages—						
Director	200	0	0			
Assistant Director	200	0	0			
Naturalist	175	0	1			
Salaries and Wages	639	0	2	1,214	0	3
Travelling Expenses				47	5	6
Library.....				101	6	5
Journal.....	132	11	3			
<i>Less</i> Sales of Journal	23	17	8	108	13	7
Buildings and Public Tank Room—						
Gas, Water, and Coal	107	12	6			
Stocking Tanks, Feeding, etc.	52	6	1			
Maintenance and Renewals	61	12	9			
Rent of Land, Rates, Taxes, and Insurance	39	17	8			
	261	9	0			
<i>Less</i> Admission to Tank Room	120	0	10	141	8	2
Laboratory, Boats, and Sundry Expenses—						
Stationery, Office Expenses, Printing, etc.....	155	8	7			
Glass, Chemicals, and Apparatus.....	£160	6	10			
<i>Less</i> Sales	46	0	0	114	6	10
Purchase of Specimens				55	14	4
Maintenance and Renewal of Boats, Nets, Gear, etc., exclusive of s.s. <i>Huxley</i> £173 14 9						
<i>Less</i> Sales	18	14	1	155	0	8
Insurance of Steamers—						
S.S. <i>Huxley</i>	£215	11	3			
S.S. <i>Oithona</i>	20	13	7	236	4	10
Coal and Water for Steamers, excluding s.s. <i>Huxley</i>				98	3	6
				814	18	9
<i>Less</i> Sale of Specimens, etc.				441	2	11
Bank Interest						373 15 10
						14 11 8
„ Extraordinary Expenditure :—						
Purchase of s.s. <i>Huxley</i> —						
Second instalment of purchase price	150	0	0			
Interest on Loan	46	5	7	196	5	7
(The balance of the purchase price is secured by a Mortgage of the vessel repayable by annual instalments.)						
By Balance, including Special Grant of £500 per contra, applicable to the years ending 31st May, 1910 and 1911						
Cash at Bank	713	10	3			
Cash in hand	14	2	3			
	727	12	6			
<i>Less</i> Loan due to Bank	500	0	0	227	12	6
This Balance is apportioned as follows :—						
General Account	£ 49	11	2			
Repairs and Renewals Account.....	178	1	4			
	227	12	6			

£2,668 14 0

An Experiment in the Transplantation of Plaice from the Barents Sea ("White Sea") to the North Sea.

By

George T. Atkinson,

Assistant Naturalist at the Lowestoft Laboratory.

ABOUT midnight on 26th June, 1908, at the close of a voyage to the White Sea fishing grounds, undertaken by Mr. A. E. Hefford and myself in the Hull steam trawler *Princess Louise*, H 837, Captain Turner of that vessel did me the great favour of taking a short haul to procure plaice for transplantation to the North Sea.

OBJECT OF THE EXPERIMENT.—The object of this experiment was to test if it were possible for plaice to survive such physical changes as are necessarily involved in this great change of habitat, and to see further if they would display any feature of growth.

The result has been that not only do the fish appear to have survived, but they have grown in a most remarkable manner. The rate of growth shown by the last five specimens recaptured is much greater than that of North Sea plaice of the same sizes and sex, which have been marked in the same way and set out again on the grounds where they have been caught. The growth has been many times faster than that indicated by the otoliths of plaice in the portion of the Arctic Ocean from which they were brought.* This unusually rapid growth has been accompanied by considerable improvement of the fish as a marketable commodity.

The object of the voyage in the *Princess Louise* was to continue the investigations, commenced in the *Roman*, of the conditions of the plaice fishery in the Barents Sea.† It had been arranged to again accompany the *Roman*, H 948, but as Captain Leighton sailed a day earlier than was intended, he kindly arranged for Mr. Hefford and

* As an illustration of the extremely slow growth which obtains in these northern waters, I have in my possession a photograph by my colleague, Mr. R. A. Todd, of ten otoliths of as many fish of the VIII group (in these cases fish just nine years old), five of these fish are from the North Sea and five from the Barents Sea; 47, 48, 52, 52, and 54 cm. were the lengths of the former, which were all mature females: the lengths of the latter three immature females and two mature males were only 30, 30, 32, 27, and 29 cm.

† *Journ. Mar. Biol. Assoc.*, VIII, 1908, p. 71.

myself to leave Hull with Captain Turner and to transfer to his own ship on the fishing grounds.

