A PHYSIOLOGICAL AND BIOCHEMICAL APPROACH TO THE TAXONOMY OF *MYTILUS EDULIS* L. AND *M. GALLOPROVINCIALIS* LMK. FROM S. W. ENGLAND

by

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Résumé

L'auteur montre que les populations de *Mytilus edulis* L. et *M. galloprovincialis* Lmk. qui coexistent au S.-W. de l'Angleterre sont assez différentes. Dans cette région, *M. galloprovincialis* se reproduit approximativement huit semaines plus tard que *M. edulis*. Le cycle annuel de *M. galloprovincialis* est également moins net, ce qui indique vraisemblablement que cette Moule est ici au voisinage de sa limite nordique. Au laboratoire, il est possible de « faire des hybrides » des deux espèces.

A Rock, les deux espèces se comportent très différemment vis-à-vis de l'infection par *Pinnotheres pisum* (elle atteint en moyenne 30,3 p. 100 chez *M. edulis* et seulement 1,4 p. 100 chez *M. galloprovincialis*). La croissance de *M. edulis* au laboratoire, est quatre fois plus rapide que celle de *M. galloprovincialis*).

Bien que la couleur du manteau soit généralement un caractère taxonomique sûr (pourpre-violacé chez *M. galloprovincialis*, jaune-brunâtre chez *M. edulis*), aucune différence biochimique n'a été établie jusqu'ici. L'électrophorèse des protéines du muscle adducteur postérieur a montré des différences faibles mais importantes.

Donc, en plus des différences morphologiques et anatomiques existant entre ces Moules du S.-W. de l'Angleterre, il en existe d'autres qui renforcent l'idée qu'elles appartiennent à deux espèces distinctes.

Introduction

In an earlier publication (Lewis and Seed, 1969), the existence of two quite distinct forms of *Mytilus* on the coasts of Devon and Cornwall in S.W. England was confirmed; *Mytilus edulis* L., and a form which accorded closely to the Mediterranean mussel *M. galloprovincialis* Lmk., previously described from this region as the "Padstow mussel" by Hepper (1957). In relatively sheltered localities such as harbours and estuaries the two types are easily recognised and their separation even on gross shell morphological characters presents no real problems (Plate I). *M. galloprovincialis* from such localities can generally be identified in the field on the following characters.

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a) The anterior end is more pointed and slightly downturned.

b) The shells are usually higher, often with a rounded dorsal shell margin.

c) The ventral shell margin is relatively flat or slightly inrolled especially at or just behind the anterior end.

d) The mantle edge is dark purple-violet compared with the straw coloured (yellow-brown) mantles of *edulis*.

In addition to the differences mentioned above, a wide range of shell characters have been described and their value for identification purposes discussed in detail (Lewis and Seed, 1969). Of these, the shape and size of the hinge-plate and the smaller anterior adductor muscle scar in *galloprovincialis* proved to be generally more reliable than overall shell morphology.

At high tidal levels, however, where many old animals occur, and in the distinctive growing conditions of the high density populations typical of exposed wave swept shores, external shell characters of the two forms merge until criteria for separation are frequently reduced to differences in the form and relative sizes of the hinge-plates and anterior adductor scars and the colour of the mantle edge. Mantle colour can sometimes be a very subjective character to assess whilst the two shell characters are of little practical value in the field and in some cases do not permit a positive separation even after laboratory analysis. The gross shell morphology in *Mytilus* is capable of considerable variation according to age and the environmental conditions under which they are grown and indeed many of the old slow-growing mussels on the exposed N.E. Yorkshire coast bear a strong superficial resemblance to *M. galloprovincialis* (Seed, 1968).

M. galloprovincialis from a variety of Continental localities have also been examined, and whilst these too show considerable morphological variation it would appear that the "Padstow type" and *M. galloprovincialis* are probably identical forms of *Mytilus*.

In view of the wide variations in the shell morphologies exhibited by these two mussels together with the difficulties frequently involved in their separation on these grounds alone, it was decided to extend the taxonomic investigation to cover certain aspects of their physiology and biochemistry. This work falls conveniently into five main sections, namely:—

1. the reproductive cycles;
2. laboratory growth rates;
3. infection with *Pinnotheres*;
4. mantle edge pigments;
5. protein electrophoresis.

Collection of material

During 1968-1969 several visits were made to S.W. England in order to examine mussels in the field and to collect material for subsequent laboratory analysis. In addition, regular monthly samples were sent to me by Mr. J.M. Sproull to whom I am deeply indebted. Collections were made at Rock (Nr. Padstow) in the sheltered Camel estuary where the two types of mussel were found living together

in abundance and where separation presented no problems. The latter was particularly important since most of the subsequent work relied on correct initial identification.

Each month, samples of approximately 50 individuals of each type were collected from about M. L. W. N. These were opened, fixed overnight in formalin and despatched in heavy duty polythene bags containing cotton wool soaked in formalin to prevent the mussels from drying out. Throughout, *edulis* and *galloprovincialis* were kept separate. This proved particularly important when recording infections by the parasitic pea crab since once the mussels had been opened the pea crabs were easily dislodged from the mantle cavity. Between Jan. 1968 and Sept. 1969 some 2,500 mussels were sent by Mr. Sproull and in no instance had he misidentified the two species.

From the same region of the shore, random clumps of mussels were scraped from the rock and also despatched together with any substratum or shell debris adhering to them. These were subsequently sorted in the laboratory and all the small mussels (under 20 mm) were removed in the hope that some assessment of the major settlement periods might be made.

In addition to this fixed material, samples of living mussels were occasionally sent back to the laboratory in small sacks for subsequent biochemical analysis.

THE REPRODUCTIVE CYCLES AT ROCK

On receiving the fixed material the mantle from one valve was removed and the middle third transferred to Bouin's fluid in sea water in order to ensure complete fixation of the gonad. These were then stored in 70 p. 100 ethyl alcohol until required. Before the shells were discarded, measurements were made of shell length (maximum anterior-posterior axis), height (maximum dorso-ventral axis) and width (maximum lateral axis) by means of vernier calipers. The results are shown in Table 1 and indicate that *galloprovincialis* have

TABLE 1

Shell proportions in *M. edulis* and *M. galloprovincialis* from Rock

	Length (cm)	Min.	Max.	Height (cm)	Width (cm)	L/H	L/W	H/W
M. edulis	6.37	5.10	7.92	3.08	2.83	2.07	2.25	1.09
M. galloprovincialis	6.81	5.40	8.57	3.63	2.85	1.88	2.39	1.27

much higher shells than *edulis* of approximately similar size. This is in agreement with previous publications (Hepper, 1957; Lewis and Seed, 1969).

Tissues were embedded under vacuum in Paraplast wax (m.p. 56-57 °C) and sections cut at 10-15 μ were stained in Ehrlich's haematoxylin without counterstain. Details of the anatomical relations and

histology of the gonad, together with the scheme of classification of gonad condition are given in an earlier publication (Seed, 1969 a). In view of this, only a brief description of the gonad will be given in this account.

Arbitrary scheme of classification of the gonad condition.

From the examination of histological preparations four main stages in the annual reproductive cycle could be recognised; developing, ripe, spawning and spent. Developing and spawning stages were further split into four subdivisions resulting in a total of ten stages into which any animal could be assigned. For each sample a mean gonad index which more or less defines the breeding condition was determined by multiplying the number of individuals in each stage by the numerical factor of the arbitrary rating of the stage and dividing the sum of these products by the grand total of individuals in the sample. The resulting value varies from 0 when all individuals are in the spent or resting condition, to 5 when all individuals are sexually mature.

Brief description of gonad:

1 - *The resting or spent gonad.*

Stage 0: in this stage no traces of sexuality can be observed. It includes virgin animals where the reproductive system is rudimentary, and those animals which have completed spawning.

2 - *Developmental stages.*

Stage I: this stage is characterised by the onset of gametogenesis, islands of germinal tissue appearing in the matrix of dense connective tissue. In very early stages, males and females are difficult to distinguish. No ova or spermatozoa are present at this stage.

Stage II: ripe gametes begin to appear in the centre of the follicles although these are occupied mainly by early stages of gametogenesis — small numerous oocytes in the females and spermatogonia and spermatocytes in the males.

Stage III: there is a general increase in the mass of the gonad. This is a stage of rapid gametogenesis with approximately half of each follicle occupied by ripe gametes and half with earlier stages of gametogenesis. The area occupied by genital tissue is about half of the fully ripe condition.

Stage IV: maximum proliferation of genital tissue is almost attained. There is a preponderance of ripe gametes in each follicle with a general reduction in the earlier stages of gametogenesis. Gametogenesis is, however, still in progress.

Stage V: the gonad attains its fully ripe condition. It differs from the previous stage only in the reduction of early stages of gametogenesis (a few small oocytes in the germinal epithelium of the female, and a narrow band one or two cells wide, of spermatogonia and sperma-

tocytes in the male). Ova are compacted into polygonal configurations whilst in the male the follicles are distended with morphologically ripe spermatozoa.

3 - *Spawning stages.*

Stage IV: the follicles are still relatively full of ripe gametes but active discharge of these is now in progress. This is obvious from the general reduction in density of spermatozoa and the rounding off of the remaining ova as pressure within the follicles is reduced following partial emission.

Stage III: this stage is similar in some ways to developing stage III in as much as the follicles are approximately half full of mature gametes. Here, however, unlike developing stage III, relatively few early stages of gametogenesis are present. In the female, the ripe eggs are rounded rather than polygonal in appearance. There is a general reduction in the area of mantle covered by genital tissue.

Stage II: at this stage the follicles are considerably less than half full of ripe gametes and there is still further general reduction in the area occupied by genital tissue.

Stage I: residual spermatozoa and ova are still present and can often be seen undergoing cytolysis by amoeboid phagocytes. The centre of the follicles is often filled with a yellow-brown material — the result of gamete cytolysis.

The criteria that exist for separating spawning and developmental stages are thus somewhat subjective and sometimes difficult to assess quantitatively with all intermediate stages present. Yet with experience in examination of gonads throughout the year it is possible to recognise each stage with relative confidence.

From the sectioned material, it was also possible to assess the average area of mantle occupied by reproductive tissue each month. This was done by using a low power microscope fitted with an eye piece grid. Such information, expressed as percentage cover, gave useful additional information of the reproductive cycles of the two species.

The annual cycle.

The breeding data for the Rock mussels is given in Tables 2 and 3 as numbers in each of the arbitrary stages (the sexes are considered separately) together with the gonad index for each sample. Over the same period the breeding cycles of *M. edulis* for five stations at Filey on the Yorkshire coast were also investigated. This data (Tables 4-8) enables the breeding cycles of this species grown on the relatively cold N.E. Yorkshire coast to be compared with those from the much warmer Cornish waters. [Data for these Filey stations from Oct. 1964-Dec. 1967 is published elsewhere (Seed, 1969 a)].

