THE ONTOGENY OF RESPIRATION IN HERRING

AND PLAICE LARVAE

by

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CONTAINS

PULLOUTS

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GENERAL INTRODUCTION.

The study of larvae, their behaviour & physiology has gathered momentum in recent years due to the development of improved techniques of rearing during the past decade. Many marine larvae have been successfully reared in the laboratory (Shelbourne, 1964; Schumann, 1967; Blaxter, 1968,1969; Houde & Palko, 1970; Futch & Detwyler, 1970; Lasker, Feder, Theilacker & May, 1970). Fish larvae, in particular those of most marine species form an important tool for research in that at hatching they only possess the rudiments of most organs. Thus they are ideal material for ontogenetical studies.

Although the respiratory mechanisms of adult fish have been the subject of a great deal of investigation from the point of view of gas exchange (see Randall 1970) gill structure (Hughes, 1966; Hughes & Grimstone, 1965; Newstead, 1967; to name a few) and dimensions (see Muir, 1969) gill ventilation and perfusion (see Shelton, 1970) circulatory systems (see Randall, 1970) bioenergetics, (see Brett, 1970) the respiratory systems of larvae have not been investigated in any great detail. Apart from a few studies on oxygen uptake (see Blaxter, 1969) and Harder (1954) on the development of branchial elements, no detailed study of the development of respiratory mechanisms have been made in marine fish larvae.

The purpose of this study was to investigate the development of respiration in two species of marine larvae viz. the herring (<u>Clupea harengus</u> L.) and the plaice (<u>Pleuronectes platessa</u> L.) These two species are well separated taxonomically and both adults and young have very different life histories. Herring lay demersal eggs, the plaice pelagic ones. The yolk-sac larvae of both species are planktonic, later feeding on diatoms and copepod nauplii and much later copepods. Adult herring are pelagic, living in mid water as juveniles and moving into deeper water with age, ranging from offshore to about 200m. They perform migrations partly caused by the distribution and density of food organisms. In contrast to this plaice at metamorphosis show an interesting asymmetry in that one eye migrates over the head and comes to lie against its opposite number. At this stage pelagic life ceases and the young fish assumes a bottom-living existence. Other features associated with asymmetry are secondary to the migration of the eye and follow on from the adoption of the benthic mode of life. They range from the shoreline when young to 1000m feeding on bottom living organisms.

During development the mechanism of respiration changes from a cutaneous one to gill respiration typical of the adult form. There is apparently no respiratory pigment in the early stages but the blood becomes pink at

metamorphosis.

The problem was approached from a morphological and a physiological viewpoint. The main parts of the study are as follows.

(1)   The survival times in water of low oxygen concentrations.

(2)   The oxygen uptake at normal oxygen concentrations.

(3)   The oxygen uptake at low oxygen concentrations.

(4)   Measurement of the body surface area as well as the gill area available for respiration.

(5)   The appearance of haemoglobin and its quantitative measurement.

MATERIALS

Incubation of Eggs

Herring.

The larvae used for the experiments were reared from eggs. Gonads were obtained from spawning fish captured by trammel net on the Ballantrae bank in the Firth of Clyde. Gonads were dissected out immediately after capture and individually placed in small glass jars. These were stored on a small amount of ice in a vacuum flask during transportation to the laboratory. Care was taken to ensure that the ovaries did not come into contact with water as this tended to make the eggs sticky and clump together during the subsequent dispersion on to glass plates.

Eggs were fertilized in the laboratory according to the method of Blaxter (1968). Ground glass plates of approximately 20 x 50 cm were placed singly on the bottom of rectangular white plastic tanks containing sea water. The eggs from one ovary were removed in groups using a scalpel and gently dispersed in the water, care being taken to wipe the scalpel dry before each group of eggs was taken. This was repeated until the plates were more or less uniformly covered with eggs but not crowded. Some eggs were dispersed on to glass slides to facilitate easy removal of eggs for some experiments. The plates were then transferred back to back, to a second tank containing the milt suspension. The

latter was obtained from the gonads of three to four male fish. The plates were removed after about 10 minutes and rinsed several times to remove excess milt by dipping them in clean sea water. They were then placed vertically around the sides of a black Darvic tank in water which had been left standing overnight. The tanks were covered with black plastic sheeting to keep out light. Plates with eggs were transferred daily to fresh sea water until a peak of hatching was observed. The eggs from one female were kept separate for some experiments, while the rest were mixed.

Plaice.

Fertilized eggs were obtained from the White Fish Authority at Hunterston, and Ardtoe, as well as from Port Erin, Isle of Man. The eggs were transported to the laboratory in sea water contained in plastic jars, stored on a little ice in a vacuum flask. At the laboratory the temperature was allowed to rise until it reached that of the sea water in the incubation tanks, which was about 9-10°C. These tanks contained antibiotics (sodium penicillin and streptomycin sulphate) in the concentrations recommended by Shelbourne (1964). The eggs were left in these tanks until hatching occurred. Time to hatching varied from 3 - 12 days depending on the stage of development

at which they were obtained. Eggs from one female were kept separate for some experiments while the rest were mixed.

Rearing techniques.

At hatching the larvae were removed by means of wide-mouthed pipettes and glass beakers, and transferred to black plastic tanks with a matt finish. The tanks varied in size from 20 to 2000 l. The former were used for plaice due to their smaller spatial requirements, while the larger ones were used for herring. In both species the larvae from one female were kept separate and reared in 200 l rectangular tanks made of "Darvic" (an ICI plastic) in a constant temperature room at $10^{o}C$ for some experiments. About 200 plaice larvae were transferred to each small tank, while about 3000 herring larvae were placed in the large tanks. This reduced the occurrence of fish nipping at one another. Light was supplied by an 80W fluorescent tube and situated about 1 m above the tanks in an alcove in the general aquarium area. Since the light appeared to be too strong, as apparent from the tendency of larvae to swallow air bubbles, translucent paper was used to cover them. This reduced the mortality of the larvae. A circular fluorescent tube was used in the constant temperature room. Time clocks were used in the alcove and constant temperature room to turn the lights on and off about an hour before sunrise and

sunset respectively. At the end of the yolk sac stage only,
for 2 - 3 weeks
day length was increased to promote commencement of feeding.
∧

Temperature of the water was recorded during '71 and '72
and the general temperature patterns are shown in Fig.1. All
the tanks were supplied with running sea water. The flow
rate was about 2 l/h in the small tanks, the rate being
increased in the large tanks. Water entered in at the bottom
and left at the surface. A perspex ring of about 7 cm diameter
and 6 cm high which had fine meshed plankton netting glued to
its lower side, was fixed at the outlet to prevent loss of
larvae. The mesh was cleaned daily to prevent clogging.

Sea water entered the tanks in the alcove via a 2 l
constant level container (Ehrlich, 1972) containing a seive
with synthetic fibre wool. This could supply about six
tanks with an uniform flow of water.

Tanks were cleaned daily for the first few weeks after
hatching, by siphoning off the debris and dead larvae from
the bottom. The bacterial film at the surface was removed
by skimming with a beaker. Some plaice were lost from one
tank following a growth of ciliates on the bottom. The
reason for the death of the larvae is unknown. It is
possible that the ciliates may have stung the larvae or
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alternately released toxic substances into the water. The
rest of the larvae were transferred into a new tank to
reduce mortality.

Fig.1. Pattern of temperature changes in the rearing

tanks during the 1971 and 1972 season.
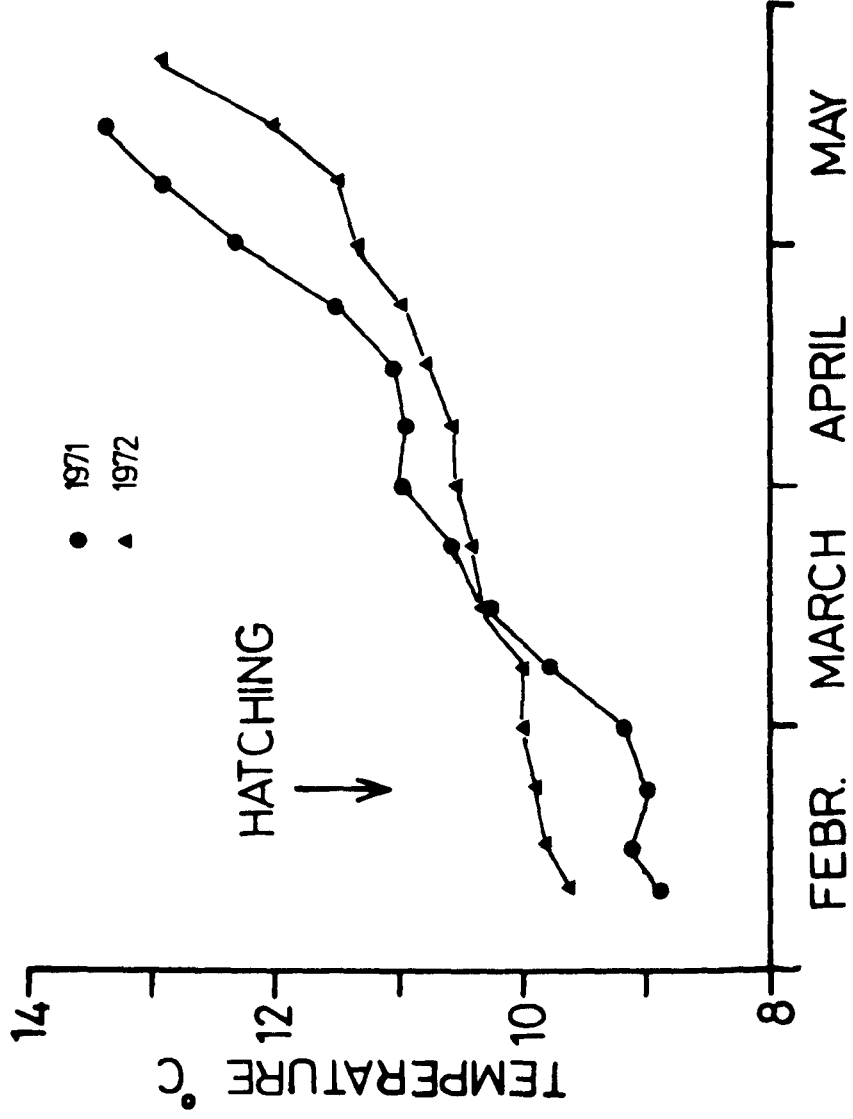
Herring larvae were fed <u>Balanus</u> nauplii initially. Egg sacs were removed from the adult barnacles and forced through a sieve into sea water. This served to break up the egg masses and release the nauplii. Later <u>Artemia</u> nauplii and sieved natural plankton was used. The <u>Artemia</u> eggs from San Fransisco Bay were incubated in 2 1 beakers of sea water at $26^{O}C$ with vigorous aeration. Nauplii were separated from the egg cases using their phototactic properties. Plaice were fed on <u>Artemia</u> nauplii and natural plankton up to metamorphosis. Both species were fed on a diet of chopped mussel, squid and mysids after metamorphosis.

PART ONE.   TOLERANCE AND SURVIVAL AT LOW OXYGEN TENSIONS

I.1. Introduction.

The dissolved oxygen requirements of marine fishes have not been adequately investigated. Most of the work has been limited to freshwater and anadromous species (see Doudoroff & Shumway, 1970). Bishai (1960) however studied the effect of reduced oxygen on herring larvae as well as that of salmonids, while Saksena & Joseph (1972) determined oxygen requirements of the larvae of the striped blenny (Chasmodes bosquianus), naked goby (Gobiosoma bosci) and skillet fish (Gobiesox strumosus).

The effects of a decreasing environmental oxygen tension on metabolic rate of teleost fish have been adequately dealt with by many authors such as Fry (1957), Winberg (1960), Brett (1962) and more recently by Fry (1971). Below a critical level, the incipient limiting level, the rate of oxygen uptake is directly dependent on the oxygen tension of the environment (see Fig.2).

Oxygen tensions above this level form the zone of respiratory independence while tensions below it form the zone of respiratory dependence. At a second critical level in the zone of respiratory dependence the maximum rate of oxygen uptake is reduced to a point where it is

Fig.2.   Relation between standard and active (maximum)
         rates of oxygen uptake at different oxygen
         concentrations.   Lines at top of graph delimit
         the zones of respiratory dependence and tolerance.
         L, incipient limiting level;   I, incipient lethal
         level;   R, minimum residual level;   M, maximum
         rate of oxygen uptake;   S, standard rate of
         oxygen uptake (after Shepard, 1955).
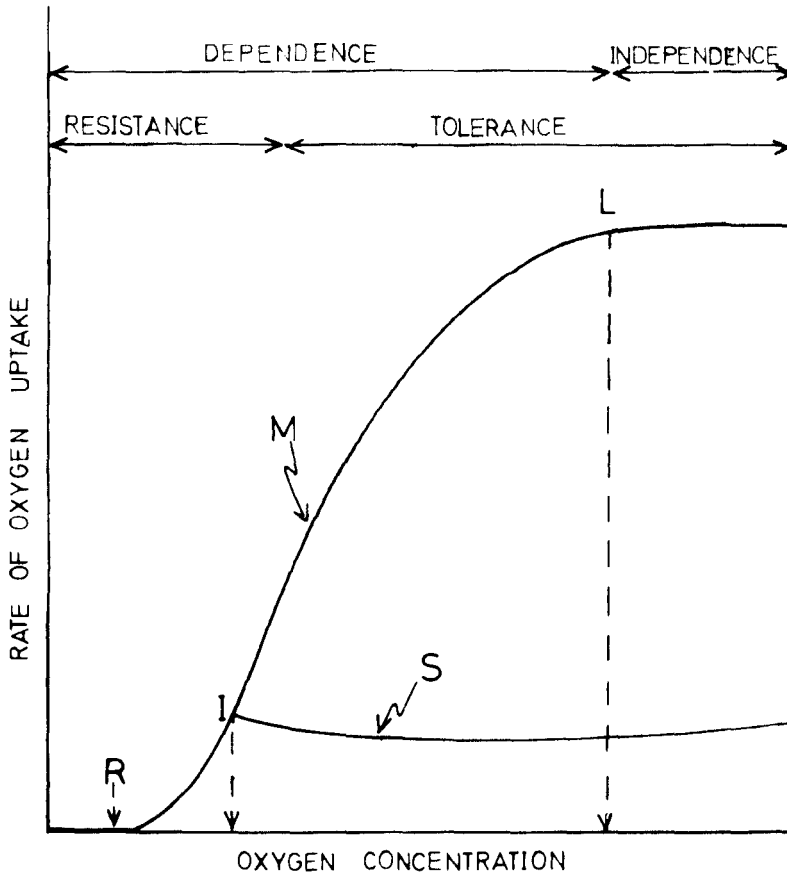
no longer sufficient to meet the minimum needs of the animal. This level has been termed by Fry (1957) the incipient lethal level LD_{50} or the median lethal dose. Above the median lethal dose one gets the zone of tolerance, below it the zone of resistance. Resistance is the ability of the animal to survive for a limited period in an environment that will eventually exert a lethal effect. ∧According to Fry Factors such as decreasing oxygen where the metabolic rate exerts an influence on the rate of dying, death is usually brought about by the interaction of limiting and controlling factors and is not considered to be a pure lethal factor like temperature according to Fry (1971). He also goes on to say that an analysis of the interaction is more valuable than a determination of the lethal level. The LT_{50} ie. the time for 50% of the animals to die at a particular oxygen concentration is another useful criterion. The experiments were designed to determine both the LT_{50} and LD_{50} for different stages of development in the two species considered.

I.2. Methods.

Five stages of herring were studied viz yolk-sac, 2-3 wks feeding, 5-6 wks feeding, 7-8 wks feeding and metamorphosed fish. The stages of plaice studied were

Developmental stages of plaice from Ryland (1966) at a rearing temperature ranging from 7 to 12 $^{\circ}$C.

Stage	Mean Length[1] (mm)	Mean Weight[2] (mg)	Description
1	7.0	0.87	Yolk-sac present
2	8.0	1.69	Notochord straight
3	9.75	4.46	Caudal extremity of notochord bent, eyes symmetrical
4	11.0	8.84	Eyes asymmetrical, flatfish shape develops
5	11.5	-	Left eye on or beyond edge of the head, pupil visible from on top

[1] Length obtained from Fig. 9.

[2] Weight averaged from Table 2.

yolk-sac, 2-3 wks feeding, (stage 2 (see Shelbourne, 1957), 5-6 wks feeding (stage 4-4b), and 3-4 wks after metamorphosis (see Ryland, 1966, for duration of stages). All the experiments were performed during the 1971 rearing season except those on metamorphosed plaice which were performed in the 1972 season.

Larvae which were abnormal looking or deformed, for example blue finned yolk-sac herring larvae were not used for experiments. The larvae were transferred by pipette in the early stages and later using a beaker into a rectangular chamber (25cm x 11cm x 7cm) into which deoxygenated water entered from a fractionating column. Fry's (1951) system was used where purified N_2 gas was bubbled in at the bottom of the column and aerated water entered at the top (Fig.3). The flow rate of nitrogen was measured using a GAP flowmeter while a Beckman model No.777 laboratory oxygen analyser was used to monitor the deoxygenated water (as % of air saturation) flowing out of the column. Oxygen content was calculated as mlO_2/l at NTP, by reference to a standard table of oxygen saturation values (Carpenter, 1966).

A sample of fish ranging from ten to fifteen was used in each experiment. A few experiments were repeated at oxygen concentrations well below the LD_{50} level and there was good agreement between the LT_{50} values in the replicate

Fig.3. Apparatus used for the LD_{50} experiments.

← AERATED WATER

FRACTIONATING
COLUMN

N₂ →

FLOW
MET.

N₂ ↓

GLASS
MARBLES

FISH
CHAMBER →

pO₂ ELECTRODE

CUVETTE →

→
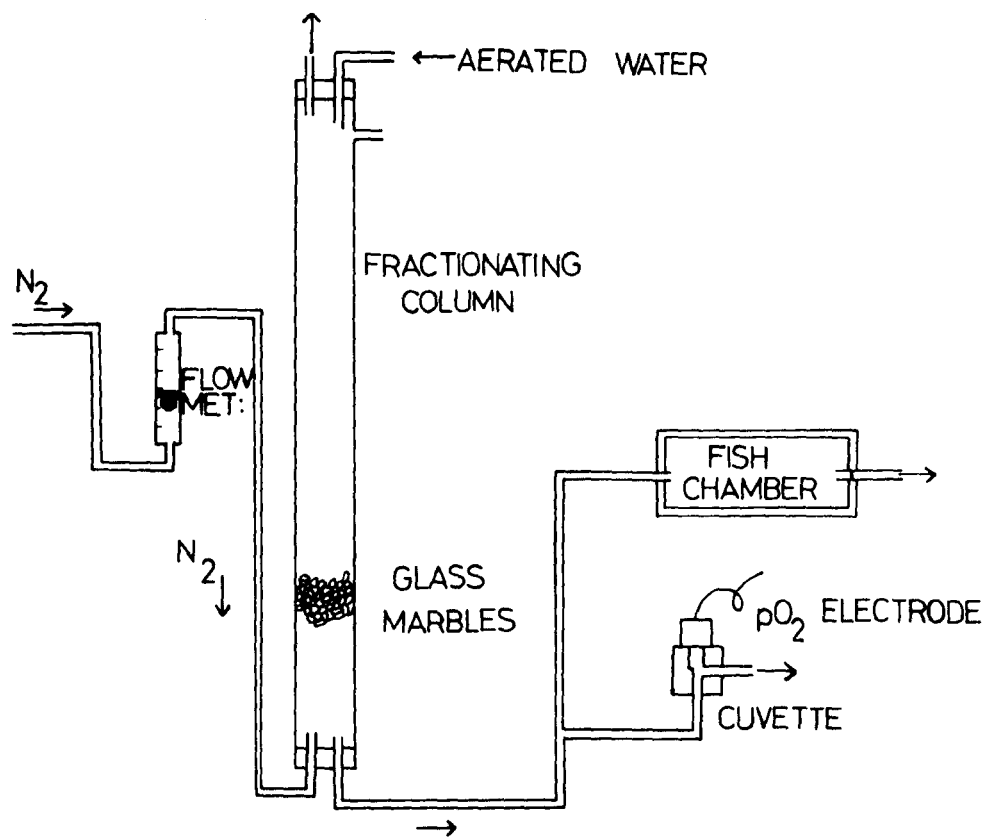
experiments. The temperature range of the experimental water corresponded to the temperature at which the larvae were reared and ranged from 8° - 13°C over the full rearing period. Temperature changes throughout the experimental period of 12 h was 0.5°C which was about equivalent to the temperature ranges of the rearing tanks during the same time. Fish were observed continuously during the lethal experiments and at half-hourly intervals during the higher oxygen levels. An experimental period of 12h was chosen because of the problems of rapidly growing larvae and as such a dosage period of 96h which is postulated as being the best time interval for large fish (Fry, 1971) would not be practical.

I.3. Results.

I.3.1. Statistical procedures for lethal oxygen experiments

When larvae are exposed to lethal levels of oxygen the pattern of mortality was found to be similar to that of other time-effect relations e.g. bioassay work of Bliss (1952), studies of lethal temperature relationships by Brett (1952), lethal oxygen level by Sheppard (1955).

The mortality curves are basically of 3 types (see Fig.4). At very lethal levels of oxygen, mortality occurs rapidly and uniformly throughout the experiment (Curve A). At slightly higher levels of oxygen

Fig.4.　Resistance times of herring larvae at the yolk-sac

　　　　stage exposed to various low levels of oxygen.

　　　　A,　0.06 ml/l;　B, 1.38 ml/l;　C, 2.07 ml/l.
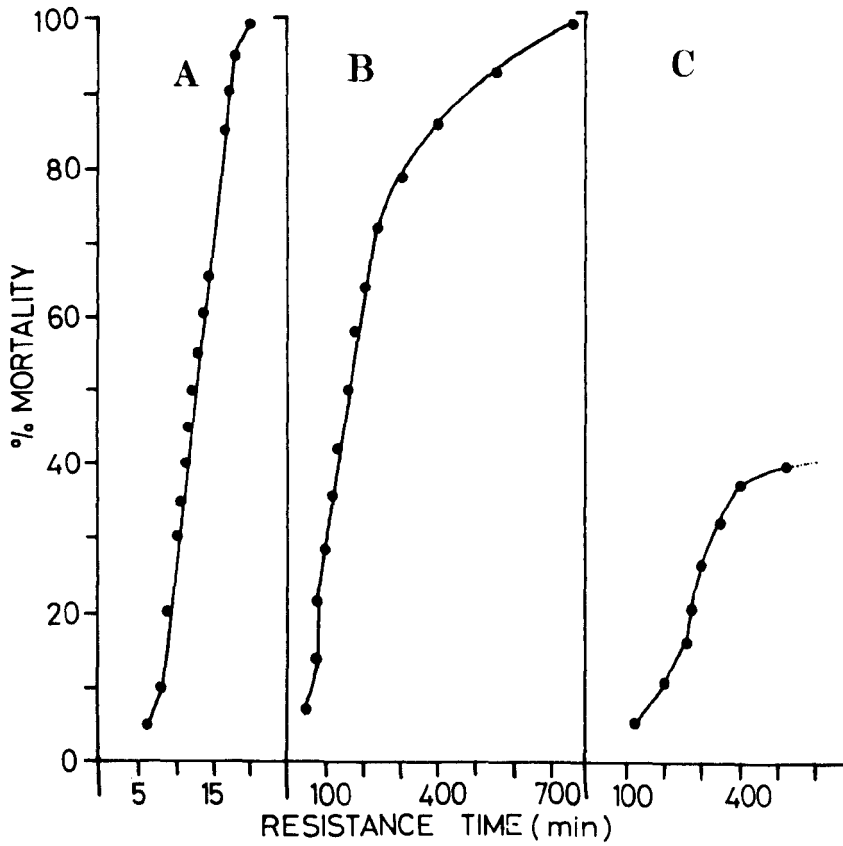
(Curve B) 100% mortality takes place but the intervals between successive deaths increases throughout the experiment. This set of points shows the typical asymmetrical sigmoid curve characteristic of many experiments where the animals are exposed to lethal conditions. At relatively higher oxygen concentration (Curve C) there is incomplete mortality during the experimental period. When the data in Fig.4 is replotted with the cumulative mortality expressed as units of standard deviation (probits) and the resistance times transposed into logarithms of time the points can be resolved into approximate straight lines (Fig.5).

As is evident the best fit is obtained in the range of 4 - 6 probits i.e., between 16 and 84% mortality. The slopes of the lines tend to decrease as the resistance times increase. From this graph the LT_{50} or the time to 50% mortality for each dose can be obtained. The median effective dose i.e., the dose which will produce a response in half the population in 12 hours, which is thus the mean tolerance limit or LD_{50} and its confidence limits were determined by the bioassay technique of Litchfield & Wilcoxen (1947 & 1949). The method is comparable to the dosage mortality of Bliss (1935, 1937, as cited by Sheppard, 1955).

Fig.5. Time mortality curves for herring larvae at the yolk-sac stage exposed to various dosages of lethal oxygen concentration. Note that mortality is expressed as cumulative % dying and as a probit. Broken line indicates incomplete mortality. Oxygen concentration is given in ml/l.

HERRING YOLK-SAC
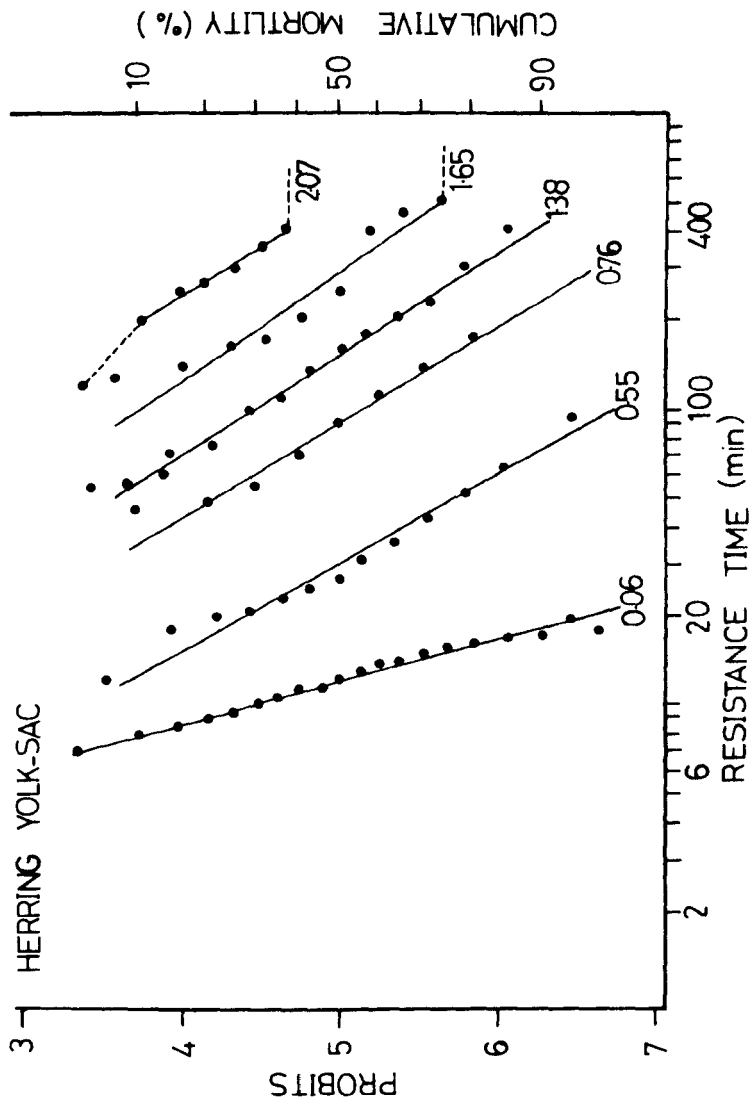
I.3.2. Behaviour of larvae during lethal experiments

When exposed to lethal oxygen concentrations the larvae tended to swim around very actively. As the oxygen content decreased further the rate of ventilation increased in the older larvae, the operculae described wider arcs and in the later developmental stages (mainly metamorphosed fish) both herring and plaice tended to swim upside down. Following cessation of respiratory movements in the metamorphosed fish the opercula fanned out and the mouth was opened to its widest extent. The metamorphosed plaice which tended to lie on the bottom on exposure to lethal oxygen concentrations tended to swim in short bursts, lying on the bottom between each successive burst of swimming, but with time tended to show continuous active swimming movements finally turning upside down. The latter is repeated a number of times before cessation of respiratory movement. The larvae were considered to be 'dead' when the brain became opaque and there was no movement.

I.3.3. Lethal oxygen level and resistance time

In general the resistance to low oxygen varied directly with the oxygen concentration of the environment and at very low oxygen concentrations mortality occurred rapidly. The results of a typical series of experiments for herring and

plaice are shown in Figs 5 and 6 and Appendix I and 2.
The full LT_{50} results are given in Table 1 for herring
and Table 2 for plaice. The LD_{50} values and their
confidence limits for both species are given in Table 3.

From Table 3 it is evident that there is a change
in the incipient lethal level or LD_{50} with development in
both species. An increase in the LD_{50} value is obtained
up to 5 wks of feeding in herring and a decrease thereafter.
In plaice there is a decrease in the LD_{50} levels with time
but the LD_{50} levels are not very significantly different
in the young larvae when compared to the later stages.
The survival time below the LD_{50} in the later stages was
much lower than in the earlier developmental stages in
both species. This appears to be a contradiction but can
be explained on the basis of the difference in size at
these stages. In the early developmental stages larvae
are much smaller and their oxygen requirements are small
and therefore they will be able to survive for a longer
time below the median lethal dose. In the older larvae
the oxygen requirements will be greater resulting in a
shorter survival time below the LD_{50} level.

I.4. Discussion.

The results show a considerable degree of tolerance
to low oxygen especially in herring. It is interesting

Fig.6. Time mortality curves for plaice at 2-3 weeks of feeding exposed to various dosages of lethal oxygen concentration. Mortality is expressed as cumulative % dying and as a probit. Broken line indicates incomplete mortality.

Oxygen concentration is given in ml/l.

Table 1. *Median resistance times* (LT_{50}) *and extent of mortality in herring larvae exposed to various low oxygen levels.*

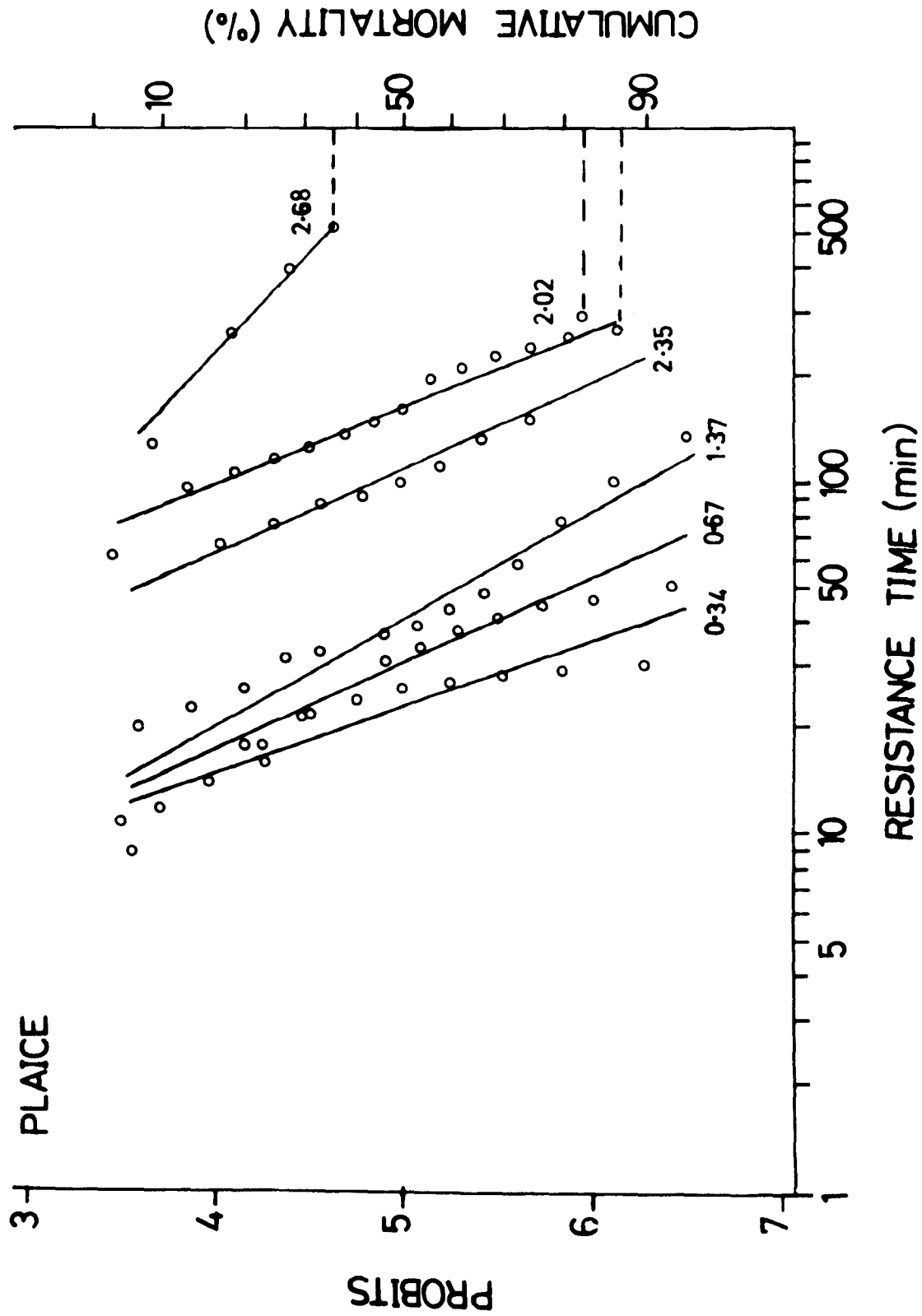| Stage	Oxygen conc ml/l	LT_{50} (mins)	Slope	Number tested	Number of individuals dead	Temp. ($^{\circ}$C)
Yolk-sac	.06	12	1.39	20	20	8.3
	.55	30	1.97	14	14	8.3
	.76	90	2.05	10	10	8.2
	1.38	155	2.14	14	14	8.0
	1.65	285	-	13	9	8.0
	2.07	-	-	19	7	8.2
2-3 weeks feeding	.06	7.6	1.93	31	31	8.5
	.66	56	1.47	32	32	8.4
	1.47	345	1.32	13	11	8.5
	2.06	430	-	11	9	8.6
	3.45	-	-	11	4	8.4
	4.09	-	-	11	3	8.5
5-6 weeks feeding	.67	6.7	1.98	13	13	8.8
	1.36	20	1.34	30	30	8.9
	2.02	27.5	1.90	12	12	8.8
	2.68	175	1.94	12	12	9.0
	3.39	380	-	11	7	8.9
	3.70	-	-	10	4	9.1
	4.04	-	-	12	3	9.4
8-9 weeks feeding	1.28	8	1.29	14	14	9.9
	1.50	24	1.20	11	11	10.1
	1.93	84	1.83	11	10	10.3
	2.47	228	2.15	29	21	10.1
	3.27	-	-	12	4	10.5
	3.93	-	-	12	2	10.3
Metamorphosed	1.27	48	1.44	11	11	10.5
	1.51	74	1.87	8	8	11.2
	1.94	135	-	10	8	10.7
	2.23	-	-	11	5	11.3
	2.58	-	-	10	1	11.4

Table 2.   Median resistance times (LT_{50}) and extent of mortality in plaice larvae exposed to various low oxygen levels.

Stage	Oxygen conc ml/l	LT_{50} (mins)	Slope	Number tested	Number of individuals dead	Temp. (°C)
Yolk-sac	0.60	27	1.95	12	12	8.5
	1.37	61	1.73	16	16	8.2
	2.05	114	1.63	12	11	8.4
	2.44	182	-	12	9	8.4
	2.71	270	-	11	7	8.5
2-3 weeks feeding	.34	23	1.53	11	11	8.5
	.67	30	1.72	13	13	8.8
	1.36	41	2.00	15	15	8.8
	2.02	160	1.62	16	15	8.9
	2.35	110	1.74	12	9	8.9
	2.68	-	-	11	4	8.8
Stage 4-4b	.64	14	1.29	12	12	9.7
	1.68	52	2.0	12	12	9.4
	2.22	90	2.34	13	12	9.5
	2.55	-	-	12	3	9.6
	2.85	-	-	12	2	9.6
3-4 weeks after metamorphosis	1.06	40	1.72	12	12	11.3
	1.53	135	2.50	12	10	11.2
	1.60	605	-	12	6	11.5
	2.00	-	-	12	2	11.7
	2.30	-	-	12	3	11.5

Table 3. Incipient lethal levels (LD_{50}) of herring and plaice larvae.

Herring

Stage	Age (days)	LD_{50} (ml/l)	.95 C.L.
Yolk-sac	1-8	1.93	$1.69 \ll LD_{50} \ll 2.20$
2-3 weeks feeding	21-28	3.08	$2.52 \ll LD_{50} \ll 3.75$
5-6 weeks feeding	42-49	3.57	$3.35 \ll LD_{50} \ll 3.80$
7-8 weeks feeding	56-63	2.91	$2.64 \ll LD_{50} \ll 3.19$
metamorphosed	70-80	2.17	$2.03 \ll LD_{50} \ll 2.32$

Plaice

Stage	Age (days)	LD_{50} (ml/l)	.95 C.L.
Yolk-sac	1-8	2.73	$2.53 \ll LD_{50} \ll 2.95$
2-3 weeks feeding	21-28	2.66	$2.43 \ll LD_{50} \ll 2.91$
stage 4-4b'	42-49	2.52	$2.35 \ll LD_{50} \ll 2.71$
metamorphosed 3-4 weeks	77-84	1.69	$1.60 \ll LD_{50} \ll 1.80$

that the incipient lethal level or LD_{50} changes with
development in both herring and plaice. The herring
are more resistant than plaice at the yolk-sac stage.
At 2-3 wks of feeding in herring the resistance appears
to have decreased while in plaice there is little
change. A further decrease was observed in herring
larvae at 5-6 wks of feeding. At the pre-metamorphic
stage in herring the resistance increased slightly and
again after metamorphosis. The same pattern was seen
in the pre-metamorphic (stage 4) and metamorphosed
plaice.

Bishai working on Atlantic herring obtained a LD_{50}
value between 2.23 - 2.67 mg/l for an experimental period
of 12h for newly hatched larvae comparable with 2.75 mg/l
in the present study. Saksena et al. (1972) obtained LD_{50}
values of 2.50 mg/l for striped blenny and lower values
for the naked goby and skillet fish, which they related
to the larger size at hatching of the blenny. All three
species lay demersal eggs. Holeton (1971a) observes that
trout larvae show a bradycardia only below an oxygen level
reported lethal by Bishai, (1960) and Silver, (1963) for
some salmonids. The low resistance of the plaice larvae
at hatching when compared to that of herring (2.73 ml/l
and 1.93 ml/l respectively) could possibly be related to
the modes of spawning of the two species, the former

laying pelagic and the latter demersal eggs. Thus there is a greater chance of yolk-sac herring larvae finding themselves in water of low oxygen concentration in and around the layers of eggs on the spawning ground.

The decrease in the survival time (LT_{50}) below the LD_{50} levels in the older larvae is associated with the increased oxygen requirements at these stages. The changes in LD_{50} with age in both species could be explained on the basis of development of the branchial apparatus. In herring the secondary lamellae appear on the gill filaments at about the 20mm stage (Harder, 1954; see Part four of this study) corresponding to about 5 weeks of feeding. In plaice it appears at about 4 weeks of feeding (stage 3). There is a rapid growth of the gills of both species up to metamorphosis, after which it slows down. The increased resistance after metamorphosis could be explained in terms of the development of respiratory pigment at this stage (see part five of this study).

PART TWO.   OXYGEN UPTAKE AT NORMAL OXYGEN CONCENTRATION

II.1. Introduction

The respiratory rates of demersal and planktonic eggs and larvae of marine fish give an assessment of oxygen requirements. Yet measurements of oxygen uptake of planktonic fish eggs and larvae have been confined to very few species e.g. the plaice (Burfield, 1928). Lasker & Theilacker (1962) measured oxygen consumption in relation to salinity changes in the embryo of the Pacific sardine (Sardinops caerulea), while Alderdice & Forrester (1968) studied oxygen uptake in eggs of the English sole (Parophrys vetulus). Demersal fish eggs and larvae have been studied by a few workers such as Hymen (1921) and Amberson & Armstrong (1933) on Fundulus heteroclitus. Of particular interest to the study are the measurements of oxygen uptake of herring larvae made by Marshall, Nicholls & Orr (1937) who compared the uptake in relation to light and darkness. Holliday, Blaxter & Lasker (1964) compared O_2 uptake in relation to rearing salinity to test the effects of osmotic stress. More recently Stelzer, Rosenthal & Siebers (1971) measured the effect of dinitrophenol (inhibitor of oxidative phosphorylation) on the oxygen uptake of the herring embryo.

A number of observations have been made on freshwater species. Nakano (1953 cited by Blaxter, 1969) worked on oxygen uptake during maturation and fertilization in Oryzias latipes. Ivlev (1960) studied metabolic intensity in salmon fry (Salmo salar), while Hayes, Wilmot and Livingstone (1951) studied the uptake of salmon eggs.

Oxygen consumption measurements have been used almost universally to determine the metabolic rate of fish. Nevertheless, Fry (1971) says that "it cannot be assumed that all fish are obligate aerobes and that a measure of oxygen consumption is always a measure of the metabolic rate". Fry (1957 in Brown) distinguished between three levels of oxygen consumption.

(1) Standard

(2) Routine

(3) Active

Standard oxygen consumption is the oxygen consumption in the post absorptive state and when exhibiting zero locomotor activity.

Routine oxygen consumption is the rate of oxygen consumption of an organism when exhibiting natural spontaneous locomotor activity.

Active oxygen consumption is the rate of oxygen consumption at maximum locomotor activity.

In this study measurements of routine uptake was made for both species of larvae.

II.2. Methods

The oxygen uptake of both species was measured in a Gilson differential respirometer at $10^{\circ}C$ ($\pm$ $0.1^{\circ}C$). A temperature of $10^{\circ}C$ was chosen as this seemed to be the average temperature experienced by the larvae from hatching to metamorphosis. The larvae were reared at the experimental temperature of $10^{\circ}C$ in a constant temperature room. The temperature never varied by more than $\pm$ $0.2^{\circ}C$.

In the early stages flasks of 16 ml capacity each containing 10 larvae in 5 ml of water were used. The number was decreased as the larvae grew older. An equilibration time of 1 hour was allowed prior to the start of the experiment. Thermoblanks were run with each experiment to correct for ambient temperature changes (Carver & Gloyne, 1971). Flasks of 85 ml capacity containing 30 ml of water were used for the larger herring larvae and for the metamorphosed plaice, flasks of 25 ml capacity with 7 ml of water. Readings were taken hourly for a maximum period of 5 h. The larvae were transferred to filtered (Whatman glass filters GF/C) seawater overnight to defaecate. A second transfer was made before final

transfer to the experimental vessels. Uptake over the 5 h period was fairly uniform (Figs 7A & B), at all the stages, except for the pre-metamorphic herring where the experiments were terminated after 1 or 2 readings, the larvae being very active at these stages. In fact it was necessary to anaesthetize the larvae before transfer to the experimental vessel, readings being taken after recovery from the anaesthetic.

In an attempt to obtain a measure of the basal metabolism the larvae were placed in an anaesthetic solution (1:20,000 MS 222 SANDOZ). The concentration was varied with age, the level of anaesthesia produced corresponding approximately to stage 1 in the classification proposed by McFarland (1960). Routine metabolism (Fry, 1957) was obtained from unanaesthetized larvae in flasks which were only shaken during a brief equilibration period at the end of each hourly interval.

Many authors (Fry, 1957; Winberg, 1960; Brett, 1962; Paloheimo & Dickie, 1966) have drawn attention to the need for homogeneity of material in any measurements of oxygen uptake in fish. For herring, all the larvae used were from one female even though egg size and larval size from one female may vary by a factor of 1.5 (Blaxter & Hempel, 1963). Larvae from two different stocks were used in the case of plaice, due to the lack of large

Fig.7. Uptake of oxygen over a 5 h period of unanaesthetized

larvae at the yolk-sac stage. Each flask is denoted

by a different symbol. Each point represents the

average value for a larva for that particular flask.

A. Herring

B. Plaice
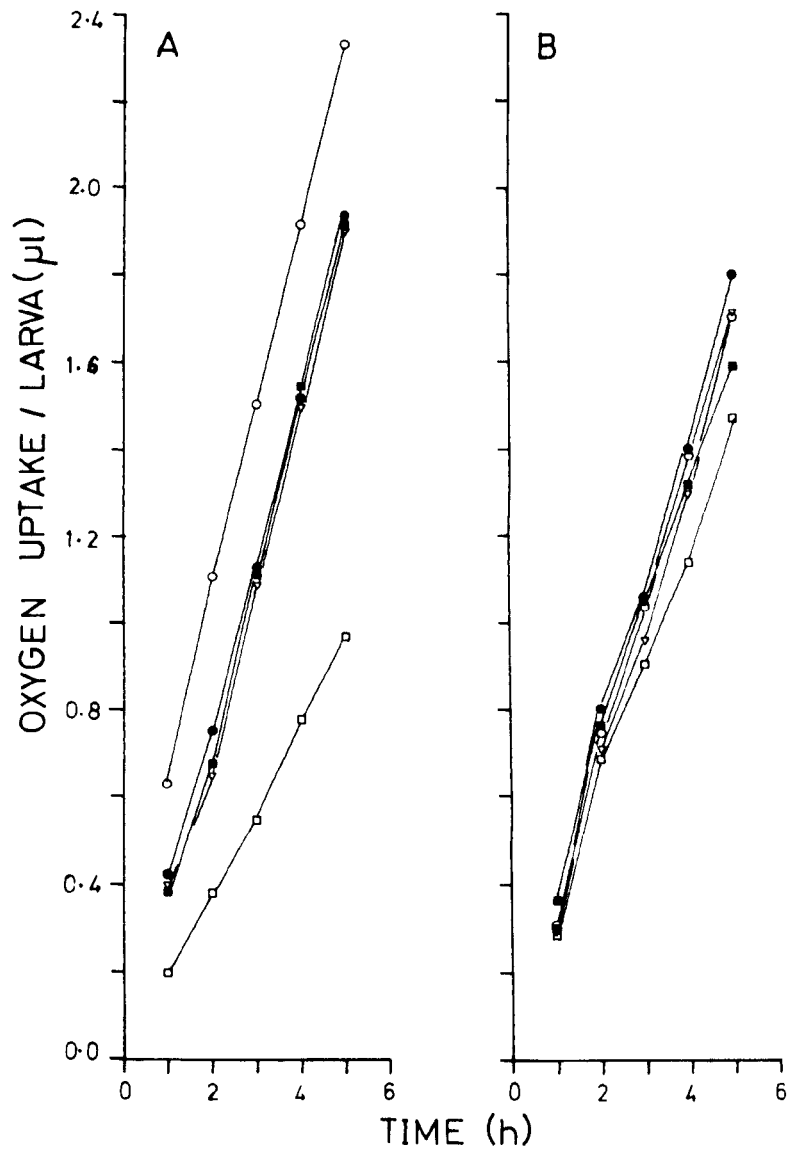
numbers of larvae from one stock. Larvae were matched for size in the experiments.

At the end of the experiments the organisms were weighed after drying to constant weight in an oven at $60^{\circ}C$.

In the early eggs the chorion only was removed, and therefore the dry weights included both embryos and yolk. In later eggs and in yolk-sac larvae, the yolk was dissected out after fixation of the larva in formalin. The larval bodies were then washed in distilled water and dried to constant weight. Weighing of eggs and small larvae were made on a Beckman Microbalance Model EMB1 and larger larvae on a Mettler Balance Model H10/H20. Thus the QO_2 (ulO_2/mg dry wt/h) could be calculated based on actively respiring tissue except in the case of early stage eggs.

II.3. Results

The combined results obtained from eggs, unanaesthetized and anaesthetized larvae of herring are shown in Fig.8. An increase in uptake per larva was observed with development, there being an increase in the slope of the relationship after the commencement of feeding. Variability also

Fig.8. Oxygen uptake of eggs and larvae of herring with
age at $10^{O}C$. Upper line, unanaesthetized larvae;
lower line, anaesthetized larvae. Vertical lines
indicate $\pm$ 2 S.E. Larvae started feeding between
5 and 10 days post hatching. Egg dry weight includes
yolk.
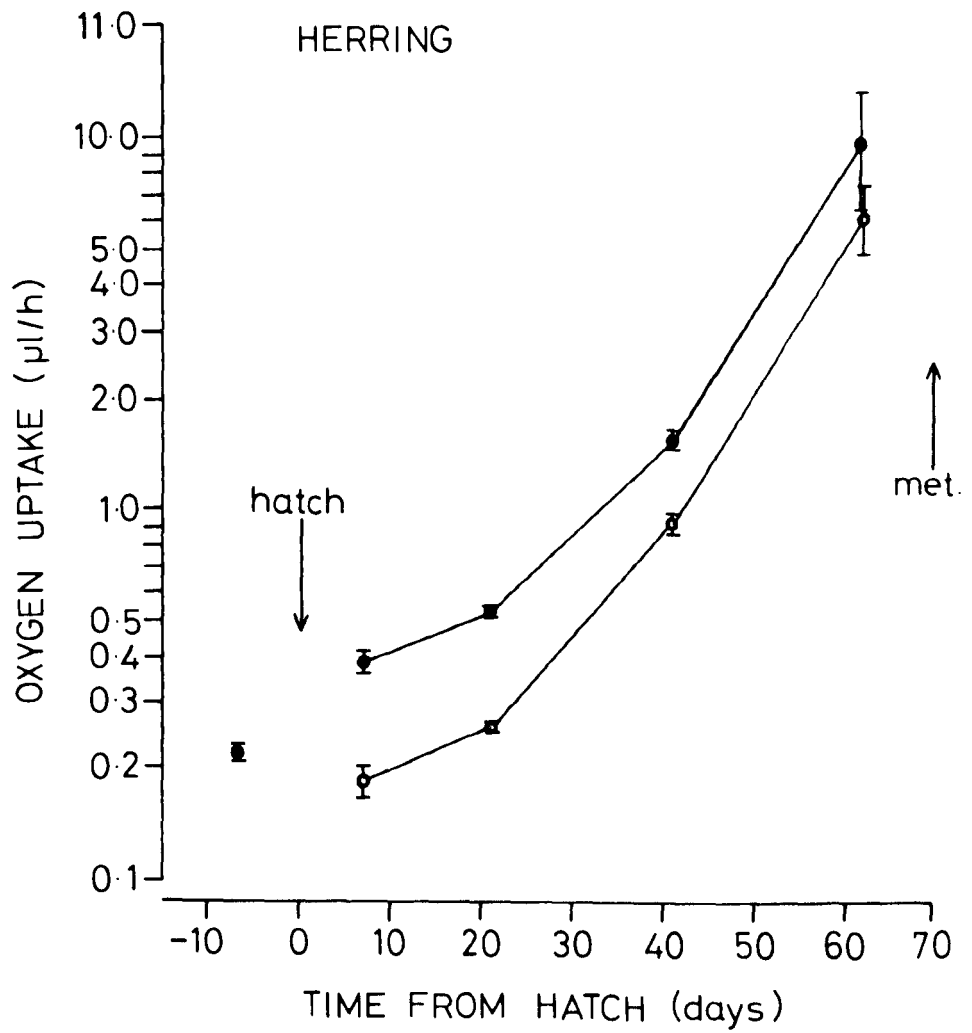
increased as development proceeded causing an overlap
in the ranges for anaesthetized and unanaesthetized
larvae. The differences between each group is highly
significant (Table 4).

The uptake for eggs and larvae in plaice is shown
in Fig. 9 and Table 5. As expected uptake/larva increased
as development proceeded, the variability being less
marked than in herring. The means are highly significant
(at the 0.1% level) in unanaesthetized larvae. In both
species the basal rate of metabolism (as judged by
comparisons between anaesthetized and unanaesthetized
larvae) was about half the routine metabolic rate.

The oxygen uptake results converted to QO_2 are
shown in Figs 10 & 11 and Tables 6 & 7. QO_2 generally
falls during development with a tendency to stabilize
after metamorphosis in plaice. