ABNORMAL PIGMENTATION AS A POSSIBLE TOOL IN THE STUDY OF THE POPULATIONS OF THE PLAICE (*PLEURONECTES PLATESSA* L.)

By

J. F. DE VEEN

Rijksinstituut voor Visserijonderzoek, IJmuiden,
Netherlands

Abnormal pigmentation in flatfish may be a useful tool in the study of populations. Since 1963 its merits as a population character have been investigated in the plaice of the central and southern North Sea. In this plaice stock the existence of at least four separate populations had been demonstrated already by means of meristic and otolith features and tagging experiments.

In general, the results obtained with abnormal pigmentation confirmed what had been found before, but in some instances more detailed information could be obtained e.g. on recruitment. Moreover the use of pigment anomalies opens possibilities in assessing natural mortality rates in pre-recruit plaice from different nurseries.

The results obtained are in contradiction with the hypothesis generally held, that pigment deficiencies are innate or develop during or before metamorphosis only. It was found that in one class of abnormal pigmentation a trematode worm living in cysts in the skin of juvenile plaice is responsible for the development of pigment anomalies.

INTRODUCTION

In flatfishes pigment deficiencies are not rare. On the dorsal or eye-side of the fish white spots or areas devoid of the normal pigmentation can be found, whereas pigmented spots or patches do occur on the normally white ventral or blind-side. This phenomenon is well known and has attracted the attention of a great number of scientists such as BREDER (1938), CHABANAUD (1957), CUNNINGHAM (1891, 1893, 1895a, b), DAWSON (1962, 1967), EISLER (1963), ELMHIRST (1911), FRANZ (1910), GUDGER (1934, 1935, 1941, 1946), NORMAN (1927, 1934), OSBORN (1939, 1940, 1941 a, b), RILEY (1963, 1966), RILEY and THACKER (1963), SCHNAKENBECK (1923), SHELBOURNE (1963 a, b, 1964), SHELBOURNE, RILEY and THACKER (1963), and VON UBISCH (1951).

Most authors have reported on a limited number of specimens collected, some experiments have been carried out in order to throw more light on the causation of pigment anomalies, but only a few workers have made a quantitative approach to abnormal pigmentation in flatfishes.

During our studies of the plaice populations in the North Sea, started in 1957, we were in the opportunity to handle a very great number of fish for "racial" studies as well as for tagging purposes. We were struck by the fact that abnormal pigmentation is a fairly common feature among our plaice and at the suggestion of Dr HARDEN JONES of the Fisheries Laboratory, Lowestoft, we started an investigation into the possibilities of pigment anomalies as indicators of plaice populations to complement our routine programme for population study in the year 1963.

| J. Cons. int. Explor. Mer | 32 | No. 3 | 344–83 | Copenhague, Avril 1969 |

On account of differences in meristic and otolith features the existence of separate populations in the plaice in the North Sea had already been demonstrated, which was later confirmed by tagging experiments (DE VEEN and BOEREMA (1959), DE VEEN (1962, 1965)). This facilitated the interpretation of the results of our present pigment studies.

If pigment deficiencies can be used as population characters, this would be of considerable help in the study of plaice populations in general, provided the phenomenon is not rare. As large numbers of plaice can be examined in only a fraction of the time normally required for preparing and counting vertebrae, fin-rays or gill-rakers or measuring otolith structures, the use of abnormal pigmentation as an additional criterion becomes very attractive indeed.

In the years 1963, 1964, 1965 and 1966 the pigmentation pattern of every mature plaice, tagged on the spawning grounds, has been recorded. In total 4,644 adult plaice in the Flamborough area, 5,672 plaice in the Cleaver Bank area, 13,825 in the German Bight and 10,167 in the Southern Bight were used for this purpose. In addition 5,145 juvenile plaice were examined on the nursery ground in the Dutch Waddensea in 1965 and 1966 and 3,207 juvenile plaice on the beach near IJmuiden in 1965.

CLASSES OF ABNORMAL PIGMENTATION

Several classes of abnormal pigmentation occur. CUNNINGHAM and MACMUNN (1893) use the name ambicoloration for all pigment anomalies. According to VON UBISCH (1951) this is not correct; the term ambicoloration has to be applied to the intense, brown-green pigmentation on the blind-side solely. In the following we shall use the word ambicoloration for the blind-side pigmentation only and call albinism the absence of pigment on the eye-side. Both ambicoloration and albinism follow fairly regular patterns.

According to CUNNINGHAM and MACMUNN (1893) ambicolorate Pleuronectidae demonstrate a deformation of the head. GUDGER and FIRTH (1936) describe the relation of incomplete eye rotation and "hooking" of the anterior dorsal fin with complete or nearly complete ambicoloration in a number of flatfish species. In the plaice we never observed this deformation in adult fish with complete or nearly complete ambicoloration (0 out of 18 fish), and found only one out of thirteen nearly totally ambicolorate juvenile plaice with incomplete eye rotation and hooking of the dorsal fin. This is in confirmation with SCHNAKENBECK (1923) who did not find any deformations in his wholly ambicolorate plaice near Heligoland.

SCHNAKENBECK (1923) distinguishes a third class of pigment deficiency, *viz.* a diffuse pigmentation of rather small specks on the blind-side, mostly irregular in its distribution. This diffuse pigmentation is less intense than ambicoloration and has a grey-yellow, grey or pale brown colour, never bright green or brown-green as in ambicoloration, and lacks the special pattern (orange, white and black spots) of the eye-side so nicely repeated on the blind side in ambicolorate plaice.

Another class of pigment deficiency, called xanthochroism, is the case in which the melanophores are missing on both sides of the fish. This class is, however, so rare in the plaice we examined, that we left it out of consideration in our present study.

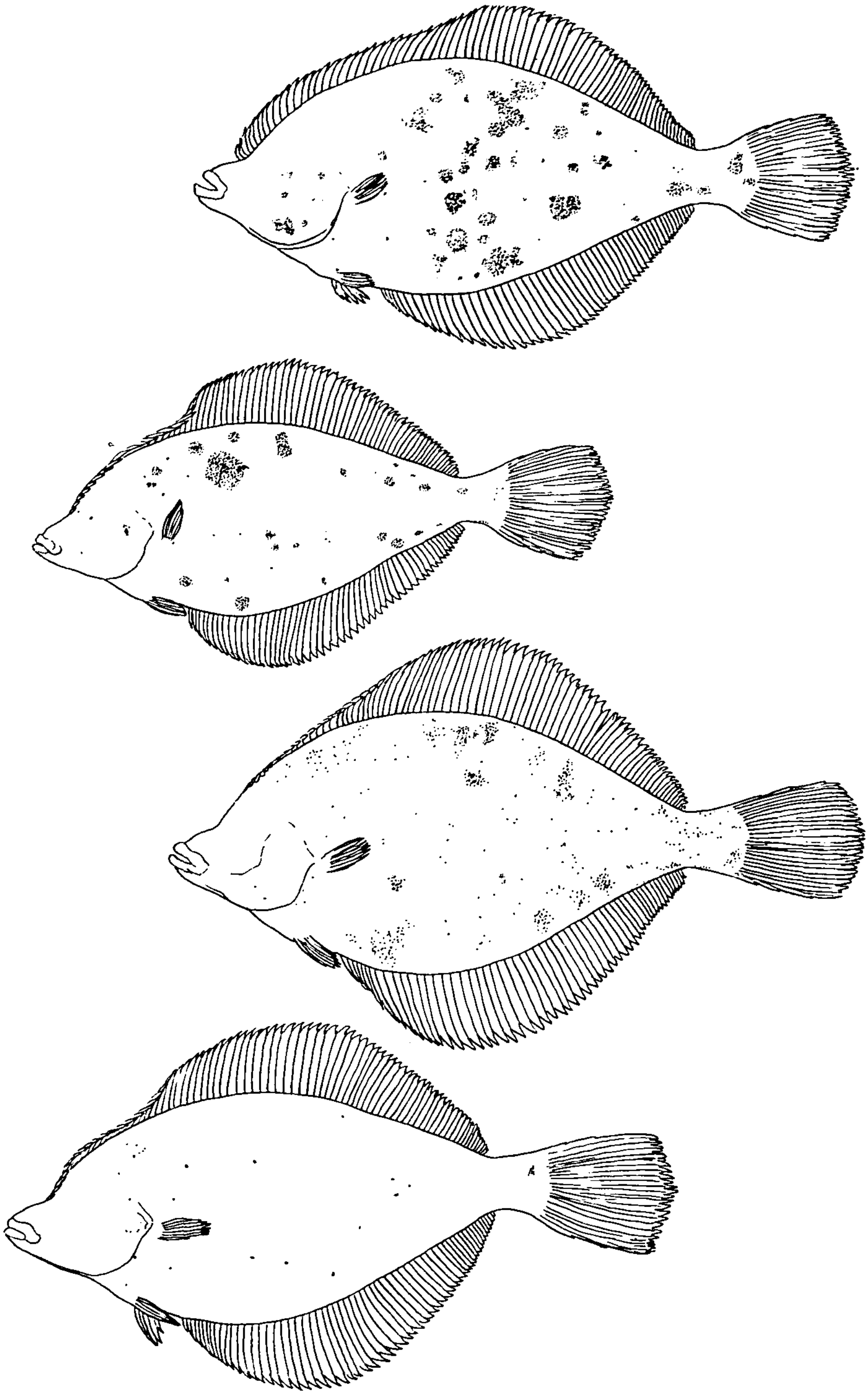


Figure 1. *Slight diffuse pigmentation* on the blind-side ranges from one or more tiny spots as shown in the plaice at the bottom to a spotted appearance as demonstrated in the specimen at the top.

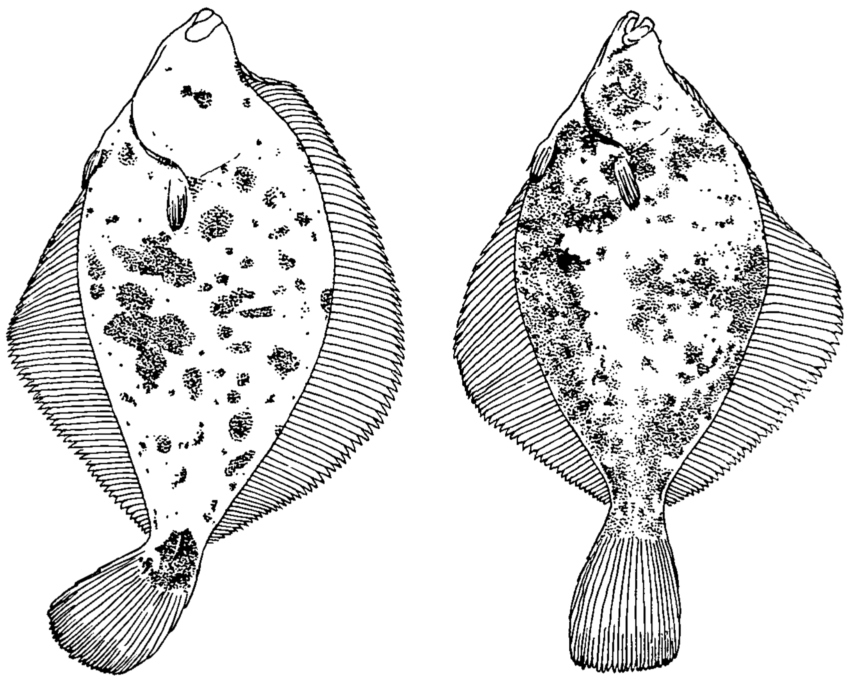


Figure 2. In *moderate diffuse pigmentation* the area pigmented ranges from 10% to 50%.

At sea most of the abnormal pigmentation is of the diffuse type; ambicoloration and albinism are relatively scarce in the sea, as was the case in former times (HOUTTUYN, 1764; VAN DEN ENDE, 1849; VAN BEMMELEN, 1866). This is in marked contrast to the plaice reared in hatcheries ((VON UBISCH, 1951; RILEY, 1963, 1966; RILEY and THACKER, 1963; SHELBOURNE, 1963a, b, 1964; SHELBOURNE, RILEY and THACKER, 1963), where these abnormalities do occur frequently.

RESULTS

IS THE DEGREE OF ABNORMAL PIGMENTATION CONSTANT THROUGHOUT LIFE?

Before proceeding to compare degrees of abnormal pigmentation in different plaice populations we should determine whether the degree of pigment deficiency is constant or varies during the life of the adult plaice. In the former case it would be simpler to use as a criterion than in the latter.

For reasons of abundance (mature females are much less abundant on the spawning grounds than mature males) we confined our attention to male plaice and left the females out of consideration for the time being.

The degree of *diffuse pigmentation* was assessed by eye in three categories viz. *slight* – from one or more tiny specks to a spotted appearance in which the area pigmented is about 10% (see Fig. 1), *moderate* – the area pigmented

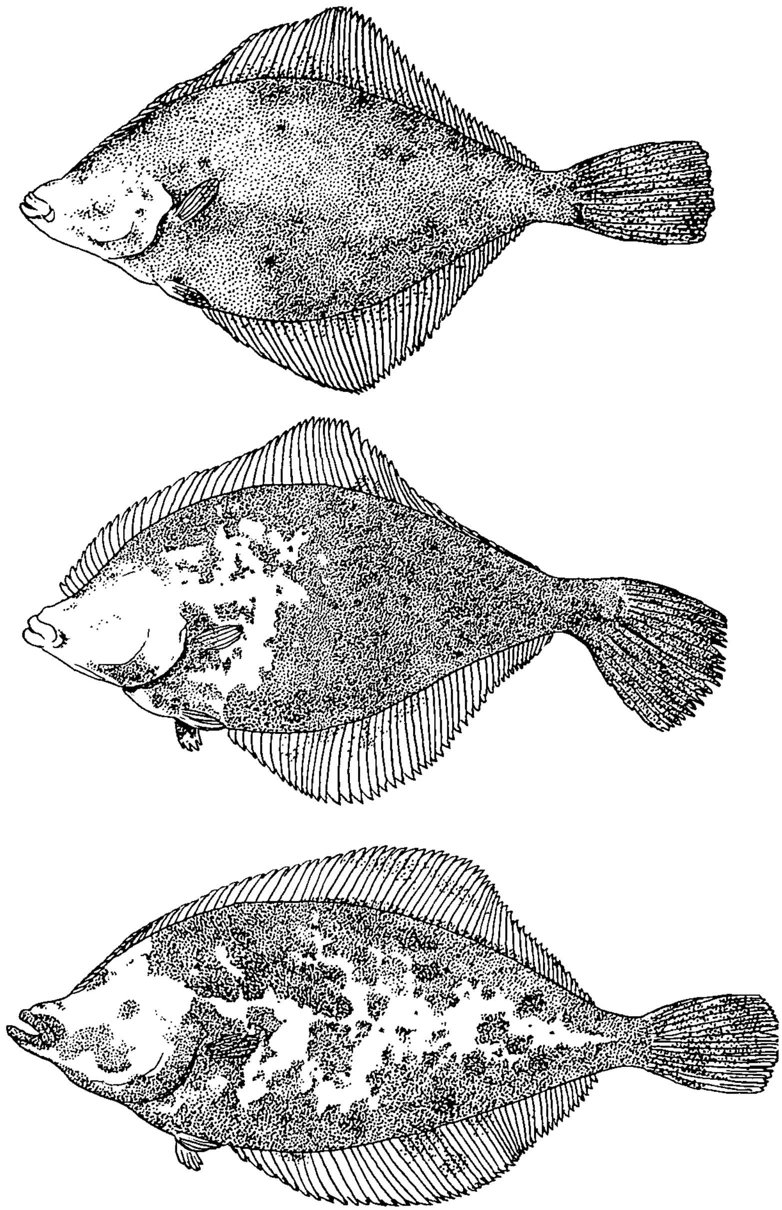
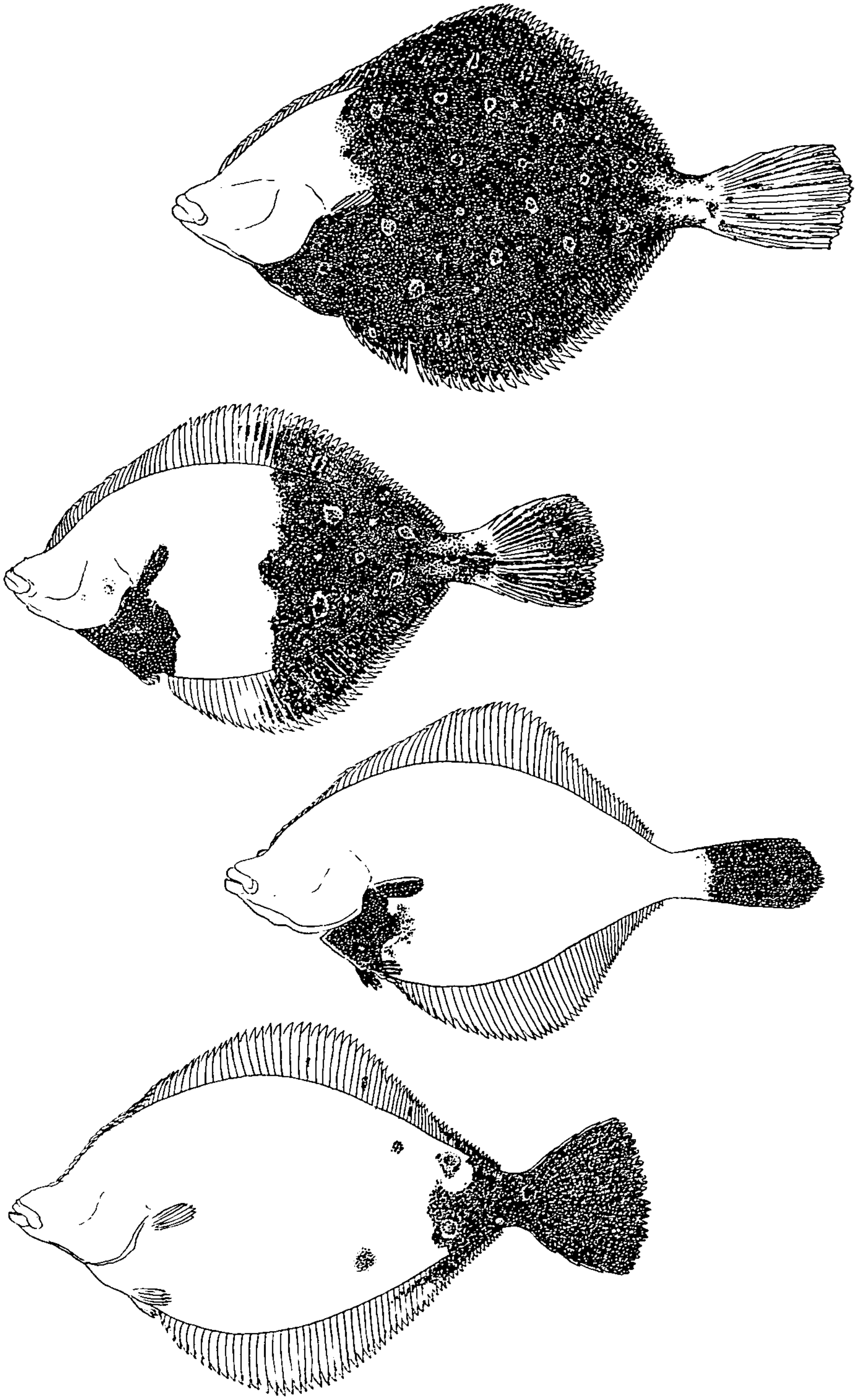