Unfortunately we were unable to meet as arranged, and this mishap deprived us of the use of two tanks which I had sent on board the *Roman*.* Had these been available a larger number of fish could have been as easily dealt with. To take the place of these tanks, there were improvised five tubs, made from halves of the casks which the trawlers take to sea for the reception of fish livers. Each of these was scrubbed out and filled with water to the depth of eighteen inches, holding in this manner 15–20 gallons apiece. Changes of water were effected by means of buckets and the use of the ship's hose at intervals. This primitive method was continued during the seven days occupied in steaming 1540 miles from the Barents Sea fishing grounds to the N.W. Rough of the Dogger, where the twenty-three surviving fish were marked and liberated.

A much larger number had originally been placed in these tubs, but owing to the very limited space available and the lack of means for adequately changing the water the mortality at first was very heavy.

We were fortunately favoured with moderate weather for the journey, except for some hours after coming out of the Norwegian fiords by the Lofoten Islands. Here the vessel, driven full speed in the face of a strong head wind, had the main deck frequently swept by the seas from the bows to the winch; however, the fish appeared to suffer no inconvenience and the tubs received no damage, being lashed on the after deck.

Besides my colleague Mr. A. E. Hefford, and Captain Turner, I have also to thank the chief engineer of the *Princess Louise*, Mr. Gardner, to whose resourcefulness in providing the tubs, and to whose interest, the successful issue of the experiment was in great measure due.

CHANGES OF TEMPERATURE ON THE VOYAGE.—One of the most striking changes accompanying this journey south was naturally that of the temperature of the water in which the fish were being kept alive. On June 26th, on the fishing grounds, the bottom temperature varied between 34° and 35° F., whilst that of the surface was between 37° and 38° F. On June 27th, before reaching Nordkyn, the temperature was between 40° and 42·3° F., and on the 29th, at Tromsø, had risen to 45° and 47·0°, and reached 48·9° on the 30th, 49·5°–52·0° on July 1st,

* I suggested to Captain Leighton that he should on the following voyage attempt to bring back some living plaice in these tanks, and brought to his notice the precautions to be observed to obtain a successful result. In correspondence he informs me that he left the White Sea fishing grounds with about sixty fish, of which forty-two were alive on his arrival at the Humber. These were then iced and finally distributed amongst the members of the crew.

54.3°–55.7° on the 2nd, and on July 3rd, up to the time of setting out the fish, the temperature had ranged between 54.5° and 58.0° F. The extremes of temperature these fish experienced thus ranged over 24° F., without their appearing to have suffered from the rapidity with which the changes occurred.

RECAPTURE OF THE FISH.—The following table gives particulars of the recapture of the individual fishes (see next page).

The object in selecting the N.W. Rough as the point of liberation for these fish was that, in addition to being in the direct track of our vessel between the Norwegian and English coasts, it was a ground which offered a fair prospect of some of the fishes being returned if they survived. Unfortunately some Grimsby, Hartlepool, and Scarborough trawlers, engaged in fishing for cod and haddock, chanced at once to visit the area of liberation, and in the first month eight fish were returned. Five more being subsequently recaptured gives the result that within one year 13 or 56.5% have been returned.

The latter five were caught in the fourth (two specimens), seventh, tenth, and eleventh months after liberation, and without exception show important and unusually rapid growths compared with those which have been observed in the case of North Sea fish of corresponding size and sex.

These growths were accompanied by considerable improvement in the condition from the point of view of the market value of the fish.

MOVEMENTS OF THE FISH.—A feature connected with the movements of the last five fish is that all but one had migrated from the deeper water (33 fms.) in which they were liberated, short distances on to the Dogger Bank (20 fms. and less).

The furthest migrant was E 3880, which was taken on the Easternmost Shoal, about sixty miles from the point of liberation. Another fish, E 3876, had moved about forty miles in the direction of the Middle Rough and was retaken by a Dutch steam trawler. It is curious to note that the Grimsby trawler which effected the recapture of the former specimen also took, at the same spot, a plaice (E 778), which I had myself transplanted to the Dogger from the Dutch coast in May, 1907. This fish had grown 18.5 cm.

All the female fish brought from the White Sea appeared to be immature, the contrast between such and spent ones, so soon after the northern spawning season, being in most cases very marked without internal examination being absolutely necessary.*

* A new feature in the biology of the plaice lies in the enormous depth at which the Barents Sea plaice spawn. In May, 1909, Captain Leighton informs me, they were found by our trawlers to be in spawning condition in great masses in 90 to 106 fathoms.

TABLE I.

Table showing the particulars of liberation and recapture of the Plaice transplanted.

PARTICULARS OF LIBERATION.

July 3rd, 1908. 23 Plaice (E 3871-E 3893). Lat. 55° 8' N., 1° 10' E., 33 fms.
Transplanted from Barents Sea, Lat. 69° 0' N., 41° 23' E., 44 fms.; carried 7 days in tubs ca. 1540 miles.

PARTICULARS OF RECAPTURE.

Date of Recapture.	No. of Label.	Locality Reported.	Depth (fms.).	Calculated Position.	Vessel and Port of Registry.	Original Length (cm.).	Ultimate Length (cm.).	Weight (grs.).	Sex and Maturity.	No. of Days at Liberty.
1908										
July 5	E 3873	Lat. 55° 14' N., 1° 15' E.	40	—	GY St. tr.	37·8	36·8	491	♀	2
„ 6	E 3887	Lat. 55° 0' N., 1° 0' E.	32	—	SH St. tr.	34·7	33·8	371	♂	3
„ 5-9	E 3889	(Found on Pontoon, Grimsby)	—	—	—	35·3	34·7	309	♂	ca. 4
„ 11	E 3881	Lat. 54° 58' N., 1° 0' E.	31	—	GY St. tr.	40·3	39·8	446	♂ sp.	8
„ 12	E 3883	Lat. 54° 52' N., 1° 22' E.	—	—	GY St. tr.	35·2	34·7	353g	♀ im.	9
a „ 13	E 3874	75 miles E ½ S of Hartlepool	30	54° 54', 0° 53' E.	HL St. tr.	41·7	39·4+	523	♀ im.	10
„ 20	E 3871	75 miles E ½ S of Hartlepool	28-30	54° 54', 0° 53' E.	HL St. tr.	35·6	35·1	407	♀ im.	17
„ 24-26	E 3890	(Found on Pontoon, Grimsby)	—	—	—	33·9	33·0	289g	♂ sp.	ca. 22
Nov. 6	E 3876	Lat. 55° 20' N., 2° 20' E.	20	—	Dutch St. tr. IJM	34·4	38·1	590	♂	126
b „ 18	E 3880	Lat. 54° 39' N., 2° 45' E.	11	—	GY St. tr.	35·8	39·6	742	♀	138
1909										
Feb. 27	E 3884	Lat. 54° 50' N., 1° 5' E.	35	—	GY St. tr.	43·5	47·3	1095g	♂ sp.	239
May 14	E 3893	Lat. 54° 57' N., 1° 20' E.	18-20	—	GY St. tr.	33·9	39·9	702	♂	315
c June 20	E 3875	105 miles N.E. from Spurn	17-20	55° 5', 1° 45' E.	GY St. tr.	42·5	50·3	1535	♀	352

NOTES.

- (a). Very stale. Tail rays damaged, probable length, 41 cm.
 (b). Caught by same boat, on same day, and in same position as Plaice E 778. Apparently X years old.
 (c). Very fat. Apparently commencing to mature for the first time. Noted "im.?" on liberation.

Fish which have died or are still at liberty are E 3872 ♀ 44·4; E 3877 ♂ 34·6; E 3878 ♀ 36·7; E 3879 ♀ 33·0; E 3882 ♂ 34·1; E 3885 ♂ 37·3; E 3886 ♀ 27·8; E 3888 ♀ 39·3; E 3891 ♂ 35·7; E 3892 ♀ 46·4. It may be noted of these that slight abrasions were noted at the time of liberation in seven instances (70%). Similar abrasions were nevertheless noted in six instances, 46% of the fish which have already been recaptured.

All the males, on the other hand, were above the average size at which this sex is found mature in the Barents Sea; two were actually found to be spent on being returned to the Laboratory after a few days of liberty. This fact makes the growth observed all the more a matter of surprise, as we usually find large male plaice grow very slowly.*

E 3884, caught in February, was observed to be recently spent, and had thus taken part in one reproductive period in the North Sea. The ovaries of E 3875 were such as one observes in female plaice which are apparently maturing for the first time.

On the basis of these last five fish, as discussed below, it would be absurd to attempt to base any definite conclusions. In discussing them, the main desire is to bring to notice the suggestive results that this small experiment has attained, so that when the opportunity again arises similar experiments may be attempted on a larger scale, since it can no longer be doubted that a rational development of the plaice fishery of the North Sea would be possible under a carefully planned scheme of transplantations.