Fig. 1 shows the total percentages in the developing, ripe, spawning and spent conditions together with the gonad index for A) *M. galloprovincialis* B) *M. edulis* from Rock and C) *M. edulis* from Filey, between January, 1968 and September, 1969. Only the data

TABLE 2
Distribution of gonad stages in samples of *M. edulis* from Rock, S.W. England

Date	N°	MALES									Spent	FEMALES									Index
		Developing					Spawning					Developing					Spawning				
		I	II	III	IV	V	IV	III	II	I	0	I	II	III	IV	V	IV	III	II	I	
1966																					
Nov.	29	0	3	2	1	3	2	0	0	0	0	13	4	0	0	0	0	0	0	1	2.10
1968																					
Jan. 1	60	0	1	6	21	4	0	0	1	1	2	3	11	7	3	0	0	0	0	0	3.08
Feb. 18	50	0	0	2	7	10	0	1	0	1	0	1	2	8	8	10	0	0	0	0	3.98
Mar. 17	51	0	0	0	6	20	0	0	0	0	0	1	2	1	6	15	0	0	0	0	4.53
Apr. 14	76	0	0	6	14	3	9	7	0	0	0	0	1	13	14	0	5	4	0	0	3.62
May 17	58	0	0	0	8	13	8	1	0	0	0	0	0	1	1	14	9	3	0	0	4.38
Jun. 12	55	0	0	0	0	1	3	11	12	3	8	0	0	0	0	1	1	3	7	5	2.07
Jul. 10	51	0	0	2	0	2	0	2	4	7	28	3	0	0	0	1	0	0	0	2	0.92
Aug. 15	68	0	1	1	0	1	0	1	1	5	48	4	3	0	0	0	0	0	2	1	0.51
Sep. 15	25	1	1	2	0	0	0	0	0	1	12	7	1	0	0	0	0	0	0	0	0.76
Oct. 5	50	3	6	6	2	0	1	1	2	4	2	19	1	0	1	1	0	0	0	1	1.74
Nov. 2	49	2	4	7	5	4	0	0	0	2	2	14	9	0	0	0	0	0	0	0	2.15
Dec. 1	53	0	2	3	12	9	1	0	0	2	2	7	10	2	3	0	0	0	0	0	2.97
1969																					
Jan. 12	44	1	2	3	11	3	0	0	0	2	1	2	13	6	0	0	0	0	0	0	2.75
Feb. 9	47	0	2	3	19	6	0	0	1	0	1	2	4	4	5	0	0	0	0	0	3.47
Mar. 16	51	0	3	1	8	11	2	0	0	1	0	0	1	0	10	7	5	2	0	0	4.08
Apl. 20	70	0	2	15	2	13	0	2	3	0	0	0	10	9	2	6	1	1	1	3	3.24
May 17	68	0	0	1	0	14	11	4	1	0	0	0	0	0	7	25	1	1	1	2	4.34
Jun. 20	52	0	0	0	1	3	4	5	3	3	20	0	0	0	0	3	2	4	4	0	1.96
Jul. 20	57	0	0	0	1	0	0	2	0	6	44	4	0	0	0	0	0	0	0	0	0.35
Aug. 22	55	1	3	1	0	0	0	0	1	1	42	5	1	0	0	0	0	0	0	0	0.36
Sep. 3	48	1	1	3	1	3	0	0	1	4	32	2	0	0	0	0	0	0	0	0	0.81

TABLE 3
Distribution of gonad stages in samples of *M. galloprovincialis* from Rock, S.W. England

Date	N°	MALES									Spent	FEMALES									Index
		Developing					Spawning					Developing					Spawning				
		I	II	III	IV	V	IV	III	II	I	0	I	II	III	IV	V	IV	III	II	I	
1966																					
Nov.	27	0	0	0	0	3	4	1	1	2	7	1	0	0	0	0	2	3	2	1	2.26
1968																					
Jan. 1	66	0	0	0	0	22	5	7	3	2	11	4	0	0	1	2	2	2	0	5	2.97
Feb. 18	56	0	0	0	0	18	6	5	2	1	2	7	2	1	2	5	1	0	2	2	3.41
Mar. 17	53	0	0	2	4	10	2	4	4	1	6	3	2	3	2	6	2	2	0	0	3.19
Apr. 14	64	0	2	9	7	1	1	5	4	0	1	4	9	9	3	1	0	4	3	1	2.75
May 17	49	0	0	4	20	10	0	0	0	0	0	0	1	2	4	8	0	0	0	0	4.20
Jun. 12	54	0	0	1	4	12	5	2	0	0	0	0	0	1	2	23	2	1	0	1	4.50
Jul. 10	50	0	0	0	0	16	3	0	1	0	1	0	1	0	2	22	0	1	3	0	4.46
Aug. 15	68	0	0	8	6	0	0	11	1	1	5	4	6	8	5	0	1	10	2	0	2.68
Sep. 15	74	0	2	3	7	12	3	2	5	1	1	5	1	3	5	7	4	3	3	7	3.20
Oct. 5	50	0	0	0	0	8	0	4	4	1	12	2	0	0	0	6	1	4	5	3	2.44
Nov. 2	50	0	1	2	2	9	0	0	6	3	12	3	0	0	0	2	0	2	4	4	2.14
Dec. 1	49	0	0	0	3	7	0	6	4	8	10	2	2	0	0	4	0	2	0	1	2.33
1969																					
Jan. 12	52	1	1	1	4	8	1	0	2	5	10	6	6	0	0	0	0	2	0	5	2.00
Feb. 9	57	0	5	1	6	8	0	3	4	2	6	6	2	2	1	4	0	1	1	5	2.56
Mar. 16	51	0	5	6	3	3	1	2	4	0	6	9	5	4	1	0	0	0	2	0	2.20
Apr. 20	56	0	4	13	9	5	0	0	0	0	0	1	9	10	5	0	0	0	0	0	3.16
May 17	62	0	0	1	15	9	1	0	0	0	0	0	2	3	18	13	0	0	0	0	4.23
Jun. 20	67	0	0	2	6	15	6	1	1	0	0	0	1	1	3	26	2	3	0	0	4.45
Jul. 20	50	0	1	4	6	10	0	0	2	0	0	0	1	7	7	7	2	3	0	0	3.90
Aug. 22	63	0	2	11	15	1	0	1	2	2	4	0	1	4	2	4	1	5	6	2	2.95
Sep. 3	45	0	0	2	6	14	0	3	1	1	1	0	2	1	0	5	2	3	3	1	3.73

TABLE 4
Distribution of gonad stages in samples of *M. edulis* from the high shore Filey Bay

Date	N°	MALES									Spent	FEMALES									Index
		Developing					Spawning					Developing					Spawning				
		I	II	III	IV	V	IV	III	II	I	0	I	II	III	IV	V	IV	III	II	I	
1967																					
Nov. 16	43	5	1	0	1	0	0	0	0	1	21	13	0	0	0	0	0	0	0	1	0.60
Dec. 18	36	5	6	0	2	0	0	0	0	0	8	11	2	2	0	0	0	0	0	0	1.27
1968																					
Feb. 1	56	5	8	6	1	1	0	0	2	2	10	17	3	0	1	0	0	0	0	0	1.45
Feb. 29	43	5	3	4	2	0	0	1	1	4	5	15	3	0	0	0	0	0	0	0	1.42
Mar. 28	42	0	3	2	5	2	0	1	0	1	6	16	4	2	0	0	0	0	0	0	1.81
Apl. 30	49	0	1	3	9	7	1	1	2	4	3	1	7	2	1	0	0	2	3	2	2.77
May 28	49	0	0	0	0	1	1	0	4	5	34	0	0	0	0	0	0	1	1	2	0.59
Jul. 2	50	0	1	0	0	3	0	2	0	6	34	0	0	0	0	0	0	2	0	2	0.74
Aug. 9	29	0	0	0	0	2	0	0	0	0	26	0	0	0	0	0	0	0	0	1	0.38
Sep. 24	53	0	0	0	0	0	0	0	0	1	52	0	0	0	0	0	0	0	0	0	0.00
Oct. 23	41	2	1	0	0	0	0	0	1	3	26	8	0	0	0	0	0	0	0	0	0.41
Nov. 21	35	2	2	0	0	0	0	0	1	2	19	9	0	0	0	0	0	0	0	0	0.54
Dec. 19	54	9	1	0	0	0	0	0	1	3	13	27	0	0	0	0	0	0	0	0	0.79
1969																					
Jan. 22	53	3	5	3	1	0	0	0	2	6	17	14	2	0	0	0	0	0	0	0	1.02
Feb. 17	64	7	7	1	2	1	0	0	2	2	21	12	3	1	2	0	0	1	0	2	1.20
Mar. 20	33	3	6	10	2	0	0	0	0	0	0	4	8	0	0	0	0	0	0	0	2.21
Apl. 23	52	1	7	2	4	0	1	2	3	2	16	8	3	3	0	0	0	0	0	0	1.50
May 19	57	1	2	3	0	0	1	1	3	5	27	2	1	0	0	0	0	6	1	4	1.05
Jun. 18	48	1	5	0	0	0	0	0	5	6	28	1	0	0	0	0	0	0	1	1	0.65
Jul. 17	47	0	0	0	0	0	0	0	0	1	44	0	0	0	0	0	0	0	0	2	0.07
Aug. 4	51	0	0	0	0	0	0	0	0	0	51	0	0	0	0	0	0	0	0	0	0.00
Sep. 2	26	0	0	0	0	0	0	0	0	0	26	0	0	0	0	0	0	0	0	0	0.00

TABLE 5
Distribution of gonad stages in samples of *M. edulis* from the low shore Filey Bay

Date	N°	MALES									Spent	FEMALES									Index
		Developing					Spawning					Developing					Spawning				
		I	II	III	IV	V	IV	III	II	I	0	I	II	III	IV	V	IV	III	II	I	
1967																					
Nov. 16	54	10	10	4	3	0	0	1	1	0	2	22	1	0	0	0	0	0	0	0	1.54
Dec. 18	31	2	2	9	1	0	1	0	0	0	0	8	5	3	0	0	0	0	0	0	2.02
1968																					
Feb. 1	55	3	10	2	2	4	1	1	2	2	1	11	9	6	1	0	0	0	0	0	2.20
Feb. 29	50	2	1	4	11	6	3	2	1	0	0	6	5	4	5	0	0	0	0	0	3.16
Mar. 28	61	1	0	3	4	17	4	1	0	0	0	2	1	6	15	7	0	0	0	0	4.05
Apl. 30	59	0	3	11	0	0	0	8	2	0	0	3	20	5	0	0	0	0	6	1	2.34
May 29	66	1	11	13	2	0	1	8	7	1	0	4	12	6	0	0	0	0	0	0	2.40
Jul. 2	48	0	0	0	0	1	3	2	6	6	17	0	1	0	0	6	0	0	3	3	1.71
Aug. 9	43	1	1	0	0	10	1	2	2	4	4	0	0	0	0	13	0	2	3	0	3.44
Sep. 24	49	2	1	0	0	4	0	0	5	2	31	0	0	0	0	0	0	0	2	2	0.86
Oct. 23	54	12	3	0	0	4	0	2	2	0	15	14	0	0	0	0	0	0	1	1	1.20
Nov. 21	35	0	2	1	3	5	2	0	4	1	1	9	6	1	0	0	0	0	0	0	2.46
Dec. 19	54	6	8	3	2	0	0	1	3	4	5	20	2	0	0	0	0	0	0	0	1.41
1969																					
Jan. 22	51	0	0	3	3	2	4	3	0	1	2	11	11	9	2	0	0	0	0	0	2.45
Feb. 17	48	0	0	4	13	6	4	0	0	0	0	1	3	9	8	0	0	0	0	0	3.67
Mar. 20	36	0	3	3	10	1	3	0	1	0	0	2	2	5	5	0	1	0	0	0	3.31
Apl. 23	64	0	0	1	20	7	5	1	0	0	0	1	1	4	13	10	1	0	0	0	4.09
May 19	64	0	0	6	6	5	11	9	0	1	1	0	4	2	6	2	6	4	1	0	3.51
Jun. 18	47	0	1	4	1	0	0	0	6	4	16	2	5	0	0	0	0	0	1	7	1.17
Jul. 17	43	2	3	0	0	0	0	1	3	2	16	5	2	2	0	0	0	0	2	5	1.00
Aug. 4	53	0	1	0	0	0	1	2	5	3	32	0	0	0	0	1	0	1	0	7	0.75
Sep. 2	31	5	1	0	0	0	0	0	1	3	19	2	0	0	0	0	0	0	0	0	0.45

TABLE 6
Distribution of gonad stages in samples of *M. edulis* from the high shore Filey Brigg

Date	N°	MALES									Spent	FEMALES									Index
		Developing					Spawning					Developing					Spawning				
		I	II	III	IV	V	IV	III	II	I	0	I	II	III	IV	V	IV	III	II	I	
1967																					
Nov. 16	45	4	5	6	4	0	0	1	0	2	3	8	9	0	1	0	0	0	0	2	1.88
Dec. 18	38	1	3	7	7	1	0	0	0	1	0	10	6	1	1	0	0	0	0	0	2.39
1968																					
Feb. 1	54	0	2	5	9	9	1	3	0	0	2	4	4	8	3	3	0	0	0	1	3.28
Feb. 28	39	1	0	1	8	11	1	0	0	0	0	1	1	1	9	4	1	0	0	0	4.13
Mar. 28	41	0	0	1	7	6	1	0	0	0	1	2	1	4	8	8	0	1	1	0	3.85
Apl. 30	55	0	2	2	2	7	4	4	3	5	1	0	5	0	0	5	5	4	5	1	3.09
May 28	53	0	2	3	0	0	0	1	6	12	4	10	6	1	0	0	0	0	2	6	1.41
Jul. 2	62	0	0	3	1	0	0	9	10	6	8	16	6	1	1	0	0	0	0	1	1.65
Aug. 9	51	0	0	2	0	4	1	4	6	7	10	2	3	1	0	2	0	0	3	6	1.84
Sep. 24	36	1	1	2	1	1	0	0	2	1	17	1	2	2	0	3	0	0	1	1	1.39
Oct. 23	33	6	3	0	0	0	1	0	0	1	8	10	2	0	0	0	0	0	1	1	1.03
Nov. 21	34	2	2	3	2	6	0	1	1	1	1	7	6	0	2	0	0	0	0	0	2.53
Dec. 19	61	2	8	6	7	0	4	1	0	2	3	13	4	8	2	1	0	0	0	0	2.34
1969																					
Jan. 22	54	1	3	3	12	0	3	1	2	0	0	5	6	6	10	2	0	0	0	0	3.11
Feb. 17	57	0	1	5	11	5	6	1	0	0	1	1	7	1	10	5	3	0	0	0	3.65
Mar. 20	48	0	0	4	13	9	0	0	0	0	0	2	6	2	5	1	0	3	1	2	3.48
Apl. 23	52	0	2	2	7	5	3	7	2	0	0	0	4	3	2	4	7	4	0	0	3.56
May 19	60	0	5	3	5	2	1	3	4	2	5	1	11	1	3	0	1	3	4	6	2.28
Jun. 18	42	0	3	6	1	0	0	0	3	3	8	2	4	2	1	0	1	0	2	6	1.69
Jul. 17	37	0	3	1	0	0	1	1	4	7	12	0	0	0	0	0	1	0	1	6	1.16
Aug. 4	50	0	0	1	0	0	0	0	2	6	33	0	0	0	0	0	0	0	0	8	0.42
Sep. 2	31	2	0	0	0	0	0	0	1	2	23	2	0	0	0	0	0	0	0	1	0.29