Figure 3. In *heavy diffuse pigmentation* the pigmented area is more than 50% and may cover 100% of the blind-side.

Figure 4 (see page 349 facing). *Ambicoloration* follows rather regular patterns. Main areas pigmented are the tail and the region around the anus. From these places the pigmentation may extend itself and eventually may cover the whole blind-side with the exception of the head.



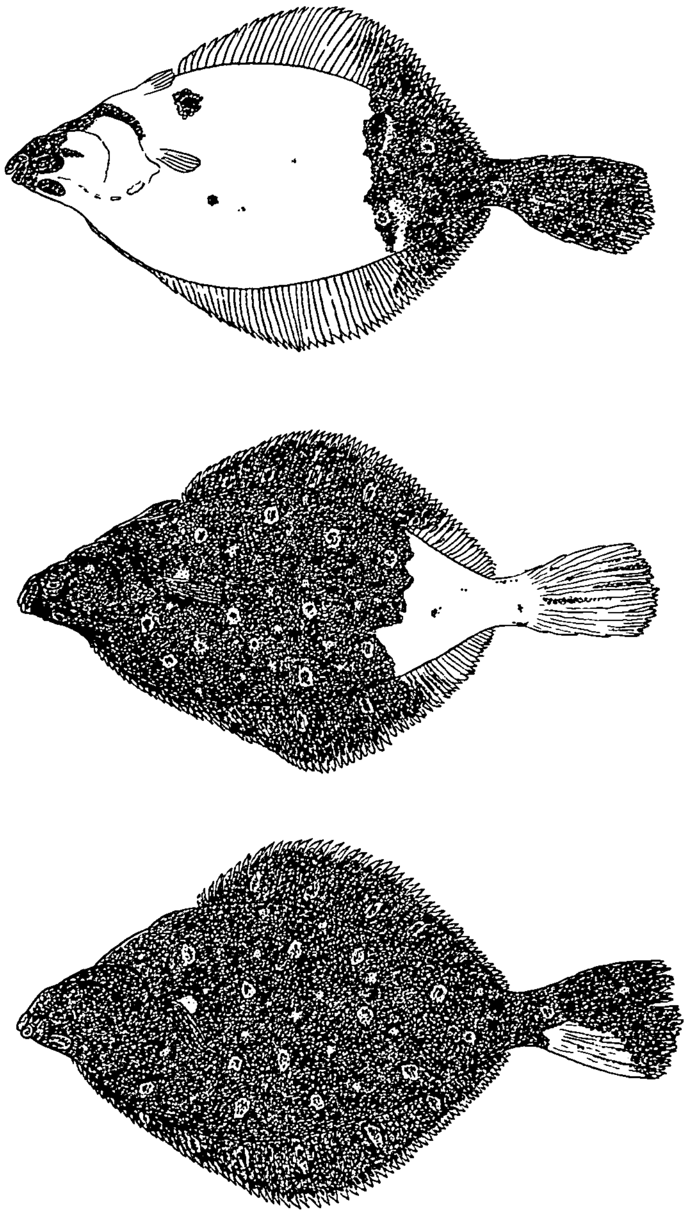


Figure 5. *Albinism* follows rather regular patterns. Main areas are the tail and the caudal part of the fish.

ranges from 10 to 50% (see Fig. 2), and *heavy* – the pigmented area covers more than 50% (see Fig. 3).

Diffuse pigmentation may occur all over the blind side of the fish including the head. This is in contrast to *ambicoloration* (see Fig. 4), in which we found that the head was never pigmented in the ambicolorated sense. Sometimes we observed plaice looking totally ambicolorate. On inspection, however, these plaice appeared to possess a combination of complete ambicoloration of the body and heavy diffuse pigmentation of the head. SCHNAKENBECK (1923) mentioned this combination.

In *albinism* (see Fig. 5) we never found a totally albinistic plaice. In the majority of the cases only white areas or spots were observed on the tail. Contrary to the pigment anomalies on the blind-side it is possible that a number of cases were not recorded because of the fact that when the white spots are too small they may easily be overlooked. Thus the incidence of albinism determined by us will be an underestimate. As, however, this will affect plaice of all the populations to the same extent, this will not influence our conclusions based on differences in relative amounts of albinism.

In order to find out whether the degrees of abnormal pigmentation are constant or not, we pooled all information of the four populations, thus having data from some 34,000 male plaice at our disposal. In Figure 6 the resulting degrees of pigmentation deficiencies with length are given.

The degree of normally pigmented plaice decreases regularly from 93% in the smallest adults to 45% in the largest fish. When comparing this decrease with the diagrams for the various pigment anomaly classes it is obvious that it is mainly caused by a steady increase in the degree of slight diffuse pigmentation with length. The pigmentation-length relation of this class is in marked contrast to that of the classes moderate and heavy diffuse pigmentation. The increase with length is low in the moderate diffuse plaice and in the heavy diffuse plaice there is no increase at all. The classes albinism and ambicoloration again do show an obvious increase with length.

Because our pigmentation studies were made on tagged fish at release we have only a limited amount of information on the age of the fish. Of the total number of tagged plaice some 15–20% were recaptured annually, of which for a number of reasons only a part could be used for age determination. For lack of more information we assumed that the trends in Figure 6 are also indicating corresponding relationships of pigment anomalies with age.

It is clear that the only classes of pigment deficiencies being more or less constant are moderate and heavy diffuse pigmentation, both having a low degree of occurrence and for that reason of restricted practical value. We therefore used, in our comparative study, not only these more or less constant pigment anomaly classes but also those that varied.

DIFFERENCES IN THE DEGREE OF ABNORMAL PIGMENTATION BETWEEN ADULT PLAICE POPULATIONS

In the Tables 1 to 5 and in the Figures 7 and 8 the relative occurrence of the various pigmentation deficiency classes is given for the four male plaice populations mentioned in the introduction. All data of the four years concerned (1963–1966) are combined. In the class slight diffuse pigmentation the highest average degree is to be found in the Southern Bight population. In the classes

TABLE 1. Percentages of plaice with *slight diffuse* pigmentation on the blind-side in four populations

Length categories cm	Southern Bight %	Cleaver Bank %	German Bight %	Flamborough Area %
16–20	6·8	2·6	4·1	0·8
21–25	14·1	14·8	15·4	6·3
26–30	24·4	23·7	24·1	12·5
31–35	35·2	33·6	34·6	23·9
36–40	42·2	43·6	45·8	43·3
41–45	56·6	46·5	45·2	39·2
Average	29·9	27·5	28·2	21·0

TABLE 2. Percentages of plaice with *moderate diffuse* pigmentation on the blind-side in four populations

Length categories cm	Southern Bight %	Cleaver Bank %	German Bight %	Flamborough Area %
21–25	0·7	1·4	0·5	0·2
26–30	1·1	0·7	1·1	0·2
31–35	1·2	1·3	0·5	0·1
36–40	1·4	2·3	0·5	0·2
Average	1·1	1·4	0·7	0·1

TABLE 3. Percentages of plaice with *heavy diffuse* pigmentation on the blind-side in four populations

Length categories cm	Southern Bight %	Cleaver Bank %	German Bight %	Flamborough Area %
21–25	0·8	0·4	0·3	0
26–30	0·9	0·6	1·1	0
31–35	0·7	0·6	0·5	0
36–40	0·5	0·5	0·3	0
Average	0·7	0·5	0·4	0

TABLE 4. Percentages of plaice with *albinism* in four populations

Length categories cm	Southern Bight %	Cleaver Bank %	German Bight %	Flamborough Area %
21–25	0·5	1·0	0·9	0·4
26–30	0·8	1·8	1·6	1·2
31–35	1·1	3·3	1·7	0·3
36–40	1·2	1·2	1·9	0·9
Average	0·9	1·8	1·5	0·7

TABLE 5. Percentages of plaice with *ambicoloration* in four populations

Length categories cm	Southern Bight %	Cleaver Bank %	German Bight %	Flamborough Area %
21–25	1·1	2·1	0·8	2·0
26–30	1·3	2·8	1·4	1·9
31–35	1·6	4·7	2·3	4·7
36–40	3·2	5·4	2·8	5·9
Average	1·8	3·7	1·8	3·6

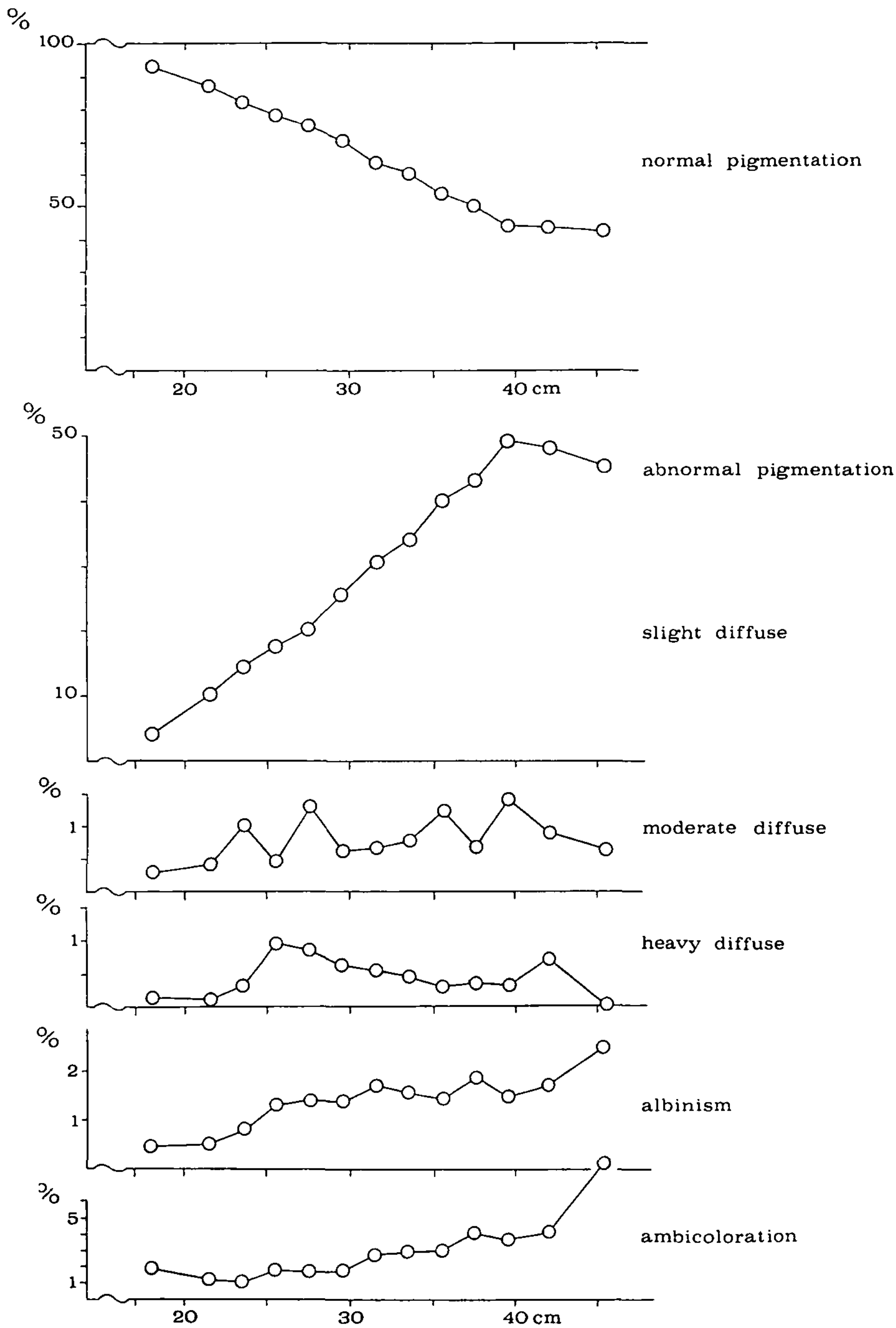


Figure 6. The percentages of adult plaice with normal and abnormal pigmentation in its various classes as functions of the length of the fish. The data of the male plaice for the four populations in the years 1963–1966 are pooled.

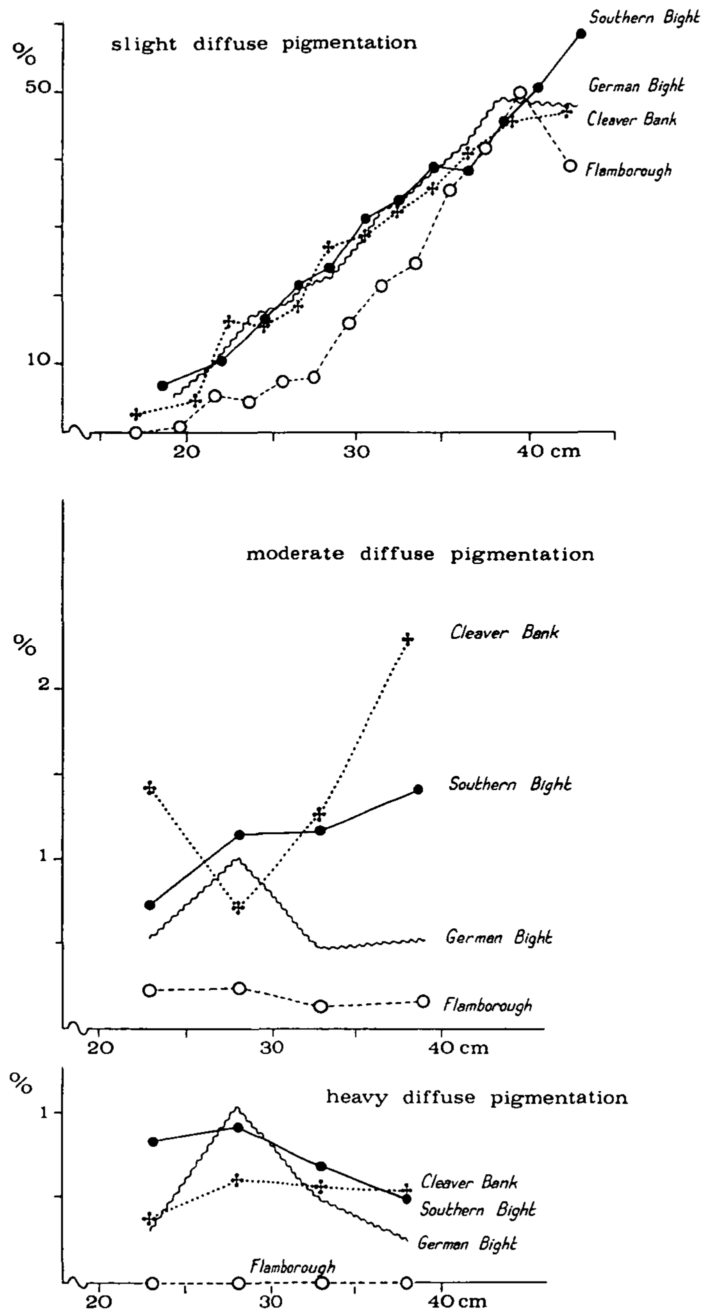


Figure 7. The relationships of the percentage of fish with *slight*, *moderate* and *heavy diffuse pigmentation* with length for the four populations of adult male plaice.