Below have been drawn up a few notes on the changes of which the last five fish returned have given evidence, regarded from the following points of view:—

1. Increase in size.
2. Increase in weight.
3. Increase in value.

1. INCREASE IN SIZE.—The eight fish caught in July all show a slight shrinkage, as is usual in marked fishes retaken shortly after liberation. It is usual with some investigators to estimate shrinkage between death and remeasurement at 0·5 cm., but in order to depress the observed growths rather than to exaggerate them this convention has been disregarded throughout.

The two specimens reported in November had increased in length from (male) 34·4 to 38·1 cm., and (female) 35·8 to 39·6, or 3·7 and 3·8 cm. respectively. The next fish was retaken in February, and the growth from 43·5 to 47·3, or 3·8 cm., is a very rapid growth for such a large male. Another male fish came back in May, and had increased 6 cm., from 33·9 to 39·9 cm. The last fish returned gives an astonishing increase for so large a fish, having grown from 42·5 to 50·3, or 7·8 cm. (female).

* It is interesting to note in comparison with this experiment, that Strodtmann transplanted plaice from the Baltic to the Elbe L.V., making the passage to the North Sea through the Kiel Canal. These were chiefly mature fish, and though many were retaken very few had grown at all after several months in their new surroundings. Cf. Reichard, *Die deutschen Versuche mit gezeichneten Schollen II*, p. 34.

How rapid these individual growths are in comparison with those of the North Sea fish can be seen by reference to any published report. To illustrate this rapidity, a number of records have been taken from the English marking experiments in various localities in the North Sea. These have been put together in the form of a table, and fish have been chosen which mostly resemble the five White Sea plaice in original size, the sex being of necessity also the same.

At the head of each of five columns is given the label number of the White Sea plaice with its original size, growth, and number of days at liberty. Below each comes a list of North Sea fishes marked in the same way, and set out again in whatever part of the North Sea they happen to have been caught. Comparison can thus with ease be made by taking any of the North Sea fish and comparing the growth, or period at liberty, given at the top of the column:—

TABLE II.

Table showing growth in the periods stated of normal North Sea marked plaice to compare with five specimens transplanted from the Barents Sea.

E 3876 ♂ 34.4 grew 3.7 cm. in 126 days.		E 3880 ♀ 35.8 grew 3.8 cm. in 138 days.		E 3884 ♂ 43.5 grew 3.8 cm. in 239 days.		E 3893 ♂ 33.9 grew 6.0 cm. in 315 days.		E 3875 ♀ 42.5 grew 7.8 cm. in 352 days.	
Growth.	Days out.	Growth.	Days out.	Growth.	Days out.	Growth.	Days out.	Growth.	Days out.
0.4	83	1.7	116	0.0	110	0.0	82	0.4	143
-0.4	136	0.9	119	0.2	142	0.8	97	0.2	148
1.4	141	1.0	121	0.3	162	3.5	112	0.1	176
1.0	142	1.0	154	0.8	230	2.4	119	0.1	197
0.7	195	2.5	156	0.9	286	0.3	123	1.8	212
1.9	196	1.0	183	1.3	293	0.6	142	1.1	234
1.0	252	1.8	193	0.3	314	2.0	262	0.7	282
2.6	292	2.5	272	0.8	321	0.2	267	0.7	296
3.0	273	5.5	277	1.1	340	3.4	287	1.6	314
4.4	275	3.1	361	0.5	365	4.7	315	2.0	326
1.9	293	4.9	373	0.5	410	2.5	338	1.8	350
5.4	453	8.1	618	3.1	676	1.6	453	4.3	353
0.3	481	10.0	649			4.3	462	1.4	360
1.2	506	4.1	802			3.1	613	1.6	414
						4.2	664	3.1	450
						1.0	694	2.5	458
						4.3	839	2.9	462
								2.1	469
								4.0	487
								1.3	489
								1.0	498
								6.5	510
								0.9	511
								1.4	556
								6.2	564
								5.0	596
								1.4	680
								5.6	781

All originally ♂ 34.0—35.0 cm.	All originally ♀ 34.9—36.5 cm.	All originally ♂ 39.0—42.7 cm.	All originally ♂ 33.0—33.9 cm.	All originally ♀ 39.0—44.7 cm.
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Although the above lists are not exhaustive and the growths quoted have been taken more or less at random, it can be clearly seen how slowly North Sea plaice of the stated lengths grew, as compared with these fish transplanted from the White Sea.