TABLE 7
Distribution of gonad stages in samples of *M. edulis* from the mid-shore Filey Brigg

Date	N°	MALES									Spent	FEMALES									Index
		Developing					Spawning					Developing					Spawning				
		I	II	III	IV	V	IV	III	II	I	0	I	II	III	IV	V	IV	III	II	I	
1967																					
Nov. 16	34	7	4	2	1	1	0	0	0	3	5	8	2	0	0	1	0	0	0	0	1.47
Dec. 18	34	3	7	5	2	0	0	0	0	0	2	9	6	0	0	0	0	0	0	0	1.80
1968																					
Feb. 1	53	2	0	6	10	2	3	1	1	0	0	2	12	8	5	1	0	0	0	0	3.00
Feb. 28	46	0	0	4	6	11	2	1	0	0	1	3	1	11	5	1	0	0	0	0	3.59
Mar. 28	60	0	2	2	20	12	0	0	1	0	0	2	1	2	12	5	1	0	0	0	3.98
Apl. 30	59	0	1	5	4	18	0	4	2	1	0	4	1	1	2	6	5	2	1	2	3.68
May 28	58	0	3	21	0	0	0	4	5	1	2	4	14	1	0	0	0	0	1	2	2.26
Jul. 2	69	0	0	0	2	6	4	8	8	6	11	1	1	0	3	7	5	0	3	4	2.60
Aug. 9	33	0	0	0	0	0	0	2	2	4	22	1	0	0	0	0	0	0	2	0	0.57
Sep. 24	51	2	0	0	0	0	0	0	2	6	29	11	0	0	0	0	0	0	1	0	0.49
Oct. 23	51	6	0	0	0	2	0	1	2	4	17	19	0	0	0	0	0	0	0	0	0.90
Nov. 21	36	2	7	1	1	0	0	1	5	6	3	10	0	0	0	0	0	0	0	0	1.17
Dec. 19	68	2	14	12	2	0	0	0	5	3	2	16	9	3	0	0	0	0	0	0	1.91
1969																					
Jan. 22	41	1	4	8	1	0	3	6	4	0	0	5	6	2	0	0	0	0	1	0	2.44
Feb. 17	51	2	6	5	5	1	1	1	2	0	3	4	7	5	5	1	0	1	2	0	2.55
Mar. 20	45	0	0	2	16	2	2	0	0	0	0	2	5	13	3	0	0	0	0	0	3.36
Apl. 23	56	0	0	4	13	3	4	9	0	0	0	1	5	8	5	1	2	1	0	0	3.45
May 19	55	0	8	5	3	3	10	3	1	1	0	3	6	2	4	1	2	0	2	1	3.00
Jun. 18	42	0	6	4	0	0	0	1	4	1	5	2	7	2	0	0	0	0	4	6	1.71
Jul. 17	42	0	0	0	0	0	1	2	3	11	16	0	0	0	0	0	0	0	5	4	0.98
Aug. 4	52	0	0	0	0	1	0	5	4	6	25	0	0	0	0	1	0	1	3	6	1.04
Sep. 2	37	0	2	0	0	0	0	0	1	5	21	3	1	3	0	0	0	0	0	1	0.70

TABLE 8
Distribution of gonad stages in samples of *M. edulis* from the low shore Filey Brigg

Date	N°	MALES									Spent	FEMALES									Index
		Developing					Spawning					Developing					Spawning				
		I	II	III	IV	V	IV	III	II	I	0	I	II	III	IV	V	IV	III	II	I	
1968																					
Jan. 17	76	1	3	7	20	7	1	0	1	1	1	9	12	10	3	0	0	0	0	0	2.93
Feb. 1	50	1	1	2	14	7	0	2	0	0	0	5	4	4	7	3	0	0	0	0	3.48
Feb. 29	39	1	1	1	8	6	1	0	0	0	0	2	4	5	8	2	0	0	0	0	3.56
Mar. 28	53	0	0	1	6	19	3	0	1	0	1	0	1	2	10	9	0	0	0	0	4.32
Apl. 30	52	0	0	1	1	16	6	3	1	0	0	0	1	0	7	16	0	0	0	0	4.46
May 28	64	0	6	10	2	0	2	10	8	1	3	3	13	3	0	0	0	0	3	0	2.33
Jul. 2	66	0	1	0	10	9	3	3	2	4	2	0	0	1	1	15	2	5	6	2	3.56
Aug. 9	35	0	0	2	0	5	2	3	1	3	9	1	4	0	0	2	0	0	1	2	2.17
Sep. 24	49	0	0	0	0	4	5	2	1	4	25	1	0	0	0	4	0	0	0	3	1.55
Oct. 23	56	0	4	1	1	2	0	3	2	7	17	6	2	0	0	4	0	1	4	2	1.58
Nov. 21	49	5	2	1	4	2	1	2	4	3	6	11	1	0	2	5	0	0	0	0	2.14
1969																					
Jan. 22	53	1	1	2	3	6	8	5	2	0	0	5	4	4	2	5	2	2	0	1	3.30
Feb. 17	41	0	3	2	5	4	4	1	1	0	1	1	5	3	4	4	0	1	2	0	3.32
Mar. 20	47	0	0	3	5	6	2	4	0	0	0	0	6	3	6	5	2	2	2	1	3.57
Jun. 18	48	0	0	3	10	0	3	3	1	0	0	0	0	9	13	2	0	3	1	0	3.58
Jul. 17	47	0	0	0	1	3	2	6	2	8	4	1	2	2	1	3	2	5	4	1	2.53
Aug. 4	52	0	0	0	2	2	2	1	11	5	4	0	1	4	0	8	3	1	3	5	2.62
Sep. 2	35	2	0	0	0	0	0	0	0	2	29	1	1	0	0	0	0	0	0	0	0.20

from Tables 5, 7 and 8 were used in constructing Fig. 1C. In Fig. 1D the area covered by reproductive tissue in transverse sections of the mantle over the same period is illustrated. Mean fortnightly surface

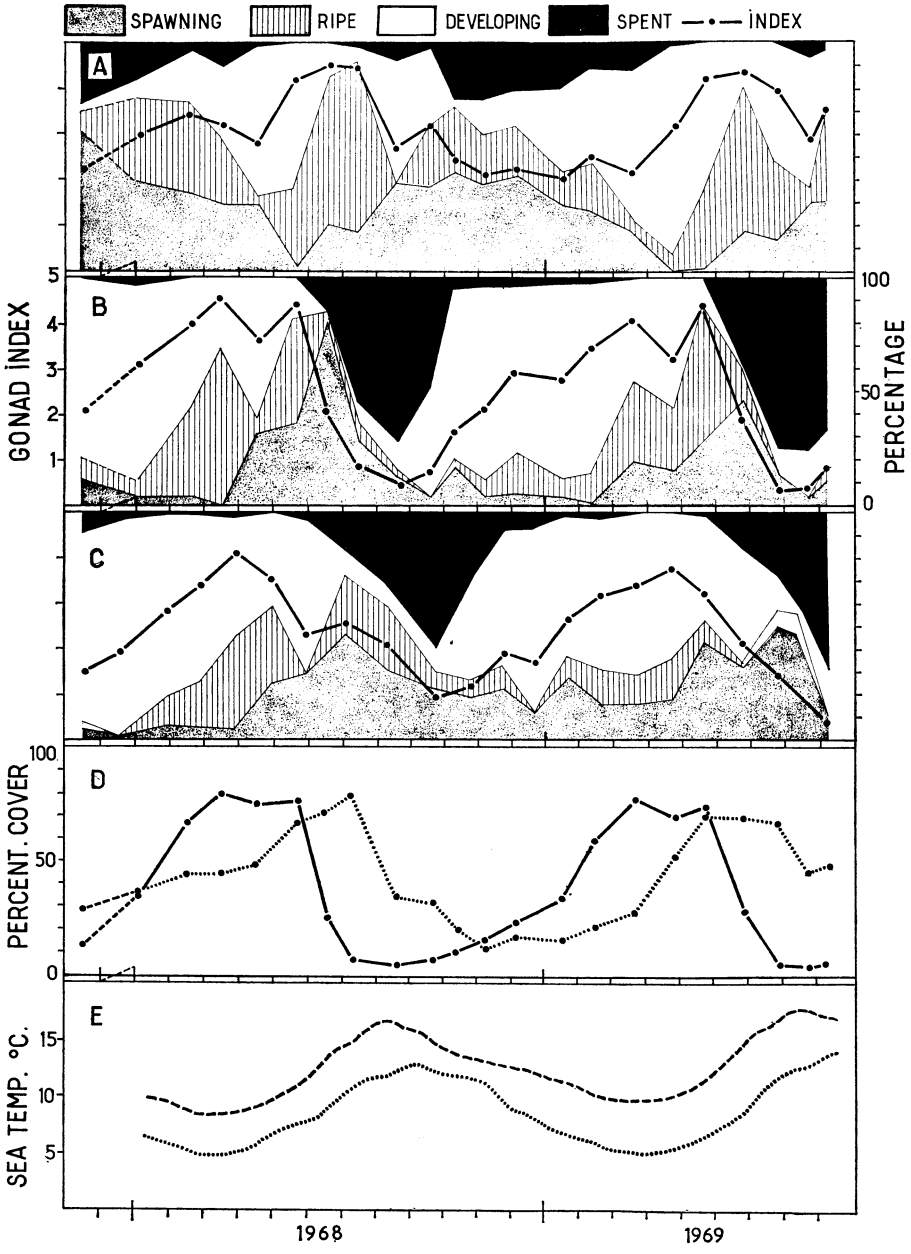


FIG. 1

The reproductive cycles of (A) *M. galloprovincialis*, (B) *M. edulis*, from Rock, S.W. England, (C) *M. edulis* from three sites at Filey, N.E. Yorkshire, (D) The area occupied by reproductive tissue in sections of mantle tissue in ..... ● ..... *M. galloprovincialis* and —— ● —— *M. edulis*, (E) Mean fortnightly sea temperature for - - - - - S.W. Cornwall, ... N.E. England

sea temperatures given in Fig. 1E are those for Longstones Light House (55°49' N, 1°37' E) and the Severnstones Light Vessel (50°04' N, 06° 04' W) (I am particularly indebted to the Meteorological Office for providing the basic data from which these graphs have been compiled). Although the Longstones data closely parallel that for the inshore waters at Robin Hood's Bay, the latter fall both slightly below and rise slightly above the Longstones temperatures. Unfortunately no data is available for the inshore waters at Rock but it is assumed that these would bear a similar relationship to the Severnstones data.

From Figs. 1A and 1B, it will be seen that considerable differences exist in the reproductive patterns of the two species. The gonad index in both shows a steady increase over the early months of the year followed by a slight drop between March and April indicating a period of partial spawning (no fully spent animals accrued from this spawning). This early spawning was more evident in *edulis* than in *galloprovincialis* and indeed did not seem to occur at all in the latter in 1969. Although it is difficult to pinpoint the main spawning periods exactly from monthly samples, this occurred between 8-12 weeks earlier in *edulis* in both years. Spawning in *edulis* commenced in late May and continued through June and into early July, whereas *galloprovincialis* did not start to spawn until late July and continued throughout August. This is also reflected in Fig. 1D (N.B. the close parallel between Fig. 1D and the gonad indices in Figs. 1A and B). Apart from spawning much later in the summer, *galloprovincialis* also showed a far less pronounced seasonal reproductive pattern. This is evident in several respects as will be seen from Fig. 1A.

a) During both years the gonad index in *galloprovincialis* never fell below 2.00.

b) There is a general absence of any well marked spent phase (though the greatest numbers of spent individuals appeared about 8 weeks later than the maximum for *edulis*).

c) The spawning period appeared to be very extended with minimum values appearing to coincide with the maximum spawning periods in *edulis*.