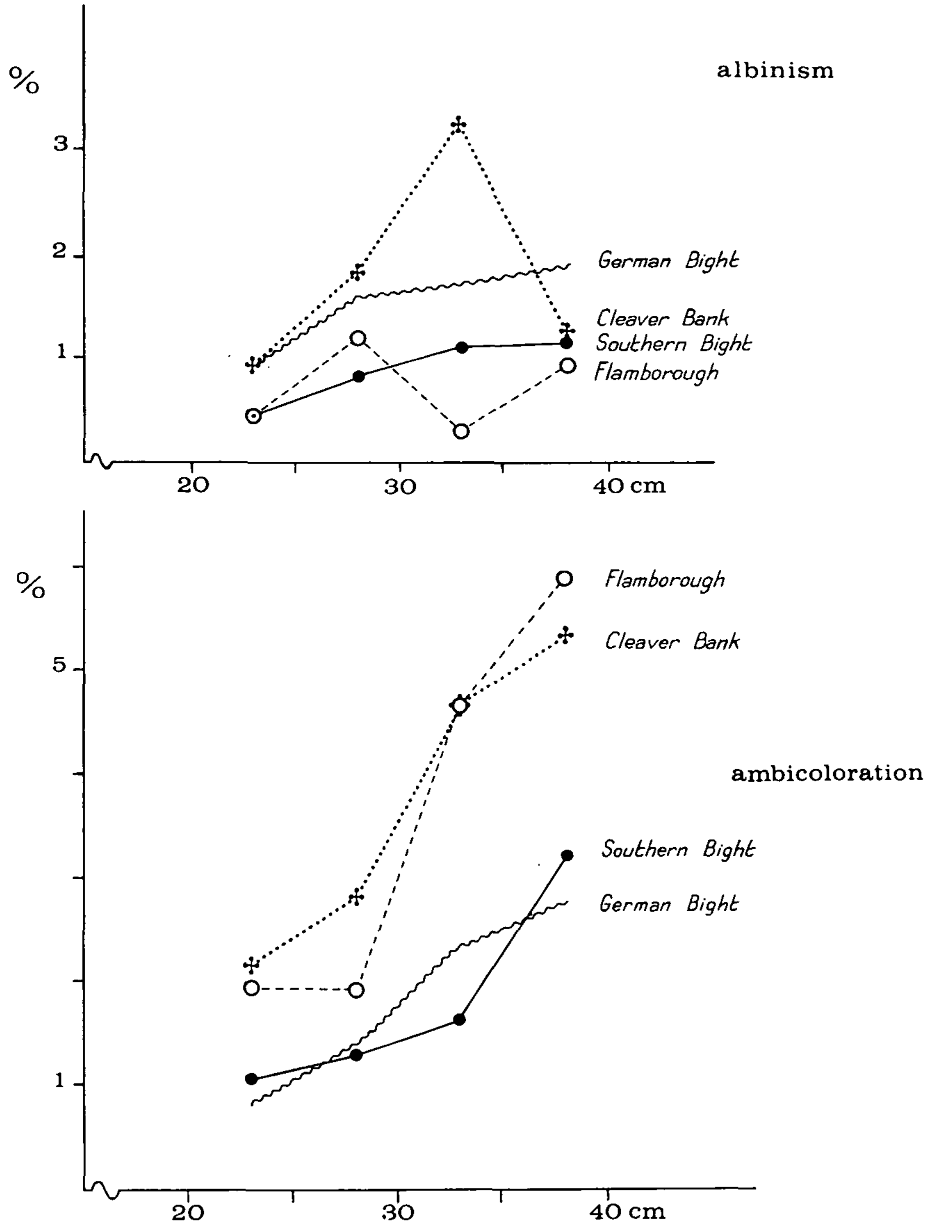


Figure 8. The relationships of the percentage of fish with *albinism* and *ambicoloration* with length for the four populations of adult male plaice.

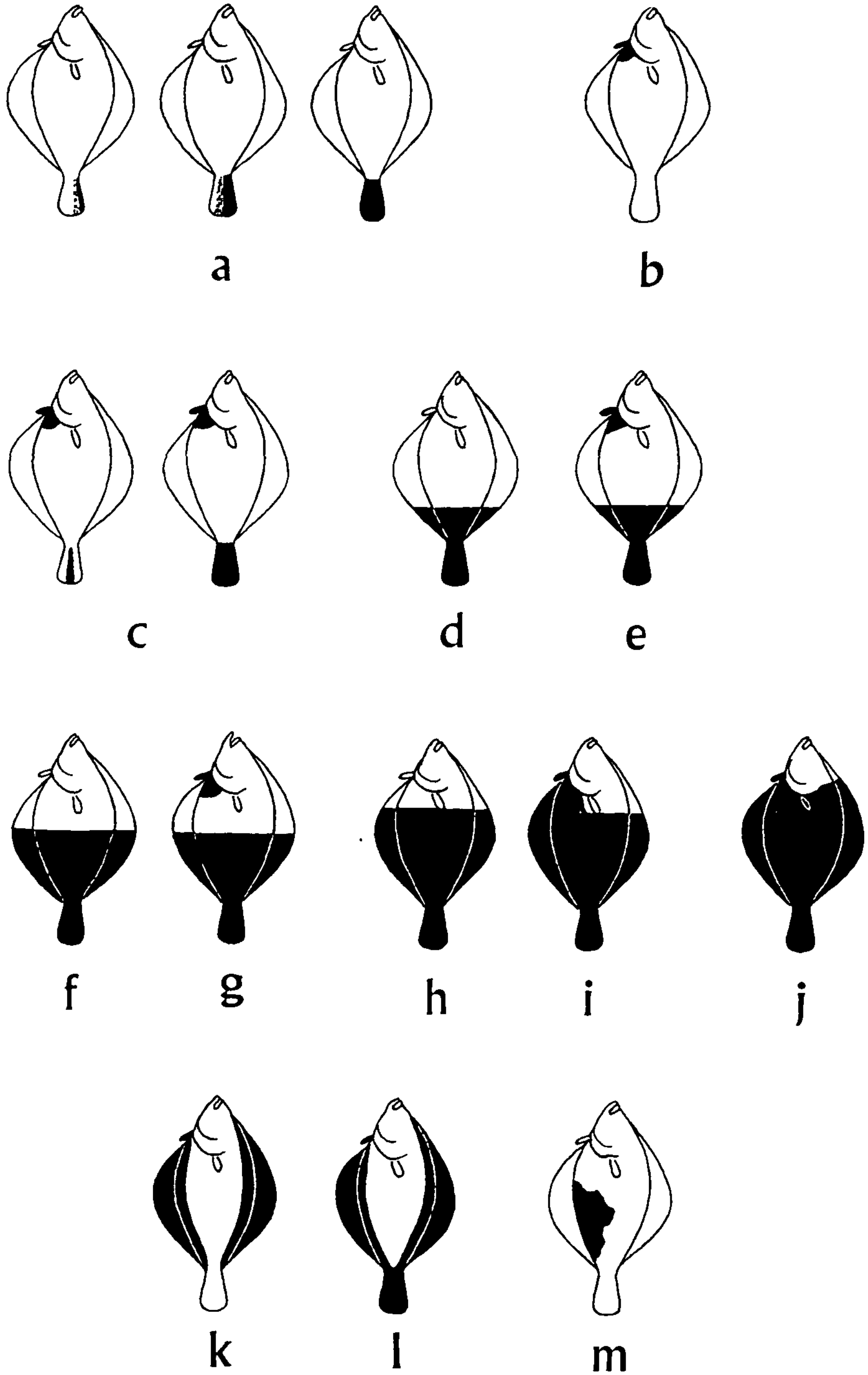


Figure 9. *Types of ambicoloration* encountered in male adult and in juvenile plaice in the years 1963–1966 in the southern and central North Sea.

moderate and heavy diffuse pigmentation the highest degree on average can be seen in the Southern Bight and the Cleaver Bank populations. The Flamborough population has the lowest incidence demonstrated in all three classes of diffuse pigmentation. In this population not one single male plaice in 4,644 fish was found showing a heavy diffuse pigmentation!

In the class albinism the Cleaver Bank population shows the highest and the Flamborough group the lowest incidence. Finally in the class ambicoloration the Cleaver Bank has the highest and the Southern Bight and German Bight plaice the lowest incidence. It is striking that the curves of the German and Southern Bight populations more or less coincide, which is also true for the Cleaver Bank and Flamborough plaice.

The differences between the populations can also be demonstrated for the separate years although annual variations do occur.

In the classes ambicoloration and albinism we can make more detailed comparisons between the populations. In Figure 9 the class ambicoloration is subdivided into a number of types we encountered during our studies. Ambicoloration is mostly located in the tail region (a) or the region around the anus (b). Combinations of these two types may occur in which an increasing part of the blind-side is pigmented (c, e, g, i and j) and also an increase of the pigmentation from the tail onwards (d, f and h). Another type is pigmentation of the base of the fin-rays, with or without tail pigmentation (k and l) and finally pigmentation may occur of a type not covered by the types just mentioned (m).

A subdivision of albinism is given in Figure 10. Most albinism is to be found in the tail region (a, c and d). Albinism of the region near the anus (b) and white edges of the fin-rays (e) are other types. The types (f, g and h) were absent in the adult plaice but were found in the juvenile fish in the Dutch Waddensea. Finally type (i) denotes white spots of a kind not covered by the other types.

For our comparisons we combined in ambicoloration the types (b, c, e and g) and did the same with the other types to get the combinations (d, f, h) and

TABLE 6. *Ambicoloration*-type composition of four populations

Population	a n	a %	b, c, e, g n	b, c, e, g %	d, f, h n	d, f, h %	i, j n	i, j %	k, l, m n	k, l, m %	Total number
Flamborough	133	*92·4*	5	*3·5*	3	*2·1*	0	0	3	*2·1*	144
Cleaver Bank	129	*93·5*	4	*2·9*	3	*2·2*	0	*0*	2	*1·5*	138
Southern Bight	69	*67·7*	13	*12·7*	10	*9·8*	8	*7·8*	2	*2·0*	102
German Bight	100	*69·0*	19	*13·2*	14	*9·7*	10	*7·0*	1	*0·7*	144

(i, j) and (k, l, m). In Table 6 the frequency and percentage per ambicoloration-type is given for the four populations. It is obvious that the Flamborough and the Cleaver Bank populations have the same composition of types which is quite different from the type composition of the Southern Bight and the German Bight populations which resemble each other.

TABLE 7. *Albinism*-type composition of four populations

Population	a n	a %	c, d n	c, d %	e n	e %	b, i n	b, i %	Total number
Flamborough	18	*75·0*	3	*12·5*	1	*4·2*	2	*8·3*	24
Cleaver Bank	46	*75·4*	9	*14·8*	3	*4·9*	3	*4·9*	61
Southern Bight	50	*82·0*	0	*0*	6	*9·8*	5	*8·2*	61
German Bight	110	*79·1*	10	*7·2*	17	*12·2*	2	*1·4*	139

In Table 7 a comparison is made between the four populations with respect to albinism. Here we took the types together to get the combinations (a), (c, d), (e), (b, i). In albinism too the Flamborough and the Cleaver Bank populations resemble each other in type composition and are different from the Southern Bight and the German Bight plaice.

It is apparent that both the degrees of ambicoloration and the ambicoloration-type compositions point to a correspondence between the Flamborough and Cleaver Bank plaice on one hand and the German Bight and the Southern Bight populations on the other. In albinism this correspondence is less clear, perhaps owing to the low frequency with which albinism is encountered.

We previously found that the relative frequency of ambicolorate and albinistic male plaice increases with length (Figures 6 and 8). It is possible that this increase is reflected in a change in the type composition with length. When we compare e.g. the ambicoloration-type composition of smaller and larger male plaice of the combined Southern and German Bight populations we find the results summarized in Table 8.

TABLE 8. *Ambicoloration*-type composition of Southern and German Bight male plaice combined

Length categories cm	a n	a %	b, c, e, g n	b, c, e, g %	d, f, h n	d, f, h %	i, j n	i, j %	k, l, m n	k, l, m %	Total number
20–30	64	*66·7*	8	*8·3*	13	*13·5*	10	*10·4*	1	*1·0*	96
31–45	101	*69·2*	24	*16·4*	11	*7·5*	8	*5·5*	2	*1·4*	146

The differences in the type compositions are not significant ($p = 0·18$). The increase with length found is thus not reflected in a change in the type composition in ambicoloration. The same is true for albinism (see Table 9). The differences in type-composition in albinism are not significant ($p = 0·80$).

TABLE 9. *Albinism*-type composition of Southern and German Bight male plaice combined

Length categories cm	a n	a %	c, d n	c, d %	e n	e %	b, i n	b, i %	Total number
20–30	64	*79·0*	7	*8·6*	7	*8·6*	3	*3·7*	81
31–45	95	*80·5*	3	*2·5*	16	*13·6*	4	*3·4*	118

DIFFERENCES IN THE DEGREE OF ABNORMAL PIGMENTATION WITHIN ADULT PLAICE POPULATIONS

The increase in relative frequency with length in the classes slight diffuse pigmentation, ambicoloration and in albinism as found in our data per population may be caused by various mechanisms.

In the first place it is possible that throughout life normally pigmented adult plaice may become slight diffuse pigmented, ambicolorate or albinistic. There is hardly any support for this view in the extensive literature on abnormal pigmentation in fish in which most authors assume that flatfish will show ambicoloration or albinism within the first months of life. FRANZ (1910), NORMAN (1934), EISLER (1963) and CHABANAUD (1957) have noted that albinism in flatfishes is frequently associated with wounds, especially those involving the vertebral column. Thus it may be possible that in our plaice the increase in albinism with length is a result of wounds inflicted e.g. by encounters with nets or predators. Partial albinism inflicted by wounds will mostly be of an irregular type and may be associated with deformations. The majority of the albinistic plaice we observed were of the regular type so that the wound factor is of minor importance. Moreover, inspection of returned tagged plaice, in which the wound caused by the tag has formed an ulcer on more than one occasion, demonstrated, that in general, these wounds did not give rise to albinism or ambicoloration.

Another explanation is that the increase of abnormal pigmentation with length is only a reflection of the fact that recruits entered the adult populations in former times with a higher degree of pigment deficiencies than at present and that there was a gradual decrease in the degrees of pigment anomalies in the successive recruiting year classes. If in adult life each year class retained its initial degree of abnormal pigmentation the result should be an apparent increase in the degrees with age (length) in the picture of the total adult population.

When we compare the diagrams of normally pigmented plaice for each population in the successive years in which we sampled we should find a gradual shift in the relation of normally pigmentation with length from smaller to bigger fish in the different years if this explanation is true. In fact none of the populations demonstrated this shift in the four years concerned. The same was true for the annual diagrams for the different pigment anomaly classes.

The third explanation is that the increase with length of ambicoloration, albinism and slight diffuse pigmentation is caused by a differential mortality between normally and abnormally pigmented plaice. This could be differential natural mortality, fishing mortality or both. If abnormally pigmented plaice are different in behaviour compared with normal plaice to the effect that plaice with pigment anomalies are more difficult to catch than normal plaice, a gradual decrease in the relative amount of normally pigmented fish with increasing age (length) and a gradual increase in the plaice with abnormal pigmentation will occur. Thus the pigmentation – length relationships found at sea might be caused by a selection through the fishery itself.