As compared with the first transplanted plaice only two growths of North Sea fish are noticed to be in excess, and these individuals had been at liberty respectively twice and three times as long after marking.

Comparing the growth of E 3880 with that of similar sized North Sea fishes, we find it only surpassed by specimens which have been out *twice, nearly thrice, four and a half, and nearly six times as long.*

No growth is observed to equal that shown in the case of E 3884, 3893, or 3875, though some of the periods of liberty are more than *twice as long.*

These growths are truly remarkable, in consideration of the probable age of the specimens concerned, and in view of the slow growth old plaice have been frequently shown to display.

It may be mentioned that further, but incomplete, investigations of the otoliths of the smallest plaice yet found on the White Sea grounds amply bear out the indications of slow growth afforded in my earlier report.

2. INCREASE IN WEIGHT.—All the fish have been weighed after their recovery by the fishermen, but, as the relation between length and weight of White Sea plaice in their normal condition is unknown at present, it is not possible to state exactly by how much the last five individuals have increased their bulk. In view of the additions which have been demonstrated as regards length, and in view of the fattened condition of the fish, the weight increments must have been very considerable. A tentative estimate can be deduced from the following data.

The weights of the eight fish caught in July compared with the average weight of Dogger plaice of the same sizes determined by Masterman* show deficiencies amounting to 17·3, 23·5, 41·5, 38·1, 29·1, 31·0, 22·9, and 26·8 per cent respectively. The average deficit amounts to just under 29%.

It cannot at present be said how closely this determination displays the actual deficiency in condition for which the White Sea plaice are noted, but it at least has the merit of bearing out the experience of practical men as to the inferiority of these fish as compared with those from the North Sea.

* Report on the Research Work of the Board of Agriculture and Fisheries in relation to the Plaice Fisheries of the North Sea. Cd. 4738. London, 1909.

I propose to estimate the increase in weight of the five fish referred to on two bases:—

- A. That the original weight of each fish was equal to that of a normal Dogger plaice.
- B. That the weights thus obtained (A) are on an average 29% too high, as was ascertained for the July fish.

Estimate A for the original weight being obviously too high, we can be satisfied that any increase shown on this basis is below that actually attained. It is further possible that increments based on Estimate B understate those actually attained.

The resulting figures are given in Table III below.

TABLE III.

Table showing estimated increase in the weight of five plaice transplanted from the White Sea to the North Sea, based on two estimates of their original weight.

- A. That the plaice were equal in weight to Dogger plaice of corresponding sizes.
- B. That estimate A gives an original weight value 29% too high in accordance with observations made on eight fish in July.

Number.	Estimated Original Weight.		Determined Ultimate Weight.	Increase in Weight.		% Increase.	
	A.	B.		Estimate A.	Estimate B.	Est. A.	Est. B.
E.	grammes.	grammes.	grammes.	grammes.	grammes.	%	%
3876	485	344	590	105	246	21·6	71·5
3880	528	375	742	214	367	40·5	97·6
3884	843	599	1095	252	496	29·9	82·8
3893	405	288	702	297	414	73·3	143·7
3875	813	577	1535	722	958	88·8	166·0

From this table it can be seen that, on the lowest possible estimate, the two fish which had been at liberty the longest (and this period less than a year and including a winter) had increased by about three-quarters of their original weight (73·3% and 88·8%); the fish which had been at liberty shorter periods also displaying corroborative increments.

The data are too small to permit of further discussion, but by kind permission of my colleague, Dr. Wallace, I am able to put forward in contrast to the above figures data from his forthcoming report.

Dr. Wallace finds from otolith investigations that, as regards the plaice of the Dogger and Flamborough region, the weight of six-year-

old males (average size 37.0 cm.) shows an increment of less than 30% on that of those five years old, whilst the seven-year-old fish of this sex, having an average length of 38.1 cm., show less than 10% weight increment on fish a year younger. This oldest group is, however, not sufficiently well represented for this result to be regarded as more than approximate.

Dealing with the females, the six-year-old fish (average size 41.0 cm.) are found to average a little over 40% heavier than those of five years, and the seven-year-old fish (average size 44.1 cm.) show an increment of just over 20% as compared with the six-year-olds.

Referring these figures, which are based on abundant material, back to the percentage weight increments of Estimate A in Table III, the indications in this table present a truly remarkable contrast in favour of the probably older plaice transplanted from the White Sea.

3. INCREASE IN VALUE.—The plaice fishery in the Barents Sea has only been conducted by our trawlers during four summers, and it would be premature to discuss the values of the product. These, however, have been adversely affected by two important considerations, the somewhat poor quality of the fish combined with excessive supplies in the summer months.

A trade expert giving evidence before the Committee on Fishery Investigations expressed an opinion that the plaice sell at less than one-tenth the value of any other plaice (*Committee on Fishery Investigations*, 1908. *Minutes*, *Cd.* 4304, *p.* 391).

The White Sea plaice have not the coarse, dark appearance, which used to characterize the old, accumulated stock at Iceland, and would, after a few months fattening in the North Sea, be indistinguishable in external appearance and doubtless too in food value from the indigenous population. Thus, if we may assume that each of the last five fish would have doubled its weight had it been at liberty a year, and basing the value of White Sea plaice at one-fifth that of North Sea plaice, each would have been worth at least ten times the price usually obtained.

It would be absurd, on the slender though corroborative evidence of the above results, to suggest that the transplantation of White Sea plaice would be practicable as a commercial undertaking. At the same time it must be admitted that even this would prove sounder economy as regards the development of the White Sea fishery than is the present plan of converting many tons of this valuable fish species into manure, as was done in the great gluts in the summer of 1909.

The fact that plaice can be carried in safety such long distances and through such varying conditions, broadens the question of trans-

plantation. Might not, for instance, plaice be carried across the Atlantic and introduced to the Canadian coasts and the Banks of Newfoundland, where its congeners in other waters, the cod, haddock, and halibut, already occur? Or, again, might not the very small halibut, which have been brought to market by the trawlers in vast quantities from certain parts of Faxø Bay, Iceland, also be brought alive and set out on the North Sea grounds, where this valuable species was without a doubt much more abundant formerly than it is to-day? Such, and many practical questions of a similar nature, proffer a wide field for future research.

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OBJECTS
OF THE
Marine Biological Association
OF THE UNITED KINGDOM.

THE ASSOCIATION was founded at a Meeting called for the purpose in March, 1884, and held in the Rooms of the Royal Society of London.

The late Professor HUXLEY, at that time President of the Royal Society, took the chair, and amongst the speakers in support of the project were the late Duke of ARGYLL, the late Sir LYON PLAYFAIR, Lord AVEBURY, Sir JOSEPH HOOKER, the late Dr. CARPENTER, Dr. GÜNTHER, the late Lord DALHOUSIE, the late Professor MOSELEY, the late Mr. ROMANES, and Sir RAY LANKESTER.

The Association owes its existence and its present satisfactory condition to a combination of scientific naturalists, and of gentlemen who, from philanthropic or practical reasons, are specially interested in the great sea fisheries of the United Kingdom. It is universally admitted that our knowledge of the habits and conditions of life of sea fishes is very small, and insufficient to enable either the practical fisherman or the Legislature to take measures calculated to ensure to the country the greatest return from the "harvest of the sea." Naturalists are, on the other hand, anxious to push further our knowledge of marine life and its conditions. Hence the Association has erected at Plymouth a thoroughly efficient Laboratory, where naturalists may study the history of marine animals and plants in general, and where, in particular, researches on food-fishes and molluscs may be carried out with the best appliances.

The Laboratory and its fittings were completed in June, 1888, at a cost of some £12,000. Since that time investigations, practical and scientific, have been constantly pursued at Plymouth. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council; in addition, naturalists from England and from abroad have come to the Laboratory, to carry on their own independent researches, and have made valuable additions to zoological and botanical science, at the expense of a small rent for the use of a working table in the Laboratory and other appliances. The number of naturalists who can be employed by the Association in special investigations on fishery questions, and definitely retained for the purpose of carrying on those researches throughout the year, must depend on the funds subscribed by private individuals and public bodies for the purpose. The first charges on the revenue of the Association are the working of the sea-water circulation in the tanks, stocking the tanks with fish and feeding the latter, the payment of servants and fishermen, the hire and maintenance of fishing-boats, and the salary of the Resident Director and Staff. At the commencement of this number will be found the names of the gentlemen on the staff.

In the summer of 1902 the Association was commissioned by His Majesty's Government to carry out in the southern British area the scheme of International Fishery Investigations adopted by the Conference of European Powers which met at Christiania in 1901. In connection with this work a laboratory has been opened at Lowestoft.

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