This apparently extended spawning period in *galloprovincialis* is probably artificial and more likely reflects the limitations of histology in analysing breeding cycles. A gonad which may seem to be in the spawning condition histologically may not in fact be in the active process of liberating gametes. The later spawning in *galloprovincialis* might be expected if in fact this is a southern species towards the northern limits of its distribution, and indeed spawning only occurred when the maximum sea temperatures for this region had been attained. It could well be that the sea water barely attains a sufficiently high temperature to induce complete spawning in *galloprovincialis* in this locality. Since the spawning temperature thresholds may differ slightly from one individual to another this could result in some individuals spawning completely but with the majority undergoing only a partial release of gametes and some possibly not releasing at all. Indeed the results in Tables 2 and 3 show that spent *galloprovincialis* start to appear from August onwards but even throughout

winter a considerable number of ripe individuals can still be found in the population. This might account for the relatively high gonad index recorded throughout late autumn and winter. It would also seem that many of the animals which do undergo a partial release may then be held in an apparently suspended spawning condition for some time, giving to the population a suggestion of a relatively extended spawning period. Fig. 1C shows the reproductive cycle of three of the Filey populations considered collectively. The cycle shows an overall similarity to Rock *edulis* but with the main spawning period about 4-6 weeks later in the year. This is undoubtedly a reflection of the later rise in sea temperature in Northern waters.

Artificial fertilisation.

During the spring of 1969 attempts were made to artificially cross fertilise *edulis* and *galloprovincialis* in the laboratory. The mantles from ripe female mussels were dissected and gently macerated in a small amount of filtered sea water in order to release the eggs. Any detritus was carefully pipetted off and the pinkish coloured eggs allowed to settle. In this way several thousand eggs were obtained. Ripe male mantles treated in a similar manner produced a creamy suspension of highly active sperm.

Initially, cleaned eggs were transferred to solid watch glasses containing fresh filtered sea water and one or two drops of sperm suspension added. These were then kept at 6°-8° C. with regular changes of sea water. In later experiments, fertilised eggs were transferred to 250 ml of filtered sea water in conical flasks and kept vigorously stirred by a mechanical stirrer. This kept the developing embryos in motion and prevented them from congregating on the bottom and producing locally unhealthy conditions.

Maximum numbers of active trochophore larvae were obtained within 24 hours after fertilisation. The proportion of unfertilised to fertilised eggs in the samples was always relatively high. This is perhaps to be expected when using gametes which have not spawned naturally and which will therefore contain physiologically unripe eggs. Cross fertilisations of male *galloprovincialis* with female *edulis* and vice versa produced large numbers of active larvae (it was also possible to successfully cross fertilise *galloprovincialis* with *edulis* from North Yorkshire). Within 3-4 days many of the trochophores had developed into healthy veligers with shell rudiments clearly visible.

Throughout these experiments no attempts were made to feed the larvae. Nevertheless, apparently normal larvae were kept alive for up to 7 days. After this the majority had died, presumably through lack of food although the possibility that they had been attacked by saprophytic ciliates, several of which were found in the cultures, could not be excluded.

Settlement of young mussels on the shore.

Between February and December 1968, clumps of mussels from Rock were sent back to be laboratory and carefully examined for

small mussels in order to determine when the maximum periods of settlement occurred and to see whether or not these could be correlated with the breeding cycles recorded from histological preparations. All animals under 20 mm were removed and separated into 2 mm size categories. In Fig. 2 these are expressed as percentages of the total number of animals in each sample.

Mussels are known to settle on adult beds when they have reached about 1.5.-2.0 mm shell lengh and it would be expected therefore that

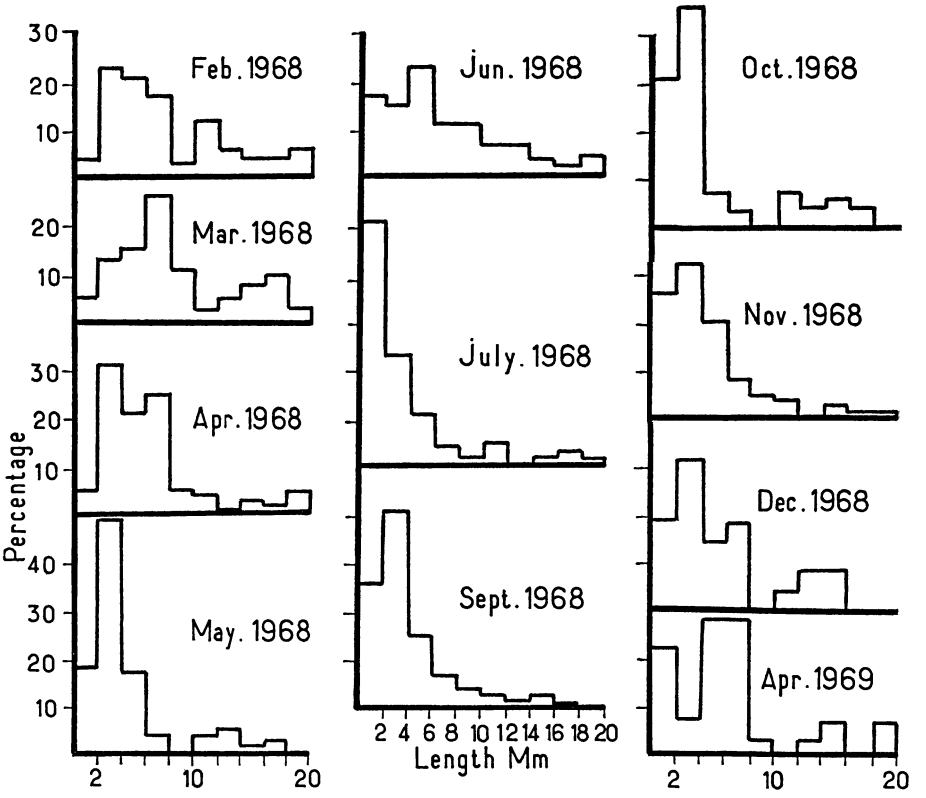


FIG. 2

The occurrence of small mussels at Rock throughout 1968 expressed as percentages of the total number of mussels present.

peaks of abundance of mussels in this size range would occur after the main spawning period. Fig. 2 shows that marked increases in small mussels under 4 mm occurred during April and May when they account for about 70 p. 100 of the total number of mussels present. It is known that settlement of young mussels onto adult beds, however, is not direct. Instead there is a temporary period of attachment spent on filamentous substrates such as red algae and hydroids (Bayne, 1964; Seed, 1969 a). The migration from these temporary sites occurs when the mussels have grown to a certain size, but those which settle later in the year and which therefore meet with poorer growing condi-

tions may remain on these sites overwinter, migrating only as more favourable conditions during the following spring return. This is generally reflected in spring settlements which frequently cannot be correlated with a particular spawning period. It is probable therefore that this increase in small mussels during April-May is due to such migrations of overwintering mussels. The possibility that they are a direct result of the early partial spawnings during March-April cannot, however, be excluded. During June the numbers of small mussels fell, followed by marked rises in July (when over 50 p. 100 of the population was under 2 mm) and October (when 80 p. 100 of the population was under 4 mm). Unfortunately it proved impossible to identify these very small mussels accurately (a view also expressed by Berner (1935) who showed that no biometric differences existed between individuals of *edulis* from the Channel and *galloprovincialis* from Marseilles, in animals under 7-10 mm). It was uncertain therefore whether the increases in small mussels particularly in July and October, could be independently ascribed to the respective spawning periods of *edulis* (May-June) and *galloprovincialis* (July-August). In view of these difficulties further attempts to monitor settlement during 1969 were abandoned.

GROWTH OF MUSSELS IN THE LABORATORY

Since it was impossible to make regular visits to the study area no information concerning the growth rates of the two species under natural conditions could be obtained. However, in June 1968, samples of small mussels were collected at Rock in order to compare their growth rates when kept under identical laboratory conditions. These were measured to an accuracy of 0.01 cm using vernier calipers, marked, and kept in plastic mesh cages suspended in the large header sea water tanks in the laboratory roof space. These tanks were constantly replenished with fresh sea water and the cages were kept free of any fouling organisms which developed on them. In all, 60 *edulis* of mean length 1.75 cm (range 1.03-2.37) and 79 *galloprovincialis,* mean length 1.82 cm (range 1.15-2.40) were used. All mussels (alive and dead) were measured in length approximately every month between July 1968 and September 1969 and the cumulative increments for each species are expressed graphically in Fig. 3. Records of the sea temperature in the tanks were also kept throughout. Mortality over the period of observation was fairly low, 7 p. 100 for *edulis* and 11 p. 100 for *galloprovincialis* but in order to allow for the maximum and minimum effects or mortality between successive measurements, growth increments were calculated on the assumption that all deaths occurred either immediately before the observation or immediately following the previous measurement. Assuming that the mortality was normally distributed, these values should vary equally from the true growth rate. An average of these values was used in calculating the mean monthly increment.

From Fig. 3, it will be seen that the growth rate was extremely

seasonal, with little or no increase over late autumn and winter. This is the characteristic growth pattern for *edulis* grown under field conditions in this locality (Seed, 1969 b). The reasons for such seasonal differences in growth will not be discussed here, but it would appear not to be due entirely to temperature since growth was surprisingly slow during July-August 1969 when the sea temperature was quite high. It was again fairly slow in late September, October and early November 1968 compared with April and May 1969, even though the temperatures during the latter period were generally slightly lower.

The significant feature in Fig. 3 is the marked difference in the growth of *edulis* and *galloprovincialis* even though both were kept

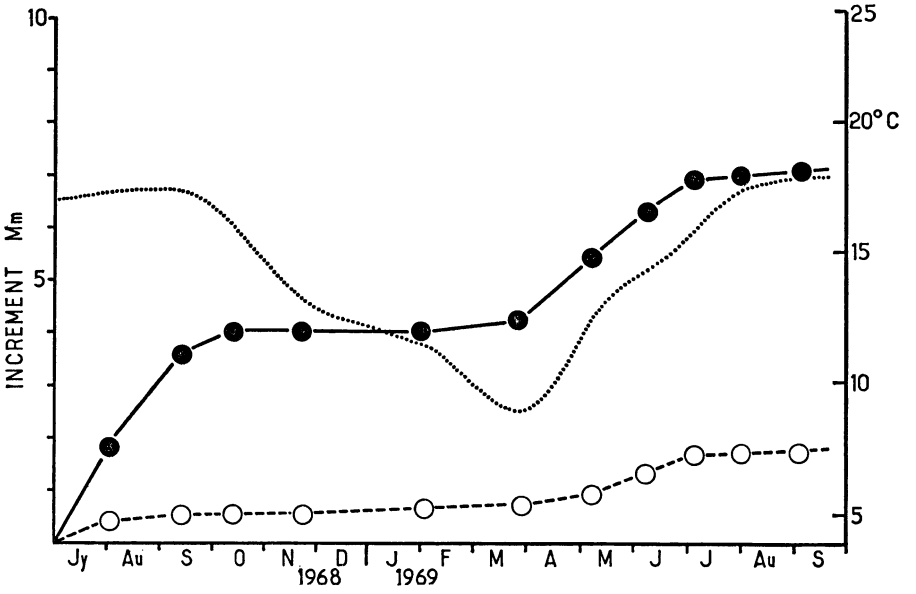


FIG. 3

Growth rates of —— ● —— *M. edulis and* - - - O - - - *M. galloprovincialis* in laboratory tanks. Water temperature is shown as a dotted line.

under identical conditions. Over the period of investigation *edulis* showed a mean increment of 7.00 mm compared with 1.75 mm in *galloprovincialis*. Although these increases may be small compared with their natural growth rates in S.W. England, such differences would seem to indicate marked physiological differences between the two species, though whether these differences would be maintained in natural conditions is perhaps doubtful. The higher sea temperatures in the S.W. may well be more favourable to *galloprovincialis* allowing it to compete more successfully with *edulis*. Certainly the largest mussels at Rock (some of which were over 10 cm in length) were predominantly of the *galloprovincialis* type (also noted by HEPPER), and on many shores in Cornwall *galloprovincialis* is the dominant mussel.

INFECTION WITH *PINNOTHERES*

The mussels at Rock, particularly in the mid and low shore, have previously been shown to be highly infected with the parasitic pea crab *Pinnotheres pisum* (Penn.)

Edulis was consistently found to have much heavier infections (mean 45.20 p. 100) than *galloprovincialis* (2.97 p. 100) from the same mid and low level sites, and possible explanations for these differences have been discussed (Seed, 1969c). Infection in *edulis* was found to increase with increase in the size of the host, the largest mussels (> 9.0 cm) having 80-100 p. 100 infection whilst the smallest size group to be infected (3-4 cm) had less than 10 p. 100 infection. The presence of the crab caused gill damage and infected mussels had considerably lower tissue weights when compared with uninfected mussels of similar size.

Since the « fixed » mussel samples sent back to the laboratory for reproductive analysis were kept separate, it was possible to assess the percentage infection of each species with *Pinnotheres* throughout the year. From Table 9, it will be seen that *edulis* showed consistently heavier infections than *galloprovincialis*. The mean infection for *edulis* over the period investigated was 30.3 p. 100 (range 8-56 p. 100) and 1.4 p. 100 (range 0-5 p. 100) for *galloprovincialis*.

TABLE 9

Seasonal variations in the infection of *Mytilus* by *Pinnotheres pisum* at Rock S.W. England

Date	N° Mussels Examined	N° Infected	percentage Infection	Date	N° Mussels Examined	N° Infected	percentage Infection
1968				1969			
May 17	*edulis* 52 *gallo.* 50	17 0	33 0	Jan. 12	*edulis* 44 *gallo.* 52	11 0	25 0
Jul. 10	*edulis* 50 *gallo.* 50	28 0	56 0	Feb. 9	*edulis* 47 *gallo.* 55	24 3	51 5
Aug. 15	*edulis* 68 *gallo.* 60	14 0	21 0	Mar. 16	*edulis* 52 *gallo.* 52	23 2	44 4
Sep. 15	*edulis* 26 *gallo.* 64	2 0	8 0	May 17	*edulis* 68 *gallo.* 61	14 2	21 3
Oct. 5	*edulis* 49 *gallo.* 50	16 0	33 0	Jun. 20	*edulis* 52 *gallo.* 67	13 0	25 0
Nov. 2	*edulis* 46 *gallo.* 49	22 1	48 2	Jul. 20	*edulis* 58 *gallo.* 47	7 1	12 2
Dec. 1	*edulis* 51 *gallo.* 48	17 2	33 4	Aug. 22	*edulis* 55 *gallo.* 63	8 0	15 0

No particularly marked or consistent seasonal patterns in the infection of *edulis* were noted, although peaks did occur in July and November 1968 and February 1969.

In sectioned mantle material, the presence of trematode sporocysts similar to those described as *Bucephalus mytili* by Cole (1935) were occasionally recorded, and in some cases appeared to cause complete castration. In samples each of over 1,000 mussels, 0.25 p. 100 of *edulis* and 1.33 p. 100 of *galloprovincialis* were infected. However, since no systematic search had initially been made for these parasites, and since only part of the mantle from each animal was sectioned, it is difficult to say whether these results are significant (a sample of 2,770 Filey mussels showed an infection of 1.23 p. 100). Parasitic castration is known to cause increased growth in some species (see for example Rothschild, 1936) and since the largest mussels at Rock do tend to be *galloprovincialis,* a more detailed study of these parasites may prove rewarding.

MANTLE EDGE PIGMENTS

In general the colour of the mantle edge proves to be a very reliable taxonomic character, being purple-violet in *galloprovincialis* and yellow-brown in *edulis.* Yet in some cases identification on this character alone can be rather subjective since animals of intermediate colour are occasionally encountered, more particularly amongst the relatively small mussels on exposed shores. In addition, certain populations of *M. edulis* are known to show a range of mantle colours from grey-white through to deep reddish purple and individuals with dark purplish mantles sometimes account for up to 30 p. 100 of the population (Lewis and Seed, 1969). It was impossible to distinguish visually between these dark Filey mussels and similar sized animals from Cornwall and indeed had they been found in the S.W. they would certainly have been regarded as *galloprovincialis* on this character. There would appear to be no adaptive significance attached to these colour varieties of *edulis* since animals exhibiting them can be found living side by side in the same population.

In the S.W. some dark mantle types from a variety of localities have been found with *edulis* characters but it is interesting to note that *galloprovincialis* types with lighter coloured mantles are exceedingly rare. Previous observations by the author have shown that *edulis* grown under well illuminated conditions tend to become deeply pigmented whereas the mantle and shell of those grown in complete darkness are very pale. Similar observations have been recorded for *Mytilus* grown in the dark grottos at Naples (Fox and Vevers, 1960 p. 40) but unfortunately no indication of the species is given (though I would suspect *galloprovincialis* since from personal observations *edulis* appears to be exceedingly uncommon in the Mediterranean). Pigment deposition would therefore seem to afford some protection from the possible harmful effects of solar radiation.

Mantle edge pigmentation is extremely superficial and can easily be rubbed or scraped from the underlying tissues. In sectioned material, it can be seen in the form of dense granules in the extreme distal portion of the outer ciliated epithelium. Slices of fresh mantle squashed between two microscope slides suggest that these granules are of four basic colours; yellow, yellow-orange, red-brown and purple (wine colour). All can usually be found in individual mussels, but variations in the proportions and distribution of each type can lead to almost any overall shade of mantle. At the light microscope level, however, it is difficult to tell whether individual cells contain one or more type of granule. Again the colour of any one particular cell appears to be modified by the presence and intensity of colour in the underlying tissues. In some cases this may be distinctly yellow and in such cases the cells with red-brown granules may appear distinctly yellowish.

In view of the subjectivity involved in using mantle colour to separate the two species in certain localities (though not in the Rock populations where mantle colours are quite distinct) together with the variability in colour in *edulis* alone, it was decided to attempt a biochemical analysis of the pigments involved. The results are far from conclusive and it is obvious that considerably more detailed research in this field is urgently required before any definite conclusions can be drawn.

Carotenoids.

Initially, it was thought that perhaps carotenoids might be responsible for the colour differences since these are widespread in the animal kingdom and are also known to exhibit considerable colour variation from yellow through orange and red to violet, according to the number of double bonds and the presence of certain radicals.

Carotenoids were extracted from the mantles of groups of 3-4 animals of each species by repeated homogenisation in cold acetone (5 °C). This resulted in a clear yellow solution irrespective of whether purple (*galloprovincialis*) or yellow (*edulis*) tissues were initially used. Pooled acetone extracts were concentrated by taking the pigment into petroleum ether (B.P. 40-60 °C) by the dropwise addition of water, and the spectral absorptions of these ethereal solutions were determined by means of an S.P. 500 spectrophotometer.

Identical curves were obtained for *edulis* and *galloprovincialis* from Rock, and for *edulis* with purple mantles from Filey. These are illustrated in Fig. 4 and show distinct peaks at 452 and 480 mμ with inflections at 425 and 400 mμ. Moreover, when equal wet weights of mantle from Rock *edulis* and *galloprovincialis* were used, virtually identical maximum absorption values were recorded, suggesting not only qualitative but also quantitative similarities in the carotenoids.

When the ethereal solutions were partitioned with 90 p. 100 methanol the pigment was hypophasic probably indicating free unesterified xanthophylls (the molecules of carotenoids such as carotene are hydrocarbons, and these together with their oxides and most of the esterified xanthophylls are preferentially soluble in the petroleum

ether phase). Scheer (1940) found that *M. californianus* Conrad selected xanthophylls from its food in preference to carotenes. Fisher et al. (1956) showed that the mantle of *M. edulis* contained oil and xanthophylls but perhaps surprisingly, in view of its probable light sensitivity, no vitamin A.

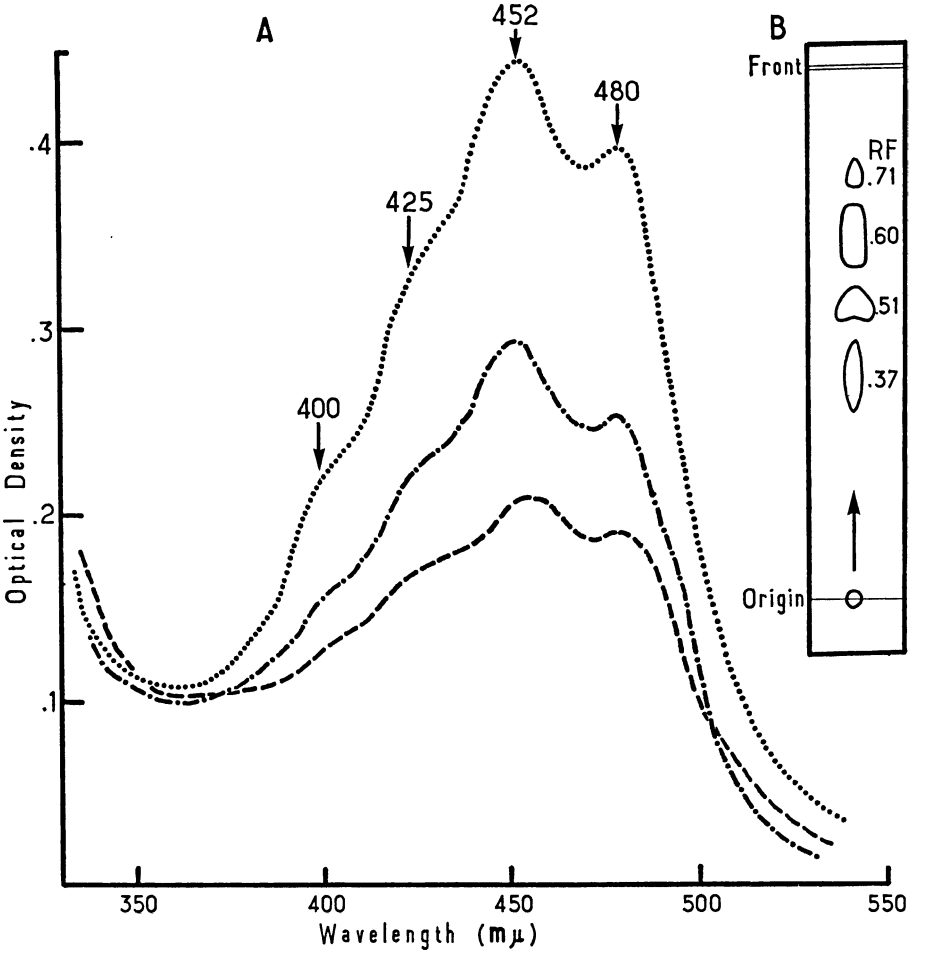


FIG. 4

(A) Non quantitative absorption spectra of the carotenoid pigments in petroleum ether, from the mantle edge of ..... *M. galloprovincialis* and —.—.— *M. edulis* from Rock and ——— *M. edulis* with purple mantles from Filey. (B) Thin layer chromatogram showing the separation of the mantle carotenoids. Rf. values are given for each of the spots.

Carotenoid extracts from the two species in petroleum ether were separately concentrated under nitrogen and spotted onto thin layer chromatographic plates (M. N. Polygram Cel 300) by means of a micropipette. These were run in an acetone-petroleum ether mixture (12:88 v/v) in the dark for one hour. Prior to the experiment the chromatographic tanks were flushed with nitrogen for an hour to

ensure a saturated vapour pressure of solvent within the tank. In addition, large sheets of blotting paper soaked in the solvent were arranged around the sides of the tanks to help retain a solvent saturated atmosphere.

In all cases, four distinct spots could be resolved with Rf values of .71, .60, .51, and .37 (these values varied slightly from plate to plate even though every effort was made to keep conditions relatively constant). Three of these spots were yellowish brown whilst one (Rf .51) was bright pink. The results obtained for *edulis* were, in all cases, identical to those obtained for *galloprovincialis*.

Since this investigation was carried out a detailed survey of the carotenoids in *M. edulis* and *M. californianus* has been published (Campbell, 1970). Both of these species were found to contain the same range of carotenoids and a total of 15 were isolated.

As carotenoids alone were apparently not responsible for the colour differences between the two species, further tests were made upon the mantle edge. The behaviour of the pigments in various solvents has been studied but many of the results are inconclusive and only a brief synopsis will therefore be given in this account. In no instance, however, were any marked differences between the pigments from the two species noted.

Pigment could be extracted with distilled water but this seemed to produce a very fine dispersion rather than a true solution since centrifugation or even prolonged standing caused considerable sedimentation. Addition of cold acetone, ethanol, 3 p. 100 trichloroacetic acid or ammonium sulphate to these aqueous extracts also caused precipitation possibly indicating the presence of protein. No carotenoid was liberated during this precipitation. Absorption curves of crude aqueous extracts showed peaks at 270 mμ (resolved only as a shoulder in *galloprovincialis* and purple *edulis*) possibly due to protein residues (trytophan or tyrosine) and 330 mμ. The latter might be due to the presence of Vitamin A which has a low water solubility. With cold concentrated sulphuric acid, an intense purple-violet colour appeared and whilst at first it was thought that this might indicate the presence of ommochrome, (Fox and Vevers, p. 51) apparently similar reactions can be obtained with certain protein residues.

The pigments showed varying solubilities in formic acid, 5 p. 100 hydrochloric acid and N/10 sodium hydroxide, all solutions again giving maximal absorptions at 270 and 330 mμ, whilst extracts in acidified methanol (5 p. 100 H. Cl) revealed peaks at 240, 315 and 360 mμ. After carotenoid extraction with cold acetone an intractible precipitate always remained which appeared to be insoluble in most solvents except formic acid, when a brownish solution was formed. Indeed, the failure to get the pigment readily into solution was the major problem encountered during this investigation. That all the carotenoid had been extracted by the acetone was confirmed by converting the material to an acetone dried powder and subjecting it to boiling 50:50 chloroform methanol. No carotenoids are known to resist such extraction. Perhaps the main criticism throughout this work is that crude extracts of all cell contents were used and many colourless compounds are known to give marked ultraviolet absorption. There is as yet no evidence that the maxima at 330 mμ has

anything to do with the pigment and it has already been pointed out that this might in fact be due to Vitamin A. Possibly we are dealing here with a mixture of substances and the colour differences are due, at least in part, to the differences in the proportions of the components in these mixtures.

A more profitable line for future work would be a closer examination of the intractible precipitate left after acetone extraction of the carotenoids since this would appear to be, or at least to contain, protein. Carotenoids, ommochromes and melanins are all known to form associations with proteins and the latter two also occur in the form of granules within the cell. Melanoproteins are known to be photosensitive and this does seem to be a feature of the mantle edge. Pigment might be released by hot hydrochloric acid-methanol treatment or, if covalently bound to protein, brought into a water or alcohol soluble form by digestion with trypsin.

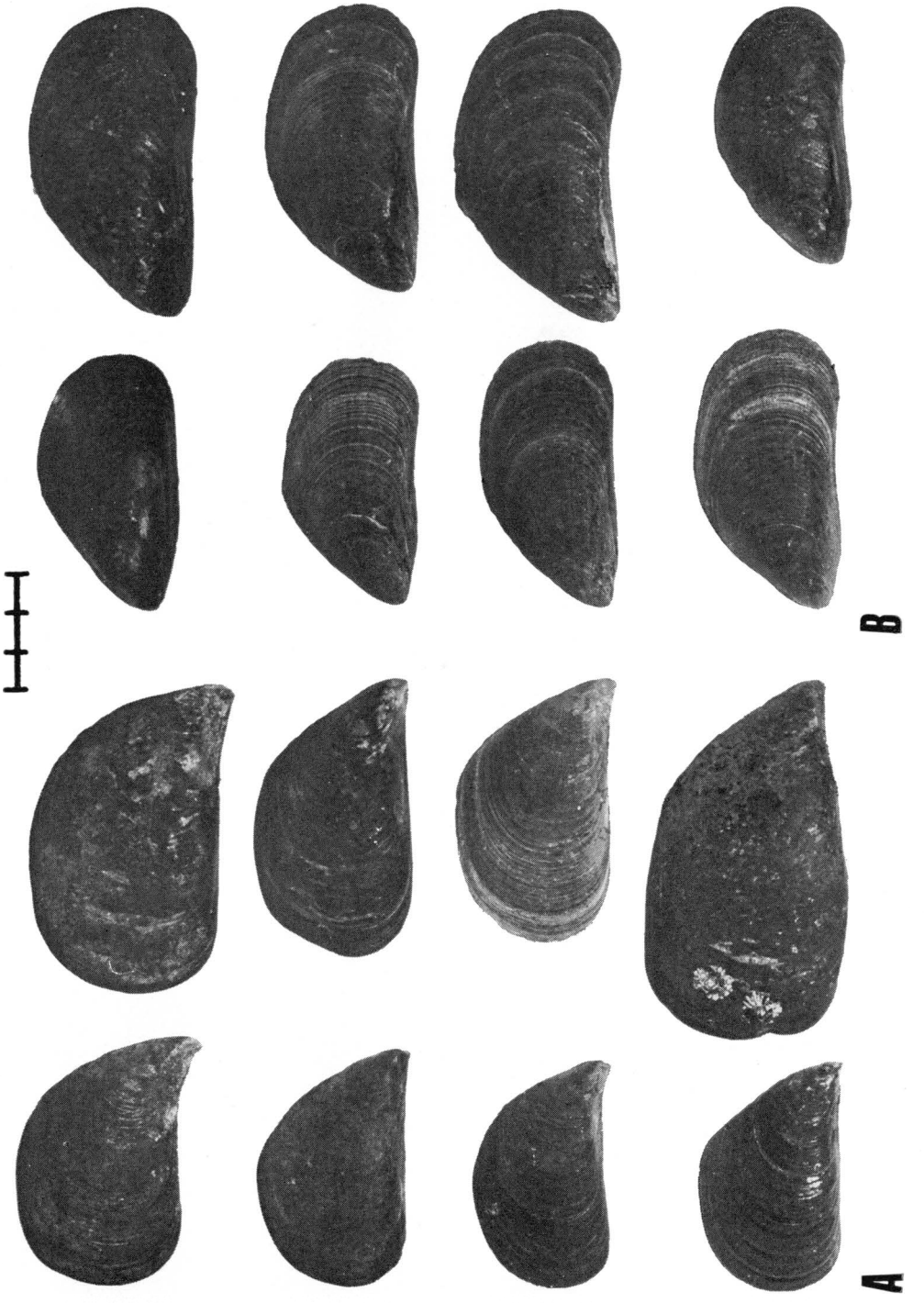
An important requirement would seem to be the isolation of a material giving a clearly defined spectrum in the visible range, and a purple pigment might be expected to show maximal absorption within the 500-600 mμ range.

Clearly much further detailed work in this field is urgently required before any definite conclusions can be drawn concerning the nature of the mantle edge pigments of these two mussels. Although no marked difference between *edulis* and *galloprovincialis* were here recorded, the failure to find any differences may simply reflect the author's limitations in this field rather than any absence of true differences between the species.

PROTEIN ELECTROPHORESIS

Electrophoretic techniques have in recent years contributed enormously to the solution of many taxonomic problems (Manwell and Baker, 1970). They have found widespread application in fisheries research (Jamieson, 1967; Cowie, 1968; Jones and Mackie, 1970) but in general invertebrates have been neglected. Manwell and Baker (1963), however, identified sibling species of sea cucumbers using gel electrophoresis and Manwell et al (1967), who also discuss the potentials and problems of biochemical systematics, found that closely related species of *Calanus* could be easily and consistently separated by electrophoresis of a number of their enzyme systems.

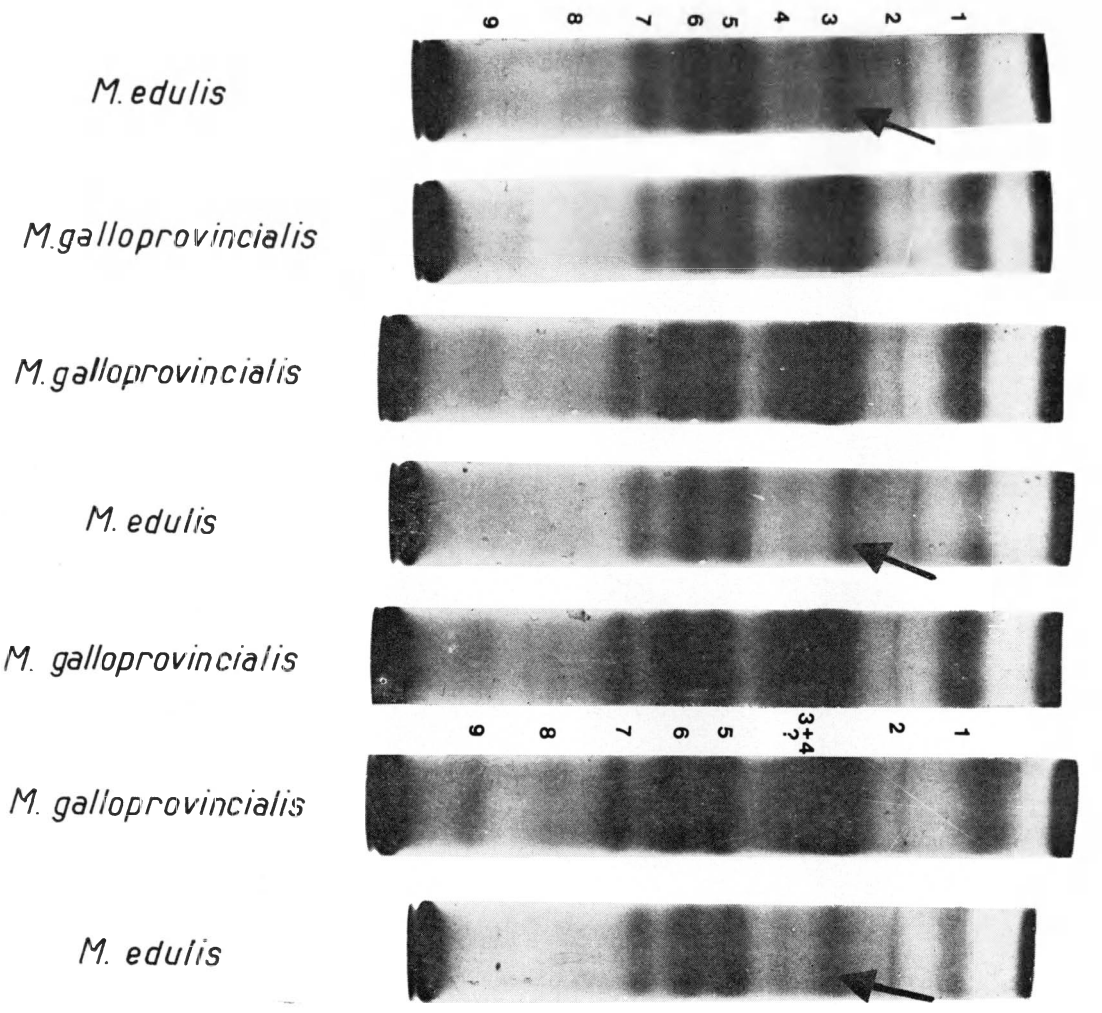
All the material used for electrophoresis was obtained from Rock. Animals were kept in large well aerated tanks and constantly supplied with a fast flow of fresh sea water. Samples were used as soon as possible after their arrival since mussels kept for long periods in the laboratory showed deterioration in their physiological condition. Posterior adductor muscle tissue was used throughout since a fairly concentrated supply of this was easily obtained without contamination from other tissues. Gills, liver and foot tissues were also used in initial trial experiments but these were found to be less satisfactory.



R. Seed

PLATE I.

(A) *M. galloprovincialis* and (B) *M. edulis* from Rock (Nr. Padstow). Note the more pointed and downturned anterior end and the generally more triangular shape of *galloprovincialis*. Scale in cm.



R. SEED

PLATE II.

Electrophoretic patterns of muscle myogens from *M. galloprovincialis* and *M. edulis*. Note the much less dense band in position 3 and the suggestion of a faint band in position 4 in *edulis*. Each gel represents a different mussel.

Adductor muscles from individual mussels were removed and homogenised in a few ml of 0.013 M phosphate buffer at pH 7.5 This was done using a glass mortar and pestle and acid cleaned sand. The homogenate was centrifuged at 12,000 g for 20 minutes and the clear supernatant collected. All operations were carried out at temperatures at or as near 0°C, as possible in order to minimise protein denaturation. The concentration of water soluble proteins (myogens) in these extracts was determined by comparing their biuret reactions against those for protein of known concentrations.

The method of electrophoresis used in this investigation was a simplified version of the system described in detail by Davies (1964). Approximately 200 γ of myogen was applied directly to the top of the polyacrylamide gel columns and electrophoresis carried out for about one hour in a tris-glycine buffer system [Tris=2 amino 2 (hydroxy methyl) 1, 3 propanediol] at 250 volts, and a current of 10 mA per tube. One ml of 0.001 p. 100 bromphenol blue was stirred into the upper reservoir in order to mark the course of the protein front during electrophoresis. Since 6 sample tubes could be accommodated in the apparatus each run, the myogens from 3 *edulis* and 3 *galloprovincialis* were always run together so that any differences that emerged must be due to protein differences rather than to any slight variations in experimental techniques. The tubes were also arranged in an alternating fashion in order to reduce any minor variations which might be set up within the apparatus itself.

After electrophoresis, gels were stained for total protein with Amido black for one hour and finally rinsed and destained in 7 p. 100 acetic acid using the same apparatus (slightly larger diameter tubes were used so that insertion of the stained gels could be accomplished without damaging them).

The characteristic electrophoretic patterns obtained for Rock mussels are shown in Plate II. In all, some 30 individuals of each species have so far been examined, and whilst some slight variations in the results from different runs were obtained, in general the protein patterns for each species were remarkably consistent. Any comparisons between *edulis* and *galloprovincialis* were made only on individuals from the same run. The patterns showed slight though consistent differences which could readily be distinguished from one another by visual comparisons of the gels. However, in order to compare gels more objectively it was necessary to have a common method of comparison. Densitometer tracings or "profiles" were obtained for each gel using a Joyce-Loebl chromoscan. The height of the profile curve at any point along the gel was assumed to correspond to the amount of protein bound dye present at that point. Representative gels of *edulis* and *galloprovincialis* together with their corresponding profiles, are illustrated in Fig. 5. Usually 10 bands appeared with consistency although some bands, especially 8 and 9 (common to both species) were often very faint and are not always resolved in photographs. Bands 5, 6 and 7 were always prominent and were again common to both species. Band 2 was always present and although still relatively dense, was invariably very narrow. Band 1, present in both species, was generally much denser and slightly more diffuse in *galloprovincialis*. The major difference between the 2 species was in the region of bands 3 and 4. In *edulis*, band 3 was strongly

developed with a relatively sharp leading edge (Fig. 5b) and a more diffuse trailing edge (c). Beyond band 3 a weak band 4 (a) could always be resolved in this species. In *galloprovincialis* on the other

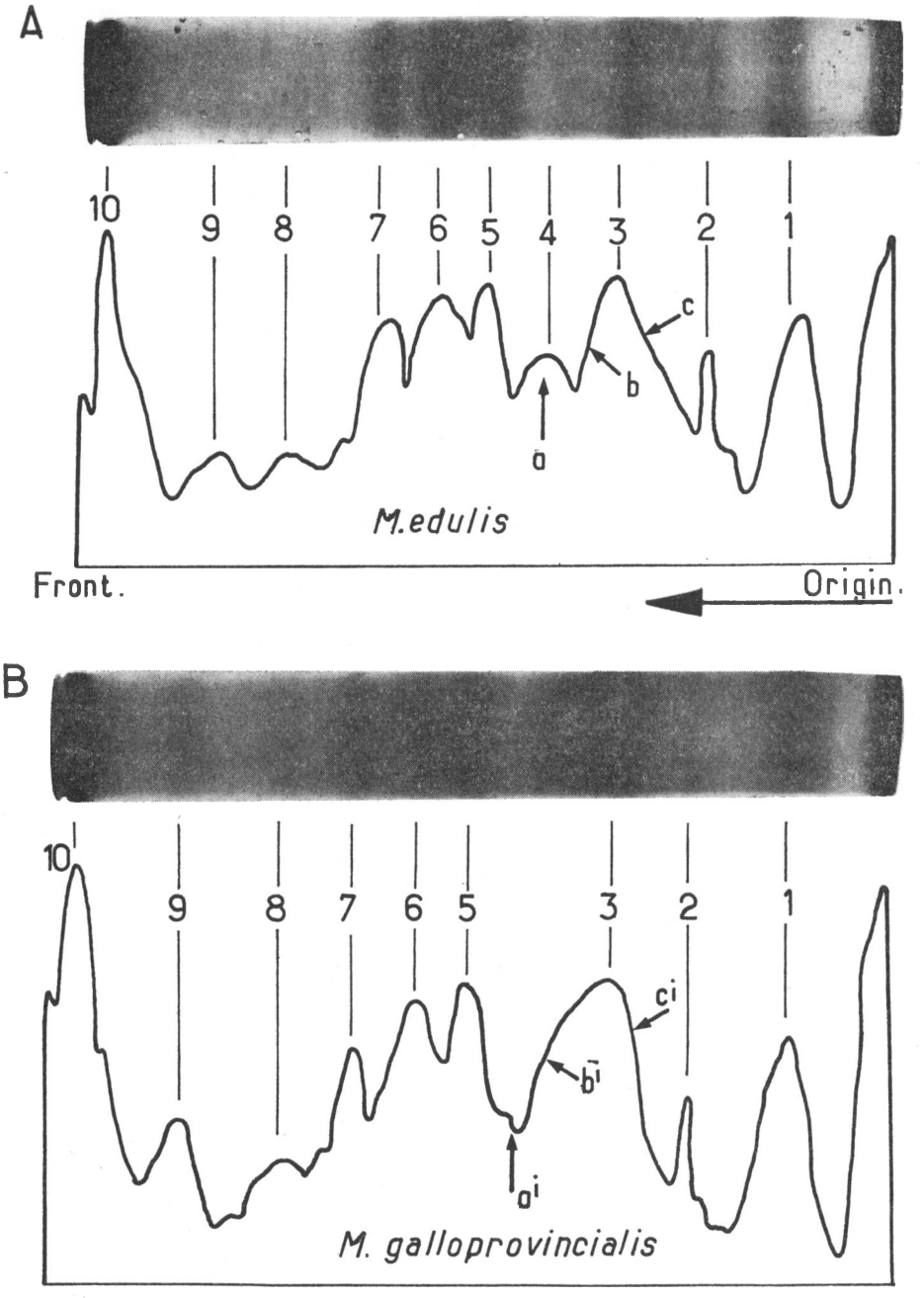


FIG. 5

Electrophoretic patterns of muscle myogens together with their densitometer profiles for (A) *M. edulis* and (B) *M. galloprovincialis*.

hand, band 3 was much broader than its counterpart in *edulis* and in this case it was the leading edge which was more diffuse (Fig. 5 b_1, c_1). No band could be resolved in position 4 but whether this implies that this band is absent in *galloprovincialis* is uncertain since it could well be obscured by, or incorporated with band 3.

Thus, although the differences between the two species are only slight, they have, nevertheless, been found to be remarkably constant in the samples so far examined (30 of each species). Jones and Mackie (1970) found that differentiation of closely related species of hake (*Merluccius*) in terms of their electrophoretic patterns could be made solely on the differences in the intensity of staining of certain protein zones of the same mobility, in contrast to differentiating widely separate species where protein zones of widely different mobilities were obtained.

Perhaps a major objection to using tissue proteins for electrophoretic studies of this kind is the relatively diffuse nature of the individual bands obtained. This might be overcome to some extent either by cutting down the amount of tissue used or, alternatively, by using blood serum proteins.

Clearly, however, these studies need to be carried out on much larger samples and on a much wider scale than here reported before any definite conclusions are drawn concerning the taxonomic significance of these results. Preferably such studies should extend over different phases in the reproductive cycle since electrophoretic patterns in the rainbow trout for example, are known to show intraspecific differences according to the state of maturity or the degree of stress to which the animals have been subjected (Thurston, 1967).

Discussion

A number of opinions have been expressed concerning the specific status of *M. galloprovincialis* since it was first described by Lamarck in 1819. Whilst some authorities believe that sufficient differences exist between *galloprovincialis* and *edulis* to regard them as distinct species, others have found insufficient evidence for separating the two and simply regard *galloprovincialis* as a variety of *edulis*. Yet most of the conclusions reached by the various authorities who have worked on this problem have rested ultimately upon morphological and anatomical characters and these are known to be subject to considerable phenotypic variation. Even in the relatively restricted geographical region of S.W. England, certain localities reveal such marked differences between these two mussels that one would perhaps have no hesitation in regarding them as distinct morphological species, whereas in neighbouring habitats the two show considerable overlap and it is often exceedingly difficult to separate them on morphological grounds alone.

The sexual cycle of *M. galloprovincialis* is well documented but rarely has any comparison been made between the cycles of *edulis* and *galloprovincialis* living in the same locality. Lubet (1957) showed that the cycles of the two mussels in the Bassin d'Arcachon are basically similar. Stage 0, however, is somewhat shorter and the cycle

is generally more extended in *galloprovincialis*. Spawning in *galloprovincialis* started in September but was then arrested by low temperatures and recommenced only at the end of March, continuing until the beginning of July. In *edulis*, feeble emissions occurred during December and early January with the main spawning period from the end of March until June (but especially in April-May). Since both species coexist in the Bassin, ecological factors alone would not seem to be responsible for these differences. During the summer of 1968 whilst studying the morphological variations in mussels from the French coasts (details of which will be published later) samples from Arcachon were collected and their mantles fixed and sectioned. These showed that all the *edulis* were not only in the spent condition, but the connective tissue was also quite thick, presumably due to stored food, suggesting that spawning had been completed for some time. Of the *galloprovincialis*, however, only 37 p. 100 were spent, 40 p. 100 were still spawning, and 23 p. 100 were either ripe or redeveloping.

Berner (1935) compared the reproductive cycles of *galloprovincialis* from Provence with *edulis* (la moule de Calais) in the Baie de l'Aiguillon. He showed that *edulis* had a single short spawning period in February-March (sometimes April) whilst *galloprovincialis* had a relatively extended period with spawnings in September-October and December-January. He considered this to be a clear distinction between the two mussels which were otherwise difficult to separate.

At Concarneau, Bouxin (1956) showed that *galloprovincialis* had an extended spawning period starting in March (sometimes February) and ending in July (exceptionally extending to mid September). At Toulon, *galloprovincialis* started to spawn in September continuing through autumn, winter and early spring and ending the following May or June (Bourcart and Lubet, 1965). They comment upon the more extended spawning periods of this species in the Mediterranean when compared with those on the Atlantic coast, but found no evidence for the existence of two distinct spawning phases (late winter-early spring and in autumn) recorded at Naples by Renzoni (1963). Lubet and Le Gall (1967) found that spawning in *edulis* at Luc-sur-Mer occurred in March-April (sometimes January-February) compared with the extended period in *galloprovincialis* from the Mediterranean (end of September until the following June). They suggest that spawning in mytilids is controlled by latitude, spawning being more extended in tropical waters but more restricted and during the summer months in cooler northerly water. That southern forms breed later in the year further north and vice versa is widely acknowledged (Eckman, 1953; Giese, 1959). Orton (1920) suggests that since no anatomical differences are sufficiently developed to warrant separation of *edulis* and *galloprovincialis*, perhaps it is in the direction of physiological characters such as temperature requirements for reproduction and adaptability to different salinities that one should look for differences between these two mussels. He points out that divergences from the breeding temperature constants at the limits of geographical distribution of a species may be one method of the origin of physiological species and suggests that there is some indication that *galloprovincialis* represents an offshoot adapted to the relatively strange conditions of the Mediterranean.

Without attempting to enter into a discussion of what constitutes a species, attention is drawn to Dobzhansky's (1935) definition of a species as, "that stage of the evolutionary process at which the once actually or potentially interbreeding array of forms becomes segregated into two or more separate arrays which are physiologically incapable of interbreeding". The species is here regarded as a stage in a process and is not necessarily a static unit. In S.W. England, the reproductive cycles of *edulis* and *galloprovincialis* have been shown to be quite distinct with the latter spawning some 2-3 months later in the summer. Cross fertilisation is certainly possible but whether this would occur under natural conditions seems most unlikely in view of the different spawning periods. If this were so, then in this part of their geographical range the two mussels would be genetically isolated, but this might not be the case throughout their range in general and in certain localities considerable hybridisation may in fact be possible. This could explain the differences in opinions previously expressed concerning the specific status of these mussels purely on morphological and anatomical grounds, for populations containing a high percentage of hybrids might be expected to show more intermediate characters than populations where the two are more or less genetically isolated.

Differences in the reproductive cycles, infection with *Pinnotheres,* and growth rates under identical conditions strongly suggest that considerable differences in the physiology of these mussels must exist. Spärck (1936) showed that boreal *edulis* from Denmark had a higher oxygen consumption than Mediterranean *galloprovincialis* at the same temperatures and he concluded that these belonged to different physiological races.

Results concerning the nature of the mantle pigments are far from conclusive. Carotenoids are certainly present but these alone are not responsible for colour variations. A major problem has been in extracting the pigment which was relatively insoluble in the majority of solvents tried. The mantle is light sensitive and prolonged exposure to light in *edulis* stimulates pigment deposition whereas animals grown in the dark are pale. The pigment is granular and there is evidence that protein is involved. Comfort (1951) points out that many bivalve pigments seem to be chromoproteins probably involving melanoid prosthetic groups for which no successful extraction techniques have yet been devised. Melanoproteins are known to be photosensitive frequently being associated with photoreceptor mechanisms. Not all melanins are black or brown and the absorption spectra of yellow, brown, red and black varieties differ only in a slight inflection at 500 mμ. No quantitative differences between these varieties have so far been established and the visible colour differences are apparently due entirely to the physical state and concentration of the melanin.

The possibility that the more intense pigmentation in *galloprovincialis* somehow evolved in response to the greater light intensities experienced by animals living in the relatively atidal, shallow sublittoral waters of the Mediterranean, might be worth consideration.

Posterior adductor myogens from Rock mussels showed consistently different patterns when separated electrophoretically. The

absence of hybrid patterns suggest an absence of interbreeding in this locality which might be anticipated from the breeding data. (Hybridisation is known to influence protein patterns in fish). Clearly it is important to determine the pattern for larger samples, possibly at different periods in the reproductive cycle, in order to see whether it is constant within the species before too much importance is attached to results from this technique in taxonomic studies. Once this is established, the method could have enormous value, not necessarily as a substitute for morphological analyses, but as a complement to them since it could reveal relationships which might not readily be discernible morphologically.

Since protein patterns are generally species specific, a single analysis of one animal should be sufficient for identification whilst on morphological grounds this might only be possible after statistical analysis of a large sample has been made. Certainly in *Mytilus* the identification of single specimens on morphological grounds alone is not always possible in some localities due to the overlap in frequency distribution between the species.

It would appear therefore that in addition to the morphological and anatomical differences between these two mussels in S.W. England, described in detail in a previous paper (Lewis and Seed, 1969) there now appear to be physiological and biochemical differences between them which strengthens the case for considering these mussels as distinct species of *Mytilus*. It would also be interesting to know whether these differences could eventually be supported by immunological and cytological differences.

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Summary

The reproductive cycles of coexisting populations of *Mytilus edulis* L. and *M. galloprovincialis* Lmk in S.W. England were investigated between Jan. 1968 and Sept. 1969. The spawning period in *galloprovincialis* (July-August) was approximately 8 weeks later than in *edulis* (May-June) and occurred only with the onset of maximum sea temperatures for this locality. The annual reproductive cycle was less pronounced in *galloprovincialis*, possibly indicating that in this region it is towards the northern limits of its breeding range. Cross fertilisation can be artificially induced in the laboratory but whether interbreeding would normally occur, in view of the different spawning periods, is questionable.

Settlement of young mussels onto adult beds was monitored during 1968 but in view of the difficulties in identifying small individuals it was uncertain whether the peaks of settlement could be exclusively attributed to the different spawning periods of the two species.

Growth rates of groups of *edulis* and *galloprovincialis* of approximately similar initial size kept under identical laboratory conditions were recorded over the period of this investigation during which time *edulis* grew at a rate four times faster than *galloprovincialis*. Whether such differences would occur under natural conditions is again uncertain.

The two species at Rock (Nr. Padstow) showed marked and consistent differences in their infections with the parasitic pea crab *Pinnotheres pisum* (Penn).

The mean infection for *edulis* was 30.3 p. 100 whilst in *galloprovincialis* the mean was only 1.4 p. 100. No marked seasonal differences in the infection in *edulis* were observed.

Whilst the colour of the mantle edge in the two species is generally a reliable taxonomic character, being purple-violet in *galloprovincialis* and yellow-brown in *edulis*, no marked biochemical differences in the pigments have so far been established. Carotenoids from the two species appear to be identical but these alone are not responsible for colour variations.

Posterior adductor muscle myogens have been separated electrophoretically using polyacrylamide gels and slight though consistent differences recorded.

Thus in addition to the morphological and anatomical differences that are known to exist between these mussels in S.W. England (see LEWIS AND SEED, 1969) there would now appear to be further points of difference which strengthen the case for considering these mussels as distinct species.

Riassunto

Il ciclo riproduttivo di popolazioni coabitanti di *Mytilus galloprovincialis* e di *Mytilus edulis* nell'Inghilterra sudoccidentale è stato dal gennaio 1968 al settembre 1969. Il periodo di riproduzione di *galloprovincialis* (luglio-agosto) cade circa 8 settimane più tardi di quello di *edulis* (maggio-giugno) e si verifica solo con l'insorgere delle massime termiche locali. L'andamento annuale del ciclo riproduttivo è men pronunziato in *galloprovincialis*, indicando forse cosi' che la specie, in questa regione, si trova ai limiti del suo ambito riproduttivo. In laboratorio si puo' ottenere artificialmente la fecondazion incrociata delle due specie, ma è discutibile se esse si possa verificare di norma in natura, data la differenza nei periodi reproduttivi.

L'insediamento di giovani cozze fino ai giacimenti adulti è stata controllata durante il 1968, ma, data al difficoltà d'identificazione degli individui più piccoli, è incerto se i massimi di fissazione si possano attribuire soltanto al diverso periodo riproduttivo delle due specie.

Il tasso di crescita di gruppi di *edulis* e di *galloprovincialis* di mole iniziale approssimativamente simile, tenuti in laboratorio in condizioni identiche, sono stati registrati per tutta la durata di questa ricerca : in tale periodo di tempo, il tasso di crescita di *edulis* è stato quadruplo di quello di *galloprovincialis*. Pure incerto rimane il fatto se tali differenze si riscontrino anche in condizioni naturali.

Le due specie, a Rock, presso Padstow, mostrano notevoli, e consistenti, differenze nell'infestazione ad opera del *Pinnotheres*. La media d'infestazione è di 30,3 p. 100 per *edulis*, contro il solo 1,4 p. 100 in *galloprovincialis*. Non si sono osservate, in *edulis*, differenze stagionali marcate nell'infestazione.

Mentre il colore dell'orlo palleale nelle due specie è generalmente un buon carattere tassonomico, poiche è violaceo in *galloprovincialis* e giallo-bruniccio in *edulis*, non si sono finora, invece, trovate differenze biochimiche sensibili tra i pigmenti. I carotenoidi di entrambe le specie sembrano esser identici, ma essi non sono i soli responsabili delle differenze cromatiche.

I miogeni del muscolo adduttore posteriore son stati elettroforeticamente separati con l'uso di gel di poliacrilamide, e si sono registrate differenze lievi, ma significative. Cosi', in aggiunta ai caratteri morfologici ed anatomici che si conoscono tra queste due cozze nel'Inghilterra sudoccidentale, cisarebbero ora altre differenze che consolidano la separazione specifica di queste cozze.

REFERENCES

BAYNE, B.L., 1964. — Primary and secondary settlement in *Mytilus edulis* L. (Mollusca). *J. Anim. Ecol.*, 33, *pp.* 513-23.

BERNER, L., 1935. — La reproduction des moules comestibles *Mytilus edulis* L. et *M. galloprovincialis* (Lmk) et leur répartition géographique. *Bull. Inst. océanogr. Monaco*, 680, *pp.* 1-8.

BOURCART, C. et LUBET, P., 1965. — Cycle sexuel et évolution des réserves chez *Mytilus galloprovincialis* Lmk. (Mollusque Bivalve). *Rapp. P.-V. Réun. Comm. int. Explor. scient. Mer Méditerr.*, 18, *pp.* 155-58.

BOUXIN, H., 1956. — Observations sur le frai de *Mytilus edulis* var. *galloprovincialis* (Lmk.), dates précises de frai et facteurs provoquant l'émission de produits génitaux. *Rapp. P.-V. Réun. Comm. int. Explor. scient. Mer Méditerr.*, 140, *pp.* 43-46.

CAMPBELL, S.A., 1970. — The carotenoid pigments of *Mytilus edulis* and *M. californianus*. *Comp. Biochem. Physiol.*, 32, *pp.* 97-115.

COLE, H.A., 1935. — On some larval trematode parasites of the mussel (*Mytilus edulis*) and the cockle (*Cardium edule*). *Parasitology*, 27, *pp.* 276-85.

COMFORT, A., 1951. — The pigmentation of molluscan shells. *Biol. Rev.*, 26, *pp.* 285-301.

COWIE, W.P., 1968. — Identification of fish species by thin slab polyacrylamide gel electrophoresis of the muscle myogens. *J. Sci. Fd, Agric.*, 19, *pp.* 226-229.

DAVIES, B.J., 1964. — Disc electrophoresis. II. Methods and application to human serum proteins. *Ann. N.Y. Acad. Sci.*, 121, *pp.* 404-426.

DOBZHANSKY, T., 1935. — Genetics and the Origin of Species. New York. Columbia University Press. 352, *pp.*

EKMAN, S., 1953. — Zoogeography of the Sea. Text-books of Animal Biology. London. Sidgwick & Jackson, 417 *pp.*

FISHER, L.R., KON, S.K. & THOMPSON, S.Y., 1956. — Vitamin A and carotenoids in certain invertebrates. IV. Mollusca, Loricata, Lamellibranchiata and Gastropoda. *J. mar. biol. Ass. U.K.*, 35, *pp.* 41-61.

FOX, H.M. & VEVERS, G., 1960. — The Nature of Animal Colours. Text-books of Animal Biology. London. Sidgwick & Jackson, 246 *pp.*

GIESE, A.C., 1959. — Comparative physiology. Annual reproductive cycles of marine invertebrates. *A. Rev. Physiol.*, 21, *pp.* 547-76.

HEPPER, B.T., 1957. — Notes on *Mytilus galloprovincialis* (Lmk) in Great Britain. *J. mar. biol. Ass. U.K.*, 36, *pp.* 33-40.

JAMIESON, A., 1967. — New genotypes in cod at Greeland. *Nature, Lond.*, 215, *pp.* 661-662.

JONES, B.W. and MACKIE, I.M., 1970. — An application of electrophoretic analysis of muscle myogens to taxonomic studies in the genus *Merluccius*. *Comp. Biochem. Physiol.*, 32, *pp.* 267-273.

LAMARCK, J.B.M., 1819. — Histoire Naturelle des Animaux sans Vertèbres. Paris.

LEWIS, J.R. and SEED, R., 1969. — Morphological variations in *Mytilus* from S.W. England in relation to the occurrence of *M. galloprovincialis* (Lmk). *Cah. Biol. Mar.*, 10, *pp.* 231-253.

LUBET, P., 1957. — Cycle sexuel de *Mytilus edulis* L. et *M. galloprovincialis* (Lmk) dans le bassin d'Arcachon (Gironde). *Année biol.*, 33, *pp.* 19-29.

LUBET, P. et LE GALL, P., 1967. — Observations sur le cycle sexuel de *Mytilus edulis* L. à Luc-sur-Mer. *Bull. Soc. linn. Normandie*, 8, *pp.* 303-307.

MANWELL, C. and BAKER, C.M.A., 1963. — A sibling species of sea cucumber discovered by starch gel electrophoresis. *Comp. Biochem. Physiol.*, 10, *pp.* 39-53.

MANWELL, C., BAKER, C.M.A., ASHTON, P.A. and CORNER, E.D.S., 1967. — Biochemical differences between *Calanus finmarchicus* and *C. helgolandicus*. *J. mar. biol. Ass. U.K.*, 47, *pp.* 145-169.

MANWELL, C. and BAKER, C.M.A., 1970. — Molecular Biology and the Origin of Species. Biology Series. London : Sidgwick and Jackson. 394 *pp.*

ORTON, J.H., 1920. — Sea temperature, breeding and distribution in marine animals. *J. mar. biol. Ass. U.K.*, 12, *pp.* 339-366.

RENZONI, A., 1963. — Ricerche ecologiche e Idrobiologiche su *Mytilus galloprovincialis* Lmk nel Golfo di Napoli. *Boll. Pesca Piscic Idrobiol.*, 18, *pp.* 187-238.

ROTHSCHILD, M., 1936. — Gigantism and variation in *Peringia ulvae* Penn. caused by larval trematodes. *J. mar. biol. Ass. U.K.*, 20, *pp.* 537-546.

SCHEER, B.T., 1940. — Some features of the metabolism of the carotenoid pigments in the Californian sea mussel (*Mytilus californianus*). *J. biol. Chem.*, 136, *pp.* 275.299.

SEED, R., 1968. — Factors influencing shell shape in *Mytilus edulis* L. *J. mar. biol. Ass. U.K.*, 48, *pp.* 561-584.

SEED, R., 1969a. — The ecology of *Mytilus edulis* L. (Lamellibranchiata) on exposed rocky shores. Part. 1: Breeding and settlement. *Oecologia* (*Berl.*) 3, *pp.* 277-316.

SEED, R., 1969b. — The ecology of *Mytilus edulis* L. (Lamellibranchiata) on exposed rocky shores. Part 2: Growth and mortality. *Oecologia* (Berl.) 3, *pp.* 317-350.

SEED, R., 1969c. — The incidence of the pea crab, *Pinnotheres pisum* in the two types of *Mytilus* (Mollusca. Bivalvia) from Padstow, S.W. England. *J. Zool., London*, 158, *pp.* 413-420.

SPARCK, R., 1936. — On the relation between metabolism and temperature in some marine lamellibranchs and its zoogeographical significance. *Biol. Meddr.*, 13, *pp.* 1-27.

THURSTON, R.V., 1967. — Electrophoretic patterns of blood serum protein from rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd Canada*, 24, *pp.* 2169-2188.