We may try to determine, in theory, the effect of a differential total mortality caused either by a differential natural or a differential fishing mortality or both.

Let the number of normally pigmented male plaice of a length p cm be $N_o{}^n$ and the number of abnormally pigmented fish of the same length $N_o{}^a$. The percentage of normally pigmented plaice of the length p is then defined by:

$$\frac{N_o{}^n}{N_o{}^n + N_o{}^a} = x \quad \text{and} \quad N_o{}^a = \frac{1-x}{x} N_o{}^n$$

Owing to mortality the number of normally pigmented plaice will decrease in the course of time. The number alive after a given period t is given by $N_t{}^n = N_o{}^n e^{-z^n t}$. For the abnormally pigmented fish this equation will be $N_t{}^a = N_o{}^a e^{-z^a t}$. In these equations z^n and z^a stand for the total mortality rates for normally and abnormally pigmented plaice respectively.

The fish increase in length from p to q cm in t years. The percentage of normally pigmented male plaice of a length q is then defined by:

$$\frac{N_t^n}{N_t^n + N_t^a} = \frac{N_o^n e^{-z^n t}}{N_o^n e^{-z^n t} + \frac{1-x}{x} N_o^n e^{-z^a t}} = \frac{e^{-z^n t}}{e^{-z^n t} + \frac{1-x}{x} e^{-z^a t}} \qquad (1)$$

Thus far we have dealt with changes in the ratio between normally and abnormally pigmented fish in the population at sea.

In practice we do not know exactly which changes take place in the population. The only information we have is derived from the catches made from this population. The picture given in Figure 6 describes what is caught and will only under certain restrictions describe the true situation in the population.

The number alive in the population after a given period t is given by $N_t = N_0 e^{-zt}$. The number caught in this period is given by

$$F\int_0^t N_t dt = \frac{F}{F+M} N_0 (1 - e^{-zt}) \text{ (BEVERTON and HOLT, 1957)}$$

in which F stands for the fishing mortality and M for the natural mortality rate. $F + M = Z$, the total mortality rate. We write $\frac{F}{F+M} = \frac{F}{Z} = E$. From the population at sea in which N_o^n individuals are normally pigmented and N_o^a are abnormally pigmented the ratio normal-abnormal in the catch after 1 year is given by:

$$\frac{E_n N_o^n (1 - e^{-z^n})}{E_n N_o^n (1 - e^{-z^n}) + E_a N_o^a (1 - e^{-z^a})} = y$$

$$N_o^n = y N_o^n + y N_o^a \frac{E_a (1 - e^{-z^a})}{E_n (1 - e^{-z^n})}$$

$$N_o^a = \frac{1-y}{y} N_o^n \frac{E_n (1 - e^{-z^n})}{E_a (1 - e^{-z^a})}$$

The ratio normally-abnormally pigmented fish in the catch after t years is:

$$\frac{E_n N_o^n e^{-z^n (t-1)} (1 - e^{-z^n})}{E_n N_o^n e^{-z^n (t-1)} (1 - e^{-z^n}) + E_a N_o^a e^{-z^a (t-1)} (1 - e^{-z^a})} =$$

$$\frac{N_o^n}{N_o^n - N_o^a \frac{E_a (1 - e^{-z^a})\, e^{-z^a (t-1)}}{E_n (1 - e^{-z^n})\, e^{-z^n (t-1)}}} =$$

$$\frac{N_o^n}{N_o^n + N_o^n \left[\frac{1-y}{y} e^{(z^n - z^a)(t-1)} \frac{E_a (1 - e^{-z^a})\, E_n (1 - e^{-z^n})}{E_n (1 - e^{-z^n})\, E_a (1 - e^{-z^a})}\right]} =$$

$$\frac{1}{1 + \frac{1-y}{y} e^{(z^n - z^a)(t-1)}} \qquad (2)$$

Although the picture given in Figure 6 is not an unbiased estimate of the stituation in the population we can use its data for an approximation of the true changes in the ratio normally-abnormally pigmented fish in the stock. If we take

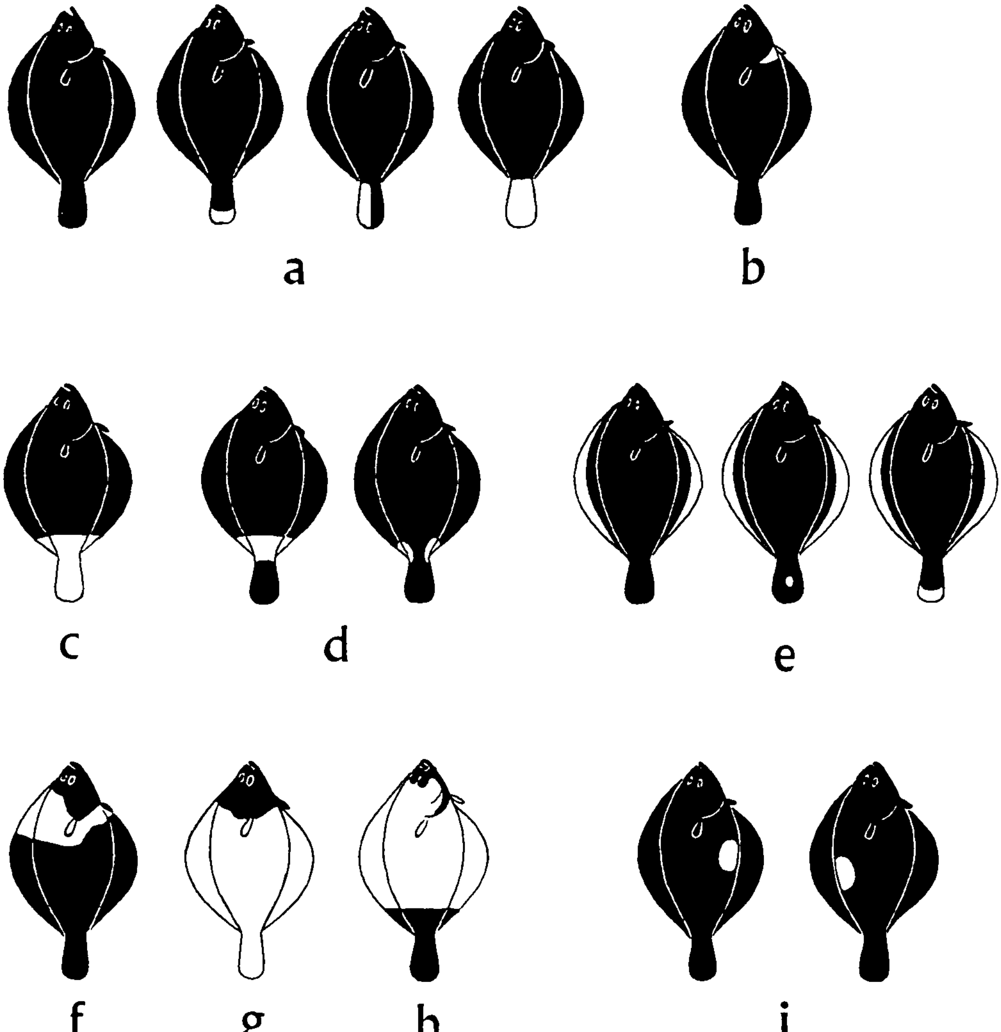


Figure 10. *Types of albinism* encountered in male adult and in juvenile plaice in the years 1963–1966 in the southern and central North Sea.

$p = 20$ cm and $q = 40$ cm, we find that the male plaice of the four populations combined grow from 20 to 40 cm in $t = 8$ years. We further take $x = 0{\cdot}9$ as the true percentage of normally pigmented male plaice of a length of 20 cm and $y = 0{\cdot}85$ for the plaice one year older. If we assume that the total mortality rate of the normally pigmented plaice is $z^n = 0{\cdot}5$ (being a fair estimate of the situation at the moment), what should be the total mortality rate of the abnormally pigmented plaice z^a? After $t = 8$ years the fraction of the normal plaice has decreased to 0·45. If we put equation (2) equal to 0·45 and $t = 8$ we find

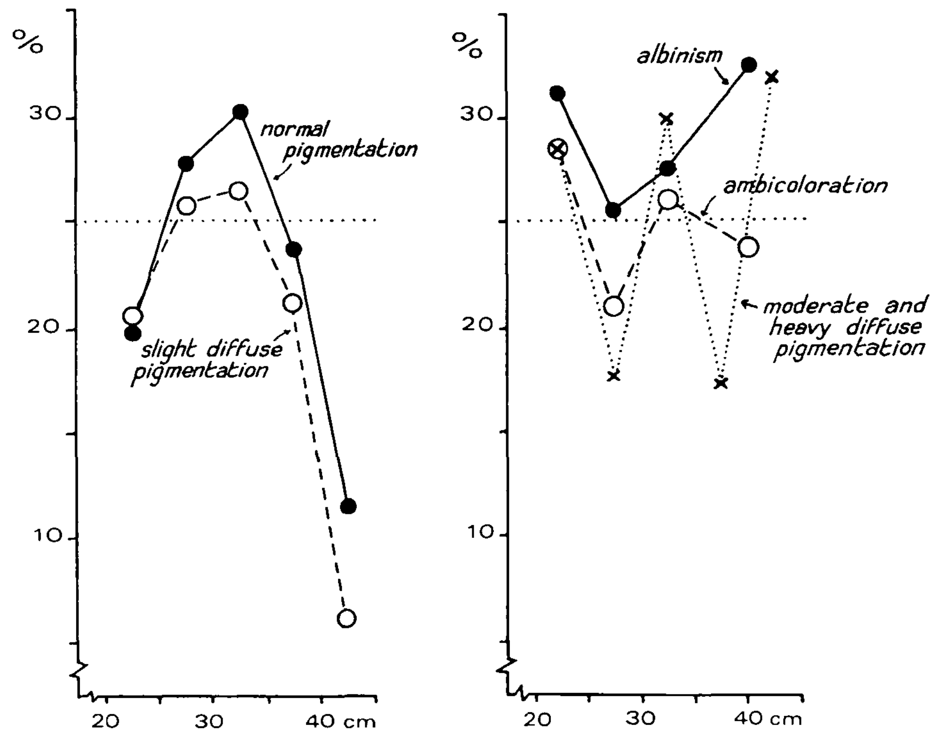


Figure 11. The percentage of returns of tagged male plaice as a function of the length at release for normally and abnormally pigmented plaice. All data for the four populations combined.

$z^n - z^a = 0{\cdot}28$. This corresponds with a difference in total mortalities of 19% annually.

Is there any evidence for a differential total mortality in our results? Fortunately we made our pigment anomaly studies as a part of the routine tagging programme of ripe plaice on the spawning grounds. If we may assume that the percentage of returns of these tagging experiments can be used as an indication of fishing mortality, we get the information on differential mortality summarized in Table 10. It is obvious that in all four populations the return rate of normal male plaice is somewhat higher than that of plaice with abnormal pigmentation. For all populations together we find a percentage of returns of the normal plaice of 26·0 and for the abnormal fish, of which 5,474 were released and 1,332 were returned, a percentage of 24.3. For the four populations combined the difference is thus 1·7% (significant, $p = 0{\cdot}025$), much lower than the 19% we expected on a theoretical basis.

The picture becomes complex when we calculate the rates of return for the various pigment anomaly classes for plaice of different lengths (see Fig. 11). The pattern for the return rates of normal and slight diffuse pigmented plaice is the same: the rate of return at first increases with length to a maximum at

30–35 cm and decreases for bigger fish. The patterns for the other abnormal pigmentation classes are quite different. We do not find the low return rates for the smaller and the bigger fish as in normal and slight diffuse plaice. In the length categories 20–25 cm and 35–45 cm the return rate of albinistic and ambicolorate fish is much higher than that for normal and slight diffuse pigmentation. At the moment it is not clear which factor is responsible for these differences.

TABLE 10. Number and percentages of returns of tagging experiments with male plaice of four populations by pigmentation classes

Pigmentation class	Flamborough area	Population Cleaver Bank	Southern Bight	German Bight	All populations together
Normal pigmentation					
Released	1,865	1,744	3,097	5,118	11,830
Returns in 3 years ..	528	472	943	1,140	3,070
% returns	*28·3*	*27·1*	*30·5*	*22·3*	*26·0*
Slight diffuse pigmentation					
Released	651	719	1,415	1,841	4,606
Returns in 3 years ..	175	171	361	381	1,118
% returns	*26·9*	*23·8*	*25·5*	*20·7*	*24·3*
Moderate and heavy diffuse pigmentation					
Released	6	53	90	108	257
Returns in 3 years ..	1	12	20	25	58
% returns	(16·7)	*22·6*	*22·2*	*23·1*	*22·6*
Albinism					
Released	16	49	61	120	248
Returns in 3 years ..	8	15	17	31	69
% returns	(50·0)	*30·6*	*27·9*	*25·8*	*27·8*
Ambicoloration					
Released	39	76	99	149	363
Returns in 3 years ..	10	23	15	37	87
% returns	*25·6*	*30·3*	*15·2*	*24·8*	*24·0*

It is clear, however, that the differential mortality as indicated by the differences found in the return rates of tagged normal and abnormally pigmented plaice is too small to explain the steady increase in relative frequency of a number of pigment anomaly classes in Figure 6 in terms of a differential fishing mortality only.

At the moment we cannot tell which mechanism is chiefly responsible for the increase in the relations between length and pigment anomaly classes. Perhaps we may find an answer if we extend our studies to the pre-recruit fish. For this purpose we have selected two nursery grounds, the Dutch Waddensea and the coastal waters near IJmuiden.

ABNORMAL PIGMENTATION IN JUVENILE PLAICE ON NURSERY GROUNDS

In Figure 12 the length composition and the rates of abnormal pigmentation are given for 5,145 juvenile plaice caught in the Wierbalg and the Malzwin, parts of the Dutch Waddensea, in 1965 and 1966. In the Waddensea normally two year groups are present, the 0-group and the I-group. Of the II-group and

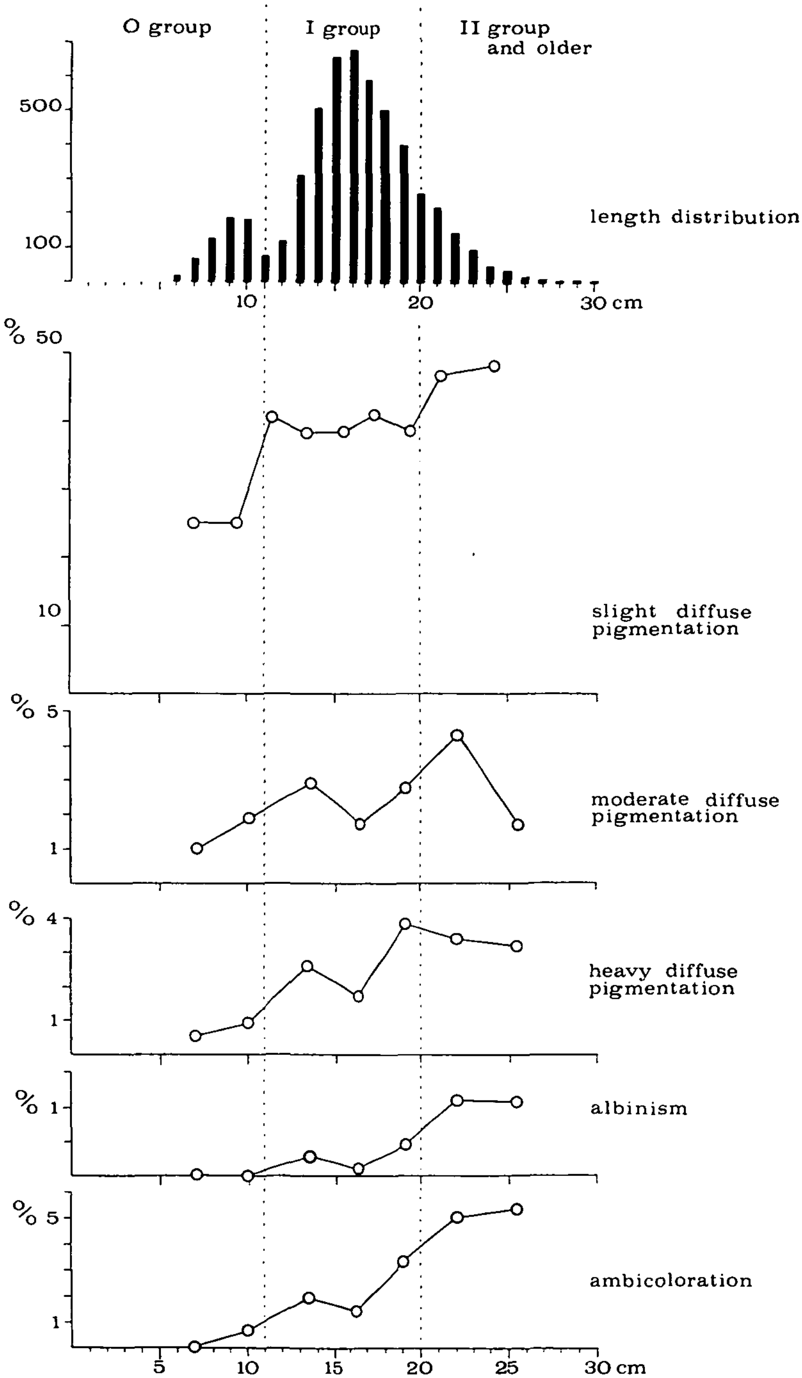


Figure 12. Length composition and degrees of abnormal pigmentation as functions of the length of juvenile plaice in the Dutch Waddensea in 1965 and 1966.

older fish, which migrate gradually to the open sea, only a part is present in the Waddensea. As can be seen in Figure 12 only the larger individuals of the 0-group were caught owing to mesh-selection.

If we look at the curves of the various abnormal pigmentation classes it is obvious that in all these classes the pigmentation rates are increasing with length. In the class slight diffuse pigmentation we can distinguish three levels. In the 0-group the rate is about 26%, in the I-group it has risen to 40% and in the older year groups the rate has increased to about 48%. This suggests that the increase in the pigmentation rate is more linked with age than with length.

The curves for albinism and ambicoloration rise quickly with length. In the 0-group albinism and ambicoloration is nearly absent, but rise in the following year groups to 1% and 5% respectively.

A peculiarity of most of the curves in Figure 12 is that they reach levels in the third year of life often much higher than those of the plaice of the corresponding length encountered in the adult populations. This is very striking in the class slight diffuse pigmentation which, in the parts of the Waddensea we sampled, reaches a level of about 48% in plaice of 25 cm whereas in all four adult populations in the North Sea we found in this class only a rate of less than 20% in fish of 25 cm. Another example is the class ambicoloration which in the Waddensea reaches a level of 5% in plaice of 25 cm as compared with 1–2% in plaice of the same length in the adult populations. This difference is still more striking when comparing the relative frequency of the ambicoloration types of the Waddensea juveniles with that of the adult plaice (Table 11).

TABLE 11. Comparison of the *ambicoloration* type composition of the Waddensea juvenile plaice with those of the four adult populations

	Ambicoloration types										
	a		b, c, e, g		d, f, h		i, j		k, l, m		Total
Population	n	%	n	%	n	%	n	%	n	%	number
Waddensea	44	*27·5*	89	*55·6*	3	*1·9*	13	*8·1*	11	*6·9*	160
Flamborough		92·4		3·5		2·1		0		2·1	144
Cleaver Bank		93·5		2·9		2·2		0		1·5	138
Southern Bight		67·7		12·7		9·8		7·8		2·0	102
German Bight		69·0		13·2		9·7		7·0		0·7	144

In the Waddensea the types containing ambicoloration of the region near the anus (b, c, e and g) are relatively much more frequent than in the adult populations in the North Sea we studied.

The albinistic juveniles reach 1% in the Waddensea at a length of 25 cm, about the same value as in the adults of 25 cm. Here too differences in type composition occur as can be seen in Table 12.

Although the number of albinistic juveniles in the Waddensea is low it is obvious that their type composition differs from that of the adults. The types (f, g and h) found in the juveniles in which fairly large parts of the eye side are white are absent in the adult plaice populations.

It is worth while to compare the data for the various abnormal pigmentation classes in the Waddensea with those obtained from a number of samples, totalling 3,207 juvenile plaice, mainly 0-group, in the other nursery ground studied, near the shore of IJmuiden (Table 13).

TABLE 12. Comparison of the *albinism* type composition of the Waddensea juvenile plaice with those of the four adult populations

Population	a n	a %	c, d n	c, d %	e n	e %	b, i n	b, i %	f n	f %	g, h n	g, h %	Total number
Waddensea	5	*35·7*	4	*28·6*	0	*0*	2	*14·3*	1	*7·1*	2	*14·3*	14
Flamborough		75·0		12·5		4·2		8·3		0		0	24
Cleaver Bank		75·4		14·8		4·9		4·9		0		0	61
Southern Bight		82·0		0		9·8		8·2		0		0	61
German Bight		79·1		7·2		12·2		1·4		0		0	139

TABLE 13. Percentages of pigment anomaly classes in 0-group plaice in the Waddensea and the IJmuiden beach nurseries

Nursery	*Normal* n	*Normal* %	*Slight d.* n	*Slight d.* %	*Moder d.* n	*Moder d.* %	*Heavy d.* n	*Heavy d.* %	*Albin.* n	*Albin.* %	*Ambicol.* n	*Ambicol.* %	Total number
Waddensea	474	*71·3*	173	*26·0*	10	*1·5*	5	*0·8*	0	*0*	3	*0·4*	665
IJmuiden beach	3,165	*98·7*	0	*0*	0	*0*	0	*0*	6	*0·2*	36	*1·1*	3,207

In the juveniles on the beach of IJmuiden no single fish was found with diffuse pigmentation, whereas in the Waddensea 28% of the 0-group plaice demonstrated diffuse pigmentation. A comparison of the type composition of the ambicolorate juveniles of the IJmuiden beach and the Waddensea is given in Table 14.

TABLE 14. Comparison of the *ambicoloration* type composition of the Waddensea juveniles with that of the IJmuiden beach plaice

Nursery	a n	a %	b, c, e, g n	b, c, e, g %	d, f, h n	d, f, h %	i, j n	i, j %	k, l, m n	k, l, m %	Total number
Waddensea	44	*27·5*	89	*55·6*	3	*1·9*	13	*8·1*	11	*6·9*	160
IJmuiden beach	4	*11·4*	1	*2·8*	2	*5·7*	0	*0*	28	*80·0*	35

In the IJmuiden beach juveniles the emphasis lies on the type (k, l and m) in contrast to the Waddensea juveniles in which the types (b, c, e and g) and (a) are important. As is the case with the Waddensea juveniles the young plaice from the IJmuiden shore demonstrate a type composition of ambicoloration quite different from those of the four adult populations studied.

As in the adult plaice we may try to find explanations for the increase in the degrees of abnormal pigmentation with length in the juvenile plaice.

As was mentioned before we find no support for the view that albinism and ambicoloration may be acquired throughout the whole lifespan of the fish. At most these classes of pigment anomalies will manifest themselves during metamorphosis but not later. However, the experiments carried out by CUNNINGHAM (1893) and OSBORN (1939, 1940, 1941 a, b) suggest that pigmentation may be induced on the blind-side by the influence of light in flatfishes other than the plaice. Thus abnormal pigmentation on the blind-side may develop after metamorphosis. In the case of diffuse pigmentation it is, however, not clear why juvenile plaice on the IJmuiden shore do not develop diffuse pigmentation whereas the Waddensea young plaice attain rather high percentages. The juvenile plaice of both nurseries are not supposed to differ greatly in their behaviour and there is no reason to assume that the Waddensea fish is much more exposed to light (e.g. reflected from the bottom) on their blind-sides than the IJmuiden juveniles.

Another explanation for the increase in abnormal pigmentation rates is that a differential mortality is at work as was suggested for the adult plaice.

TABLE 15. Number and percentage of returns of tagging experiments with I-group plaice in the Waddensea in 1965

		Pigment anomaly class				
	Normal	*Slight d.*	*Moderate and heavy d.*	*Albin.*	*Ambicol.*	Total
Released	786	654	77	4	51	1,572
Returns in 1 year ..	130	104	14	1	13	262
% returns	*16·5*	*15·9*	*18·2*	(25·0)	*25·5*	

As in the case of the adults we have the results of tagging experiments at our disposal to find out whether any differential mortality exists in juvenile plaice. In Table 15 the return rate in the first year after release is given for the I-group plaice tagged in the Waddensea in 1965 by pigment anomaly classes. Although in juvenile plaice too we find a somewhat higher rate of return in the normally pigmented plaice than in the abnormally pigmented fish the difference is not significant. This is true for all abnormal pigmentation classes. We therefore cannot explain the shapes of the curves in Figure 12 in terms of a differential mortality.

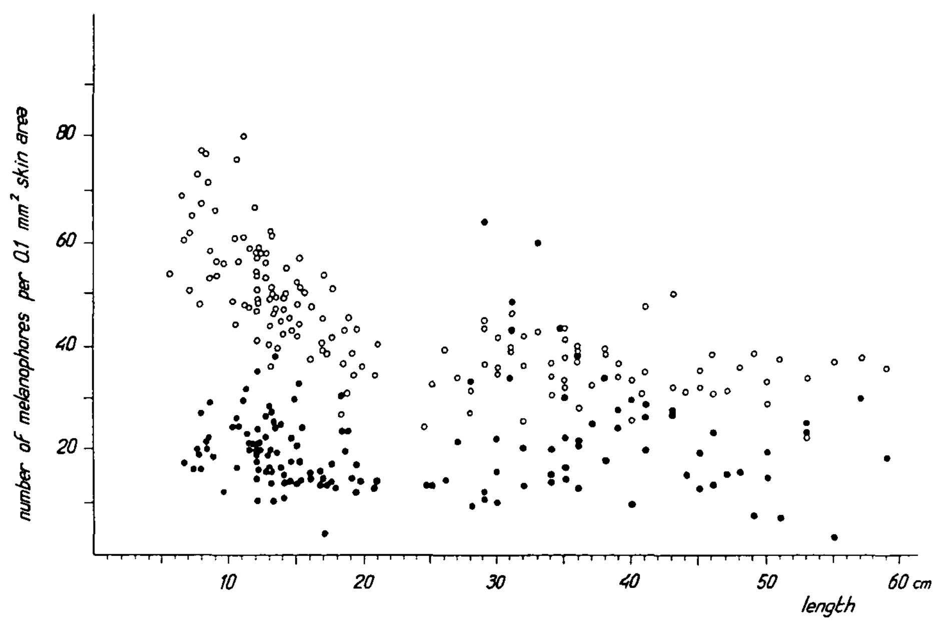


Figure 13. The average number of upper-dermal melanophores per unit skin area on the eye-side (open circles) and in pigmented parts on the blind-side (black dots) in *slight diffuse pigmentation* as a function of the length of the plaice.

The third explanation is that successive year classes in the Waddensea developed decreasing rates of abnormal pigmentation during metamorphosis and retained these rates in their juvenile life. As in the case of the adults we tested this assumption by comparing the separate diagrams for 1965, 1966 and in addition those acquired in 1967. No shift in the relationships of abnormal pigmentation with length from smaller to larger fish could be found, making this last explanation invalid.

The results obtained with the juvenile and the adult plaice have much in common. We are forced, for the time being, to accept the possibility that abnormal pigmentation including ambicoloration and albinism may be acquired throughout life. The fact, that in the juvenile plaice from two nursery grounds, geographically close together, considerable differences exist both in the degrees of abnormal pigmentation and in the type compositions of albinistic and ambicolorate fish indicates that environmental factors may be at work having different effects on the fish of the two nurseries.

If we accept the idea that pigment deficiencies can develop during the whole life span of the plaice, what information do we have on the factor(s) responsible for the causation of abnormal pigmentation?

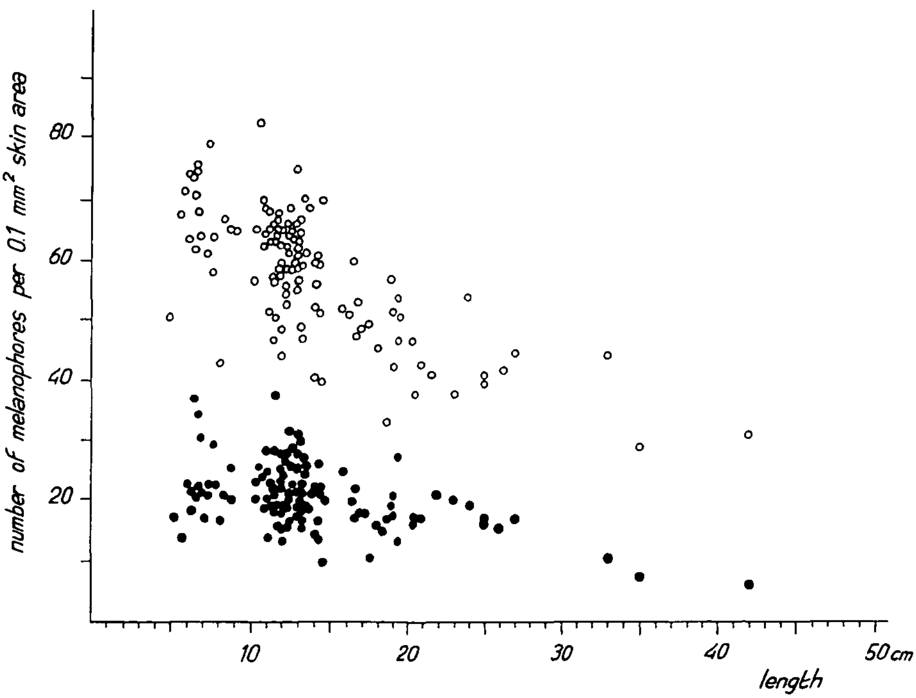


Figure 14. The average number of upper-dermal melanophores per unit skin area on the eye-side (open circles) and in pigmented parts on the blind-side (black dots) in *moderate* and *heavy diffuse pigmentation* as a function of the length of the plaice.

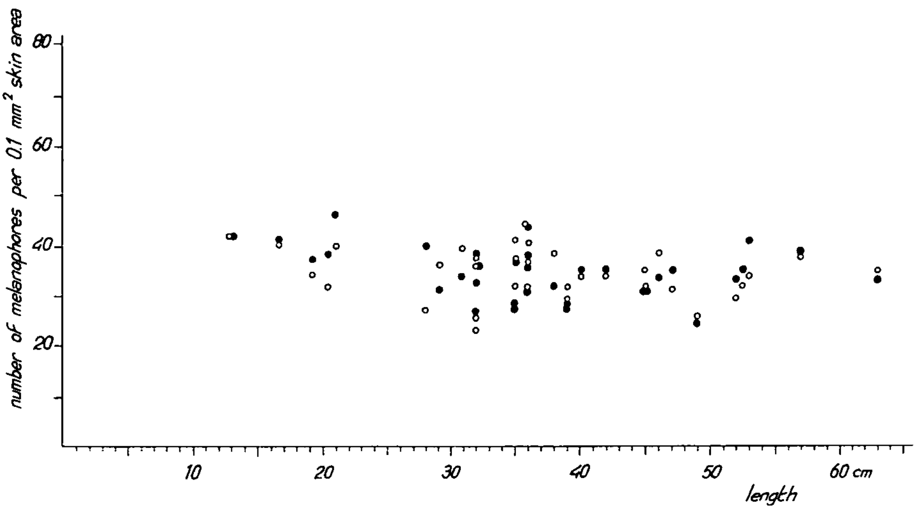


Figure 15. The average number of upper-dermal melanophores per unit skin area on the eye-side (open circles) and in pigmented parts on the blind-side (black dots) in *ambicoloration* as a function of the length of the plaice.

FACTORS RESPONSIBLE FOR ABNORMAL PIGMENTATION

In CUNNINGHAM'S (1893) and OSBORN'S (1940, 1941 a, b) experiments pigmentation developed on the blind-side of flatfishes through the action of light falling on the blind-side. From the illustrations in their papers it is not clear whether this pigmentation was of a diffuse or an ambicolorate class.

In the Greenland halibut (JENSEN, 1935) we have a nice natural experiment of the influence of light on the blind-side of the fish. At first the larvae of the Greenland halibut go through the same transformation as other flatfishes, symmetrical in the beginning and pigmented on both sides. Thereafter they become asymmetrical with the left side becoming white and their eyes wandering over towards the right side, but in contrast to other flatfish species the left eye does not migrate over to the right side but remains on the upper edge of the head whilst the left side becomes pigmented again and will gradually become as dark as the right side. There is evidence that the Greenland halibut takes up a more or less vertical position when swimming freely in the water thus allowing light to fall onto the left side of the fish. What is only a fairly rare exception in other flatfishes (incomplete eye rotation associated with nearly or complete ambicoloration) seems to be the rule in the Greenland halibut. It is, however, not clear if ambicoloration is developed in this species. From JENSEN'S illustrations it appears that this left side pigmentation at first is of a diffuse class but in later stages it may be that ambicoloration is concerned. This poses the question of the interrelation between diffuse pigmentation and ambicoloration.

The outward appearance of the different classes of diffuse pigmentation (irregular) and ambicoloration (regular) do not point in the direction of an interrelation. This is more obvious when we compare, for example, the average number of upper dermal melanophores per unit skin area on the blind and the eye-sides for the various classes of pigment anomalies (see Figs. 13–16). In ambicolorate plaice the average number of melanophores on the blind-side is equal to that on the eye-side for all lengths (Fig. 15). In diffuse pigmentation (Figs. 13 and 14) the number of melanophores on the blind-side is less than half the number on the eye-side. Moreover, there is a difference between slight diffuse and moderate-heavy diffuse pigmentation. In slight diffuse pigmentation there are a number of adult plaice in which the average number of melanophores is equal or higher than the number on the eye-side. This is not the case in the juveniles and besides, it is not found in moderate-heavy pigmentation. Those cases in slight diffuse pigmentation demonstrating an equal or higher number of melanophores on the blind-side compared with the eye-side, did not show any signs of ambicoloration (repetition of the patterns of the eye-side, such as orange, white and black spots) on the blind-side.

The relation of the number of melanophores with the length of the plaice as shown in Figure 16 for the normally pigmented plaice is in agreement with the results obtained by HEWER (1926, 1931) who found in the dab a higher number of melanophores on the eye-side in the juveniles as compared with the adult dabs.

When comparing the relation between the number of melanophores on the eye-side and length of normally pigmented plaice (Fig. 16) with those of the various pigment anomaly classes (Figs. 13–15) a new phenomenon can be observed. The number of melanophores on the eye-side of plaice with diffuse pigmentation is, in the juvenile fish, higher than in the normally pigmented

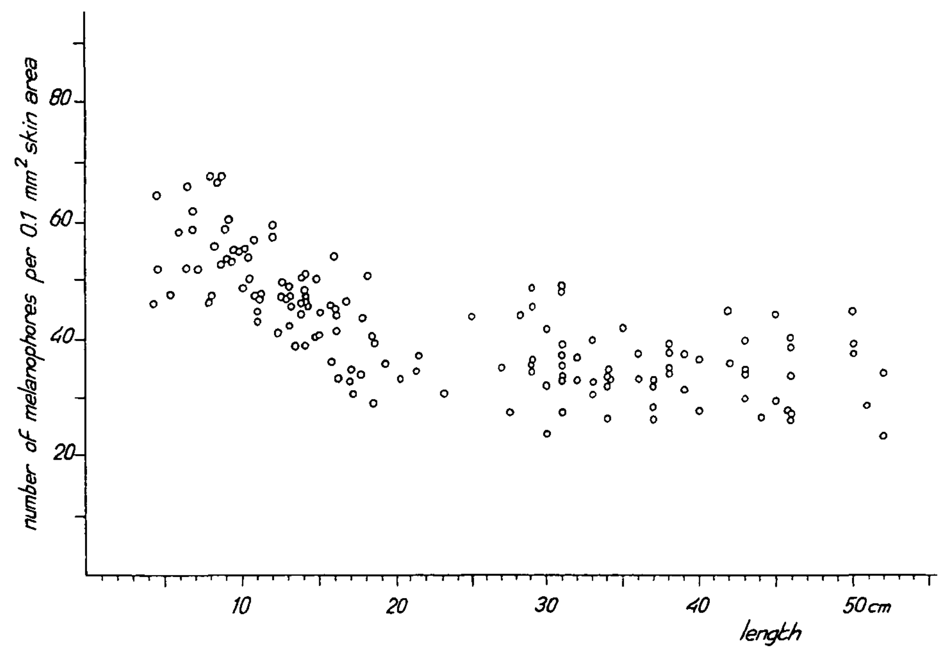


Figure 16. The average number of upper-dermal melanophores per unit skin area on the eye-side (open circles) in *normally pigmented* plaice as a function of the length of the fish.

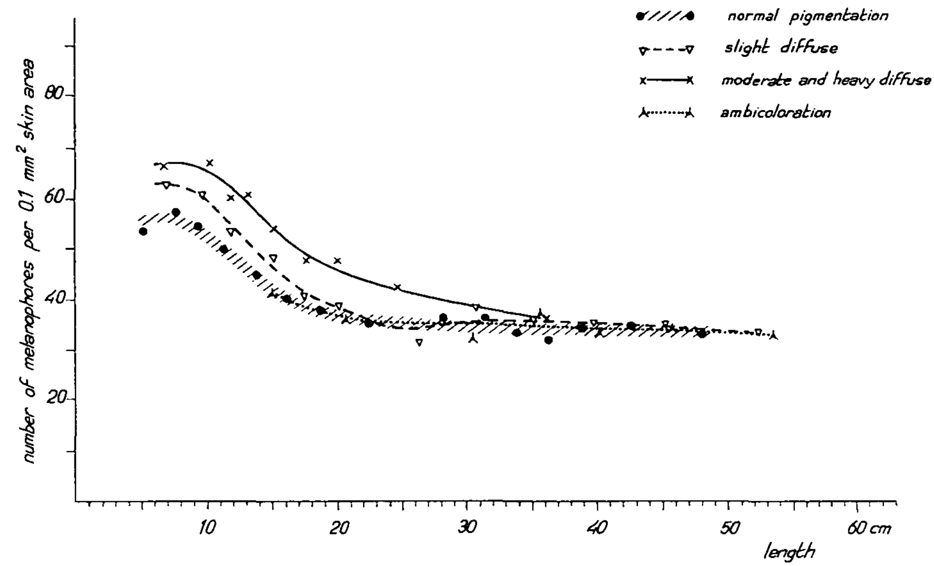


Figure 17. Curves demonstrating the relationships of the average number of upper-dermal melanophores per unit skin area on the eye-side with the length of the plaice for normally and abnormally pigmented fish.

juveniles. In Figure 17, which shows the relationships between the number of melanophores on the eye-side and length, calculated from the data in Figures 13–16, these differences at once become apparent. The curve for ambicolorate plaice coincides with that for normally pigmented fish (which is also the case for albinistic plaice of which the curve is not shown in Fig. 17). The line for the slight diffuse pigmented juvenile fish, however, lies above the line for the normally pigmented juvenile plaice and the moderate-heavy pigmented plaice line takes a position still higher. There must be a factor in diffuse pigmentation in juvenile plaice influencing the number of melanophores on the eye-side and the effect of this factor is related with the amount of diffuse pigmentation on the blind-side.

In October 1967 this factor was detected while counting chromatophores. It appeared to be a parasite living in tiny cysts in the skin on both sides of the fish. These cysts, having a diameter on average of 0·3 mm were always situated in the middle of a pigmented spot on the blind side in diffusely pigmented juvenile plaice. All kinds of transition phases could be seen. Crystal clear cysts, in which the metacercarium of the trematode (perhaps of the class Digenea (REICHENBACH-KLINKE and ELKAN, 1965; REICHENBACK-KLINKE, 1966)) could be observed alive, were mostly surrounded by a small number of melanophores and/or erythrophores and xanthophores. In larger pigmented spots on the blind side the cysts were more opaque. The cysts themselves were never pigmented (Fig. 18).

Not all diffusely pigmented plaice had cysts. We examined a large number of plaice of different lengths for the presence of cysts on the blind-side as well os on the eye-side. Figure 19 gives the percentage of fish with diffuse pigmentation infested with one or more trematodes in November–December 1967 for the

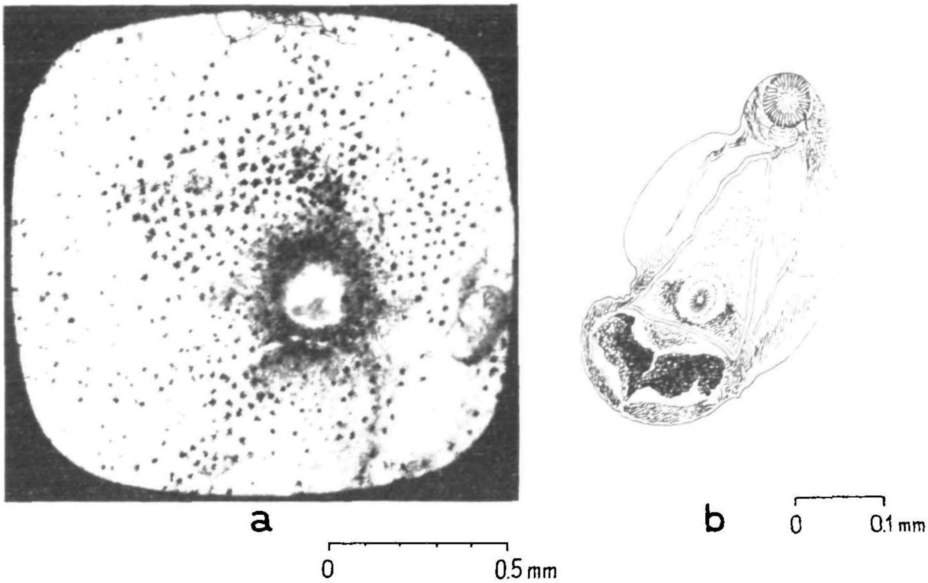


Figure 18. a. An encysted metacercarium of a still unknown trematode on the blind side of a juvenile plaice surrounded by chromatophores. (Photograph by M. KAT). b. The metacercarium after its removal from the cyst.

juveniles in the Waddensea and the adults in the North Sea. It is obvious, that the percentage infested decreases with length, at first slowly, later more quickly, and that the trematode was only found in the juvenile fish. Furthermore we found that in normally pigmented and in ambicolorate plaice no cysts could be traced.

In diffusely pigmented juvenile plaice there is a distinct relationship of the side of the fish on which the cysts are present and the total number of cysts

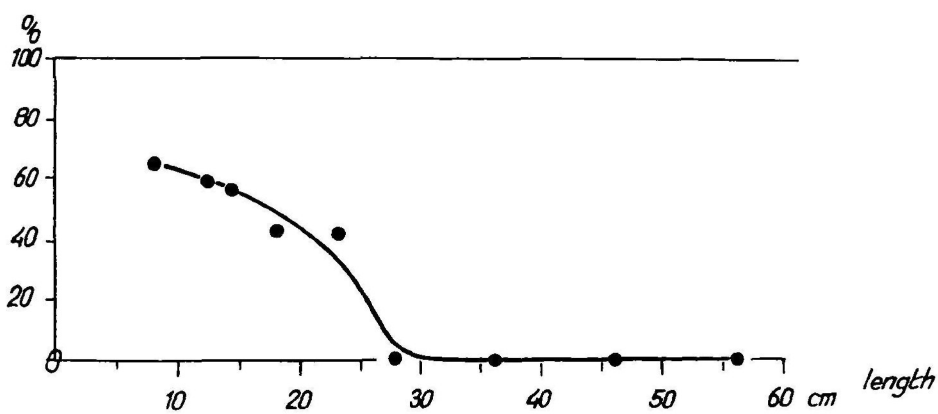


Figure 19. Percentage of plaice with diffuse pigmentation on the blind-side infested with one or more trematodes at different lengths. Waddensea November-December 1967, North Sea December-January '68.

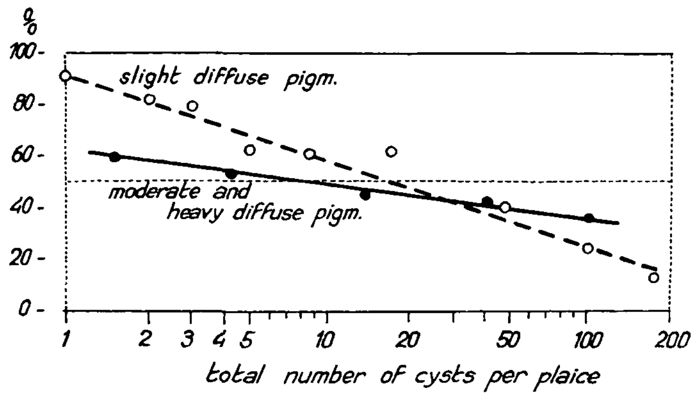


Figure 20. Percentage of cysts on the blind-side as a function of the total number of cysts on both sides of the fish for slight diffuse and for moderate and heavy diffuse pigmentation in juvenile plaice. Waddensea November-December 1967.

found on the animal (Fig. 20). In the class slight diffuse pigmentation most cysts are found on the blind-side when the rate of infestation is low. With increasing total number of cysts the ratio blind-side: eye-side changes. When the total number of cysts is about 20 the same number of cysts is present on the blind- and the eye-side. In the case of a large total number of cysts most of these are found on the eye-side. This could imply that in mild infestations the parasite enters the host by preference on the blind-side and only begins to enter the eye-side when the infestation is high. The same is true in moderate and heavy diffuse pigmented juveniles although less pronounced as in slight diffuse pigmentation.

We further found, that a number of juvenile plaice with diffuse pigmentation had a typical yellow appearance of the eye-side. This phenomenon called

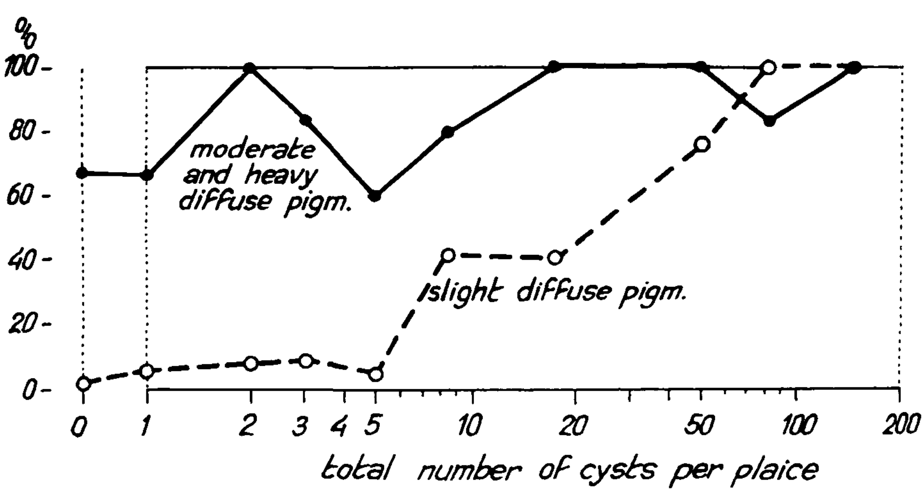


Figure 21. Percentage of juvenile plaice demonstrating the "goudschol" appearance (see text) as a function of the total number of cysts per fish for slight diffuse and for moderate and heavy diffuse pigmentation. Waddensea November-December 1967.

"goudschol" (golden plaice) has been known for a long time. It could be demonstrated (Fig. 21) that the percentage of "goudschol" was linked with the amount of diffuse pigmentation and with the total number of cysts per fish. None of the normally pigmented and the ambicolorate plaice showed this yellow appearance.

The yellow appearance of the plaice is the result of an increase and of maximal expansion of the yellow chromatophores (xanthophores) on the eye-side. Previously we found that the number of melanophores on the eye-side increased in juvenile plaice with diffuse pigmentation (Fig. 17). It is thus plausible to assume that the trematode causes a general situation of stress in an infested fish resulting in the development of chromatophores around the cysts on the blind-side and an overall increase in the number of chromatophores on the eye-side.

In November–December 1967 the average total number of cysts in juvenile plaice was: slight diffuse, 7·0 (218); moderate diffuse, 16·2 (29); and heavy diffuse, 18·7 (52). The numbers in parentheses denote the number of fish examined.

The percentage of plaice demonstrating diffuse pigmentation and infestation with the trematode varies for the different areas in the Waddensea and other estuarine waters. It was observed that this percentage increased when approaching large commercial mussel beds and the highest percentage (50 % of the plaice being diffusely pigmented) was found in samples practically on mussel beds. This might suggest that the origin of the trematode has to be sought in the benthic community typical for the mussel beds. It is clear now why we did not find diffuse pigmentation in the juvenile plaice in the IJmuiden beach nursery ground. No commercial mussel beds are present there.

We hope to get more detailed information on the trematode in the near future. In diffusely pigmented flounders in the Waddensea we found cysts of the same diameter, but it is not certain if the trematode is the same as that found in plaice.

Although we know the cause of diffuse pigmentation in juvenile plaice we are still ignorant of the way in which diffuse pigmentation is caused in adult fish. We did not find direct evidence of a parasite here and it is possible that other factors are responsible. Analogous with the "goudschol" phenomenon in juvenile plaice, diffusely pigmented adult plaice may sometimes demonstrate a dark appearance of the eye-side, a feature already mentioned by SCHNAKENBECK (1923). Maybe this points to the influence of some parasite.

We cannot, however, rule out the factor light, as it has been demonstrated by experiments that diffuse pigmentation can be induced by it in a number of flatfish species. Moreover, the nature of the sea bed may be of influence as suggested by HUSSAKOF (1914). The areas of distribution of the four adult plaice populations derived from tagging experiments (DE VEEN, 1965) as shown in Figure 22, demonstrate that mixing of the adults belonging to the various populations is only slight. Each population has more or less its own area with a special nature of the sea bed. The Southern Bight, the German Bight and to some extent the Cleaver Bank plaice live on a bottom consisting of rather fine sand and shell fragments having a higher reflecting power than the more coarse material of the bottom on which the Flamborough fish dwell. Besides, the Flamborough and the Cleaver Bank adults generally live at a greater depth (more than 40 m) than the German Bight and the Southern Bight plaice (less

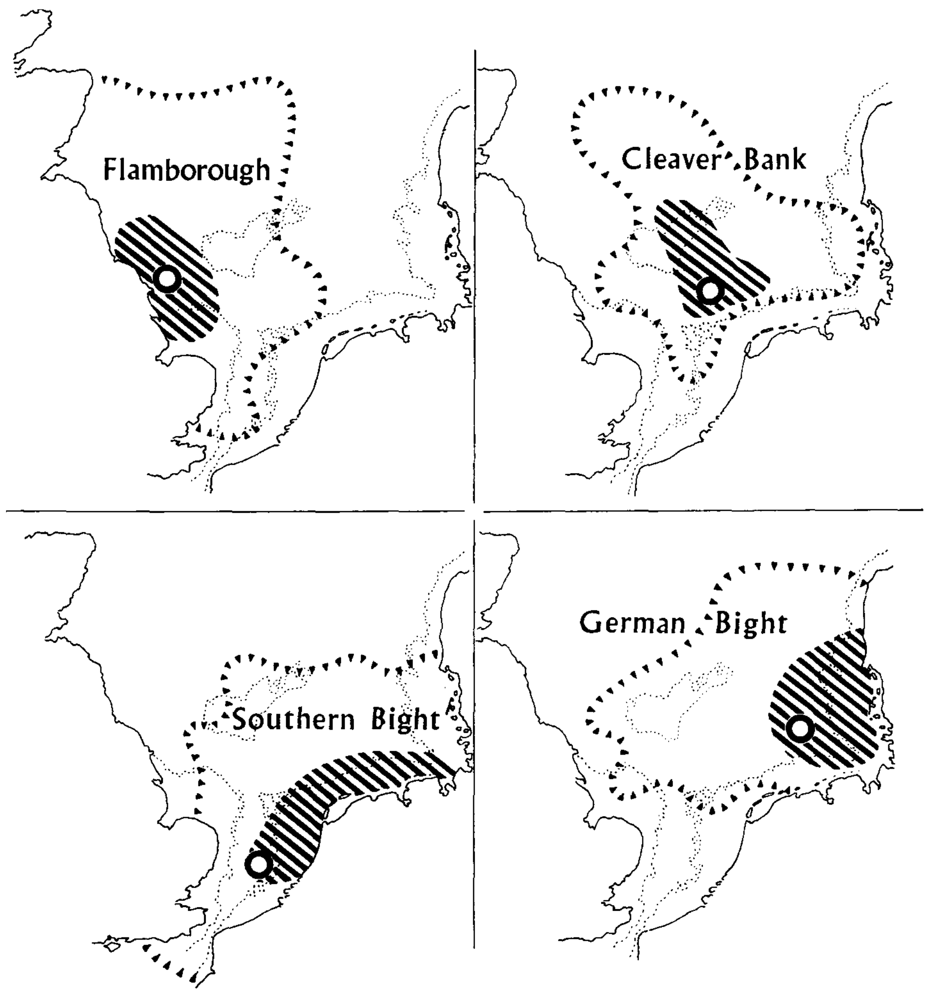


Figure 22. Distribution areas of four adult male plaice populations, obtained from tagging experiments with ripe plaice on the spawning grounds (open circles). The hatched areas contain the majority of the positions of the returned fish. The triangulated contour encloses the positions of returns at a maximum distance from each spawning ground. The 40 m depth contours are shown as dotted lines (DE VEEN, 1965).

than 40 m). There is some evidence from catches of pair-trawlers fishing with pelagic nets that migrating plaice may leave the bottom and can be exposed in the daytime to reflected light on the blind-side when swimming.

As regards ambicoloration and albinism no new arguments on the causation can be given. There are a number of cases in which plaice were recorded as normally pigmented on release and appeared to be ambicolorate when recaptured more than one year later. In addition we have started a routine programme in which every diffusely pigmented, ambicolorate and albinistic plaice to be tagged will be photographed on release and the same will be done at recovery in order to follow a possible development of the pigment anomalies under natural conditions. Unfortunately all recoveries of a pilot programme covering 150 plaice were reported at other localities than our laboratory at IJmuiden and could not be obtained for photographing. In the near future we will extend this photo-programme and hope to be able to report on the results.

DISCUSSION

Abnormal pigmentation has been taken as indicating separate populations or sub-populations in plaice earlier and VON UBISCH (1951) mentions the fact that in a certain region near Trondheim (Norway) the percentage of plaice showing abnormal pigmentation on the blind-side is obviously higher than in the adjacent areas. Fishermen used to call these fish "biologist plaice" as they thought that the plaice were descendants from plaice used in rearing experiments and afterwards put into the sea. In reality we do have here a case of a small sub-population demonstrating its own degree of abnormal pigmentation as a character induced by the environment.

In our studies covering the years 1963 to 1966 we find in general an increase in the degree of abnormal pigmentation with length, as well in the class diffuse pigmentation as in the classes ambicoloration and albinism (see Fig. 6). SCHNAKENBECK (1923) states that he could not find any increase in the degree of pigment abnormality with increasing length. He based his conclusions on plaice obtained in spring and summer near Heligoland in the years 1921 and 1922.

At present a mixture of the Southern Bight and the German Bight populations occurs in this period of the year around Heligoland, both populations demonstrating a distinct increase in albinism, ambicoloration and slight diffuse pigmentation with length. FRANZ (1910) even found that the percentage of albinistic plaice is lower in the adults (0·012%) than in the juveniles (0·84%) near Heligoland in 1908. In 1909 he found in the central North Sea, where the larger and older adult plaice are, a percentage of 0·33, which points to an increase in the degree of albinism in adult plaice. His percentages, however, are lower than those found in our studies.

It is not possible to find an explanation for this discrepancy unless one assumes that the factors responsible for the development of pigment anomalies have changed in influence since 1909. It is possible that increased pollution of the nursery grounds and coastal areas by the Rhine and other rivers in the course of this century also has some bearing on abnormal pigmentation.

A peculiarity of the results of the tagging experiments of which our present pigment studies formed a part was that plaice with abnormal pigmentation appear to behave differently from the normal adult plaice. Moreover, moderate and heavy diffuse pigmented, albinistic and ambicolorate fish on one hand and normally and slight diffuse pigmented plaice on the other, showed a different behaviour in relation to length. These differences affected their chance of being caught. CUNNINGHAM (1893) describes an ambicolorate plaice being very shy and remaining immobile in the sand. SCHNAKENBECK (1923) however, could not find any difference in behaviour between abnormally and normally pigmented plaice in his tanks. Both authors made their observations in aquaria in which fish will soon adopt a special behaviour not found in nature. It is possible that abnormally pigmented plaice do not differ in this special aquarium behaviour from normal plaice but will behave differently under normal circumstances.

In our studies we found an increase in the degrees of abnormal pigmentation with length for a number of pigment anomaly classes in adult and in juvenile plaice. We tried to explain this phenomenon on the basis of different assumptions, but none seemed to be satisfactory. For the time being, we have to accept the possibility, that in plaice abnormal pigmentation may develop throughout the whole life span of the fish. In order to understand this we have to go deeper into the problem of the causation of pigment deficiencies.

Although the majority of the workers in this field assume that abnormal pigmentation will develop in the first months of life only, there is no unanimity as to the factors causing pigment abnormalities. SCHNAKENBECK (1923) assumes that the pigment deficiencies are more innate than acquired. According to VON UBISCH (1951) light plays a predominant role. Too strong a light intensity at metamorphosis will lead to albinism and ambicoloration. DANNEVIG and HANSEN (1952) however, report that larvae hatched in total darkness seem to be predisposed towards abnormal pigmentation later on. Pigment deficiency may also bear some relation to population density at metamorphosis; moreover, a relation between temperature during incubation and larval development and the degree of pigmentation at metamorphosis seems to be present (SHELBOURNE, 1964). RILEY (1966) found that pigment deficiency in reared plaice is correlated with small size. When they had a final size at the end of the rearing experiment of less than 13 mm the plaice demonstrated a higher percentage of pigment anomalies and frequently had abnormal mandibular and opercular bone development together with incomplete eye-rotation. Moreover, in experiments in which different levels of food were supplied, the lower feeding levels gave higher percentages of pigment deficiencies and deformation in the larvae, the abnormalities only becoming obvious at metamorphosis. The results given by VON UBISCH, DANNEVIG and HANSEN, SHELBOURNE and RILEY are all derived from tank experiments in hatcheries. Here no predators are present and thus pigment abnormalities continue to survive. In nature high degrees of pigment deficiencies will be severely selected by predators and it is thus understandable why the percentage of one of the most conspicuous pigment anomaly classes viz. albinism, is very low in the 0-group plaice we studied in the nursery grounds. Besides, of the great number of varieties of albinism found in reared plaice, only a few are found in nature in juvenile and adult fish.

A number of cases point without doubt to the development of pigment deficiencies in the very early stages of life. In the dab and the turbot, for example, not only the colour pattern of the eye-side is repeated but also the structure of the skin in ambicolorate specimen. In the turbot the bony tubercles characteristic of the eye-side are repeated in ambicolorate parts of the blind-side (BOEKE, 1903; NORMAN, 1927) and in the dab the edges of the scales of the blind-side being normally smooth, become spiny in ambicolorate areas (NORMAN, 1927). Still there are indications that abnormal pigmentation on the blind-side may develop after metamorphosis. CUNNINGHAM (1891, 1893, 1895 a, b) and OSBORN (1939, 1940, 1941 a, b) showed that when the blind-side of certain flatfish was exposed to light for a given period, patches of chromatophores developed gradually covering most of the blind-side. In nature we have an example of the role of light in the repigmentation of the blind-side in the Greenland halibut (*Reinhardtius hippoglossoides* (Walb.)) described by JENSEN

(1935). Another example is the witch (*Glyptocephalus cynoglossus* L.), in which the larger specimen normally show some pigment all over the blind-side giving it a slightly smoky appearance (CUNNINGHAM, 1896). In small witches, however, say up to 12 cm, no pigmentation at all is present on the blind-side (HOLT, 1893). Although the factor responsible for the development of the pigment is unknown in this case, it is clear, that the pigmentation on the blind-side is acquired after the juvenile stage.

It is not clear in these cases which class of abnormal pigmentation is developed under the influence of light or other factors, in other words whether diffuse pigmentation or ambicoloration is concerned, and what is the relationship between both classes of pigment anomalies. To get an answer to this question we also counted the average number of upper-dermal melanophores per unit skin area in those parts on the blind-side showing pigment anomalies and on the eye-side in the area between the orange, white and black patterns, that part of the skin called "normal area" by HEWER (1926) in his study of the colour pattern of the dab.

As found by HEWER (1926) in the dab, we found that the average number of melanophores on the eye-side was higher in the juvenile than in the adult plaice. In ambicolorate plaice the average number of melanophores was the same for both sides of the fish. In diffusely pigmented plaice, however, the number of melanophores on the blind-side was less than half the number on the eye-side. This is in agreement with the results given by SCHNAKENBECK (1923) who gave for ambicolorate plaice a ratio 1 : 1, and for diffusely pigmented plaice a ratio of 1 : 0·37 for the numbers of melanophores on the eye and the blind-sides.

When counting chromatophores we detected the trematode responsible for the development of diffuse pigmentation in juvenile plaice, thus having direct evidence of the causation of a pigment abnormality in the period after metamorphosis.

The information on increased chromatophore number on the eye-side of juvenile plaice infested with the trematode and the association of the "goudschol" appearance with the presence of the cysts in the fish renders it plausible to assume that the parasite, even in restricted numbers, causes a general state of stress in the fish.

SCHNAKENBECK (1923), discussing the nature of chromatophores, mentions the fact that infection with parasites and fungi often leads to an increase and hypertrophy of pigment cells. In the "goudschol" only the yellow chromatophores are maximally expanded and demonstrate hypertrophy; the erythrophores and the melanophores behave normally: a number of the cells are contracted, others expanded to form the typical dotted appearance of the juvenile plaice as described by HEWER (1931). The highest degree of diffuse pigmentation and the phenomenon of "goudschol" was found on the mussel beds. In pure seawater blue light penetrates to the greatest depths; in turbid water, however, the wavelength showing the lowest extinction coefficient has moved to green and yellow owing to the presence of increasing amounts of "yellow substance" (KALLE, 1938; SVERDRUP, JOHNSON & FLEMING, 1952). In the water surrounding the mussel beds the turbidity is very high and consequently the bottom will be coloured yellow by the light reaching it from the surface. Skin-diving on a mussel bed in the Waddensea confirmed this; the light penetrating to the bottom was bright yellow. It is possible that trematode infested plaice, being very sensitive owing to their condition of stress, will accept a yellow appearance through expansion and hypertrophy of the xanthophores in order to make themselves as inconspicuous as possible. KRISTENSEN (personal communication) could easily tell "goudschol" (mostly heavy diffuse pigmented plaice) apart from the normal plaice by their different behaviour in his aquarium. "Goudschol" did not dig in the sand in the daytime but remained on the sand. In our aquarium in IJmuiden we found that slight diffuse pigmented plaice did not dig in the sand as deep as the normally coloured juvenile plaice obtained from the Waddensea. Thus it seems correct to suggest that diffusely pigmented plaice by their increased inability to dig in the substrate (irritation by the parasites?) has to rely more and more on colour adaptation to make themselves inconspicuous as compared with normally coloured fish which cover their eye-sides with bottom material.

On the other hand it is possible that the yellow appearance of the "goudschol" is only the result of the influence of a substance secreted by the trematode especially affecting the yellow chromatophores. SCOTT (1965), discussing the physiology and pharmacology of the color change on the eye-side in normally pigmented specimens of *Scophthalmus aquosus*, showed that a number of chemical compounds are extremely active in forcing the melanophores to maximal expansion.

The fact that trematodes give rise to the development of chromatophores in skin areas normally devoid of these pigment cells has been found before in marine fishes. SINDERMANN and ROSENFIELD (1954) described the case of the Atlantic herring (and other inshore species) in the Western North Atlantic in which cercariae of the trematode *Cryptocotyle lingua* (Creplin) invade and encyst in the fins and integument, causing the formation of conspicuously pigmented cysts or "black spots". Massive cercarial invasion may eventually lead to blindness and death of immature herring.

In flatfish trematodes in the skin were already described in the beginning of this century. *Stephanostomum baccatum* (NICOLL, 1907) for example was detected as an adult trematode in the halibut and in metacercarial forms in the flounder and other flatfish species. In Canada *S. baccatum* is locally very frequent in the winter flounder in which fish the cysts, however, do not give rise to pigmentation of the blind-side. Larger winter flounders living in deeper water are more heavily infested than smaller fish (WOLFGANG, 1954). This is in contrast with "our" trematode whose cysts induce the formation of chromatophores and in which the highest degree of infestation was found in the smaller fish.

The investigation of pigment deficiencies in plaice was primarily designed as a part of the study of populations. In general we obtained valuable information mostly confirming what had already been found by other methods. The fact that the four populations do not mix to an appreciable extent was confirmed very clearly, for example, in the results of the ambicolorate plaice.

The different types of ambicoloration and albinism make it possible to make more refined comparisons between the populations than is possible with the other methods. Thus the close resemblance of the ambicolorate Flamborough and Cleaver Bank plaice on one side and the Southern and German Bight populations on the other points to common factors, which may give us more detailed information on the recruitment to the various populations of adult plaice. In addition the uniqueness of the ambicoloration and albinism type compositions in the juvenile plaice from the two nursery grounds studied, which could not be traced in the adult populations, opens possibilities to assess the importance of different nursery grounds in recruitment. Moreover, in combination with meristic features and tagging experiments we might study natural mortality in the pre-recruit phase. The results obtained in the Waddensea juveniles and compared with those of the adult plaice suggest that we have to reckon with the possibility of a fairly large pre-recruit natural mortality. Diffuse pigmentation can be used for the same purpose and its rather high frequency is attractive.

It is important at this stage to study juvenile abnormal pigmentation in other nursery grounds. At the moment we have information from all nursery grounds along the Dutch coasts at our disposal. We hope to obtain further details from other nurseries along the continental coasts of the North Sea.

SUMMARY

1. By means of meristic and otolith characters and by tagging experiments started in 1957 the existence of at least four separate populations could be demonstrated in the Southern and Central North Sea.
2. In addition to meristic features abnormal pigmentation in plaice, being fairly common, might be of value in the study of populations. A comparison of the results already obtained with those to be revealed by the study of abnormal pigmentation make it possible to test the value of pigment deficiencies as population characters.
3. In the years 1963, 1964, 1965 and 1966 the pigmentation pattern of some 34,000 ripe plaice tagged on the spawning grounds of the four populations were recorded. In addition pigmentation data from 8,000 juvenile plaice in two Dutch nursery grounds were obtained.
4. Abnormal pigmentation can be divided into a number of classes. *Ambicoloration* and *albinism* follow fairly regular patterns. In *diffuse pigmentation* a number of small pigmented spots or areas appear on the blind-side in an irregular manner. *Xanthochroism*, in which the black pigment cells are missing, is so rare in the plaice that it was not considered.
5. For matters of abundance only the data of male adult plaice were used and all the information in the four years were combined per population.
6. The Flamborough population had the lowest percentage of fish with diffuse pigmentation, no single specimen being found with heavy diffuse pigmentation in the four years concerned. The highest degree of diffuse pigmentation was observed in the Southern Bight.
7. The degree of ambicoloration was the same in the Flamborough and the Cleaver Bank plaice and differed from that of the Southern and German Bight groups. When considering the various forms or types in which ambicoloration can be subdivided one finds that the type compositions of the Flamborough and the Cleaver Bank populations resemble each other closely and differ from those of the Southern and German Bight plaice which in their turn resemble each other closely.
8. In all four adult populations the percentage of fish showing abnormal pigmentation of the classes slight diffuse pigmentation, ambicoloration and albinism increases with increasing length. This is in contradiction with the generally accepted assumption by most authors on pigment anomalies that

their development takes place only during or before metamorphosis. A number of mechanisms is discussed in order to explain our results in this light.

9. The assumption that the increase in the degree of abnormal pigmentation with length is only apparent and results from earlier year classes recruiting to the adult stocks with a much higher degree of pigment anomalies than at present and that the intermediate year classes had in succession decreasing percentages of pigment deficiencies, cannot be maintained. The same is true for the assumption that a differential mortality between normally and abnormally pigmented plaice is causing an apparent increase in the percentage of fish with pigment anomalies with age (length). Tagging experiments showed a differential mortality for the various pigmentation classes in all four populations. The difference found, however, was only one tenth of the value expected theoretically.
10. In the juvenile plaice in the Dutch Waddensea results were obtained comparable with those of the adult fish. In all pigment anomaly classes a distinct increase in the percentage of plaice with abnormal pigmentation with length was found. As in the case of the adult plaice we tried to explain these relationships assuming that the pigment abnormalities developed during or before metamorphosis only.
11. Neither a diminishing initial percentage of abnormal pigmentation in successive year classes nor a differential mortality between normally and abnormally pigmented plaice could be found.
12. The type compositions of ambicoloration and albinism in the Dutch Waddensea juveniles differed greatly from those of the four adult populations. Moreover, in the ambicolorate juvenile plaice of the IJmuiden beach area a type composition was found completely different from that of the Waddensea juveniles and differing too from those of the adult populations.
13. Our results force us to accept the view that abnormal pigmentation may develop not only in the very early stages but also throughout the whole life span of the fish.
14. A study of the numbers of melanophores on both sides of the plaice with pigment deficiencies revealed that in ambicolorate fish the average number of melanophores is the same on both sides. In diffusely pigmented plaice the average number of melanophores on the blind-side is less than half the number on the eye-side. Moreover, in juvenile plaice with slight diffuse pigmentation the number of melanophores on the eye-side is higher than on the eye-side in normally pigmented plaice. In moderate and heavy diffuse pigmentation it is still higher than in slight diffuse pigmentation.
15. The factor responsible for the increase in melanophore number on the eye-side and related with the amount of diffuse pigmentation on the blind-side is a trematode, still unknown, living in small cysts in the skin on both sides of diffusely pigmented juvenile plaice. No cysts were found in ambicolorate, albinistic and normally pigmented plaice. Around the cysts chromatophores of all kinds develop on the blind-side and may eventually cover large parts of the skin. The pigmentation patches probably remain intact for a long time after the disappearance of the trematode.
16. Not all diffusely pigmented juveniles contained cysts. The highest percentage of trematode infested plaice with diffuse pigmentation was found in the

smallest fish studied (7 cm). With increasing length this percentage dropped and no cysts were found in plaice of 30 cm and over. The phenomenon of "goudschol" could be related with the occurrence of the trematode in the skin of the plaice and furthermore it was apparent that the source of the infestation with the trematode had to be sought in the large commercial mussel beds in the Waddensea.

17. In adult plaice the factor(s) responsible for the development of diffuse pigmentation could not yet be traced. The possible influence of light is discussed. Attention is drawn to the cases of the Greenland halibut and the witch as arguments for the view that pigment abnormalities may develop later in life.
18. There is slight evidence that ambicoloration may develop even in large plaice as found in our tagging experiments. A programme involving photographing tagged fish at release and at recovery in order to follow the development of pigment anomalies under natural circumstances is to be carried out.
19. The merits of abnormal pigmentation as a population character are discussed.

ACKNOWLEDGEMENTS

I am indebted to the following persons for their help and criticism. Dr. F. R. HARDEN JONES, Fisheries Laboratory, Lowestoft, England, for his suggestions which initiated the present study, Mr. J. J. ZIJLSTRA, of our Institute for his critical reading of the manuscript, Mr. S. J. DE GROOT, of our Institute for his continuous interest and help in dealing with the literature on the subject, Dr. I. KRISTENSEN, of the Netherlands Institute for Sea Research at Den Helder for his personal communication on the behaviour of abnormally pigmented plaice, Mr. J. J. HOOFT of the Utrecht University for his help as a skin-diver, Mr. S. SCHAAP and Mr. P. I. VAN LEEUWEN of our Institute for their close cooperation in the research. I also thank my colleague Mr. J. VAN VLIJMEN, mathematician at our Institute, for his suggestion and help in the mathematical part of this article.

REFERENCES

BEMMELEN, A. A. VAN, 1866. "Lijst van Visschen in Nederland waargenomen". *Bouwstoffen een Fauna van Nederland*, 3: 318–410. Leyden.

BEVERTON, R. J. H. and HOLT, S. J., 1957. "On the dynamics of exploited fish populations". Fishery Invest. Lond., Ser. 2, 19: 533 pp.

BREDER, C. M., 1938. "An unusual aberrantly colored pleuronectid". Zoologica, N.Y., **23**: 393–6.

BOEKE, J., 1903. "Over een bijna geheel ongekleurd exemplaar van de tarbot (*Rhombus maximus*)". Jaarb. Rijksinst. Onderz. Zee, 40–2.

CHABANAUD, P., 1957. "Etude d'une sole affectée d'une anomalie pigmentaire apparemment consécutive à un traumatisme". Bull. Soc. zool. Fr., **82**: 326–30.

CUNNINGHAM, J. T., 1891. "An experiment concerning the absence of colour from the lower sides of flatfishes". Zool. Anz., **14**: 27–32.

CUNNINGHAM, J. T., 1893. "Researches on the coloration of the skins of flatfishes". J. mar. biol. Ass. U.K., **2**: 111–18.

CUNNINGHAM, J. T., 1895a. "Additional evidence on the influence of light in producing pigments on the lower sides of flatfishes". J. mar. biol. Ass. U.K., **2**: 53–9.

CUNNINGHAM, J. T., 1895b. "A piebald plaice". J. mar. biol. Ass. U.K., **3**: 271.

CUNNINGHAM, J. T., 1896. *The natural history of the marketable marine fishes of the British Islands*. London, 375 pp.

CUNNINGHAM, J. T. and MACMUNN, C. A., 1893. "On the coloration of the skins of fishes, especially of Pleuronectidae". Phil. Trans. R. Soc. B., **184**: 765–812.

DANNEVIG, A. and HANSEN, S., 1952. "Faktorer av betydning for fiskeeggenes og fiskeyngelens oppvekst". FiskDir. Skr., Serie Havundersøkelser, **10**: (1) 36 pp.

DAWSON, C. E., 1962. "Notes on anomalous American Heterosomata with descriptions of five new records". Copeia, 138–46.

DAWSON, C. E., 1967. "Three new records of partial albinism in American Heterosomata". Trans. Am. Fish. Soc., **96**: 400–4.

EISLER, R., 1963. "Partial albinism and ambicoloration in the winter flounder *Pseudopleuronectes americanus*". Copeia, 275–7.

ELMHIRST, R., 1911. "On some ambicoloured flatfish from the Clyde". Ann. Scot. nat. Hist., 77–9.

ENDE, W. P. VAN DEN, 1849. "Eenige bijzonderheden betrekkelijk de schol". Versl. Werkzaamh. Veren. Bevord. inld. Ichtyol. Arnhem, **2**: 146–50.

FRANZ, V., 1910. "Zur Physiologie und Pathologie der Chromatophoren". Biol. Zbl., **30**: 150–8.

GUDGER, E. W., 1934. "Ambicoloration in the winter flounder *Pseudopleuronectes americanus*". Am. Mus. Novit., (717) 7 pp.

GUDGER, E. W., 1935. "Two partially ambicolorate flatfishes (Heterosomata)". Am. Mus. Novit., (768) 8 pp.

GUDGER, E. W., 1941. "A totally ambicolorate flounder *Platichthys stellatus* from Alaskan waters". Copeia, 28–30.

GUDGER, E. W., 1946. "An albino rusty dab, *Limanda ferruginea*, with notes on other abnormalities in flatfishes". J. Elischa Mitchell scient. Soc., **62**: 73–6.

GUDGER, E. W. and FIRTH, F. E., 1936. "Three partially ambicolorate four spotted flounders, *Paralichthys oblongus*, two each with a hooked dorsal fin and a partially rotated eye". Am. Mus. Novit., (885) 9 pp.

HEWER, H. R., 1926. "Studies in colour-changes in fish. II. An analysis of the colour of the dab". Phil. Trans. R. Soc. B., **215**: 177–200.

HEWER, H. R., 1931. "Studies in colour changes in fish". J. Linn. Soc., Zoology, **37**: 493–512.

HOLT, E. W. L., 1893. "Survey of fishing grounds, west coast of Ireland, 1890–91: on the eggs and larval and post-larval stages of teleosteans". Scient. Proc.R. Dubl. Soc., N.S. 5, **2**: 5–121.

HOUTTUYN, M., 1764. *Natuurlijke Historie of uitvoerige beschrijving der dieren, planten en mineraalen*. "De visschen", **1**: (7) 319–52. Amsterdam.

HUSSAKOF, L., 1914. "On two ambicolorate specimen of the summer flounder (*Paralichthys dentatus*) with an explanation of ambicoloration". Bull. Am. Mus. nat. Hist., **33**: 95–100.

JENSEN, A. S., 1935. "The Greenland halibut (*Reinhardtius hippoglossoides* (Walb.)). Its development and migrations". K. danske Vidensk. Selsk. Skr., **6**: 1–32.

KALLE, K., 1938. "Zum Problem der Meereswasserfarbe". Annln Hydrogr. Berl., **66**: 1–13.

NORMAN, J. R., 1927. "Ambicolorate flatfishes". Nat. Hist. Mag., **1**: 57–9.

NORMAN, J. R., 1934. *A systematic monograph of the flatfishes* (*Heterosomata*). British Museum, London, 459 pp.

OSBORN, C. M., 1939. "The experimental production of melanin pigment on the ventral surface of the summer flounder (*Paralichthys dentatus*)". Anat. Rec., **75**: 60.

OSBORN, C. M., 1940. "The experimental production of melanin pigment on the lower surface of summer flounders (*Paralichthys dentatus*)". Proc. natn. Acad. Sci., Wash., **26**: 155–61.

OSBORN, C. M., 1941 a. "Factors influencing the pigmentation of regenerating scales on the ventral surface of the summer flounder". Biol. Bull. mar. biol. Lab., Woods Hole, **81**: 301–2.

OSBORN, C. M., 1941 b. "Studies on the growth of integumentary pigment in the lower vertebrates. I. The origin of artificially developed melanophores on the normally unpigmented ventral surface of the summer flounder *Paralichthys dentatus*". Biol. Bull. mar. biol. Lab., Woods Hole, **81**: 341–51.

REICHENBACH-KLINKE, H. and ELKAN, E., 1965. *The principal diseases of lower vertebrates*. Academic Press, Lond. & N.Y., 600 pp.

REICHENBACH-KLINKE, H., 1966. *Krankheiten und Schädigungen der Fische*. Fisher, Stuttgart, 334 pp.

RILEY, J. D., 1963. "Farming the sea with plaice as the crop". Wld Fishg, **12**: (2) 36–8.

RILEY, J. D., 1966. "Marine fish culture in Britain. VII. Plaice (*Pleuronectes platessa* L.). Postlarval feeding on *Artemia salina* L. nauplii and the effects of varying feeding levels". J. Cons. perm. int. Explor. Mer, **30**: 204–21.

RILEY, J. D. and THACKER, G. T., 1963. "Marine fish culture in Britain. III. Plaice (*Pleuronectes platessa* L.). Rearing in closed circulation at Lowestoft 1961". J. Cons. perm. int. Explor. Mer, **28**: 80–90.

SCHNAKENBECK, W., 1923. "Über Färbungsanomalien bei Pleuronectiden". Wiss. Meeresunters. Abt. Helgoland, N.F. **15**: (10) 1–22.

SCOTT, G. T., 1965. "Physiology and pharmacology of color change in the sand flounder *Scopthalmus aquosus*". Limnol. Oceanogr., **10**: (Suppl). R230–R246.

SHELBOURNE, J. E., 1963a. "Marine fish culture in Britain. II. A plaice rearing experiment at Port Erin, Isle of Man, during 1960, in open sea water circulation". J. Cons. perm. int. Explor. Mer, **28**: 70–9.

SHELBOURNE, J. E., 1963b. "Marine fish culture in Britain. IV. High survivals of metamorphosed plaice during salinity experiments in open circulation at Port Erin, Isle of Man, 1961". J. Cons. perm. int. Explor. Mer, **28**: 246–61.

SHELBOURNE, J. E., 1964. "The artificial propagation of marine fish. Adv. mar. Biol., **2**: 1–83.

SHELBOURNE, J. E., RILEY, J. D. and THACKER, G. T., 1963. "Marine fish culture in Britain. I. Plaice rearing in closed circulation at Lowestoft 1957–1960". J. Cons. perm. int. Explor. Mer, **28**: 50–69.

SINDERMANN, C. and ROSENFIELD, A., 1954. "Disease of fishes of the Western North Atlantic. I. Disease of the sea herring". Res. Bull. Dep. Sea Shore Fish. Me. (18) 23 pp.

SVERDRUP, H. U., JOHNSON, M. W. and FLEMING, R. H., 1952. *The oceans, their physics, chemistry and general biology*. Prentice-Hall Inc. N.Y., 4th ed., 1049 pp.

UBISCH, L. VON, 1951. "Untersuchungen über Pleuronektiden. II. Ambikoloration, Inversion und Bilateralität". Wilhelm Roux Arch. EntwMech. Org., **145**: 61 pp.

VEEN, J. F. DE, 1962. "On the subpopulations of plaice in the southern North Sea". ICES CM 1962, (94) 5 pp. (mimeo).

VEEN, J. F. DE, 1965. "Schollen op stap". Viss. Nieuws, **18**: (2) 39–49.

VEEN, J. F. DE and BOEREMA, L. K., 1959. "Distinguishing southern North Sea spawning populations of plaice by means of otolith characteristics". ICES CM 1959, (91) 4 pp. (mimeo).

WOLFGANG, R. W., 1954. "Studies of the trematode *Stephanostomum baccatum* (NICOLL, 1907). II. Biology with special reference to the stages affecting the winter flounder". J. Fish. Res. Bd Can., **11**: 963–87.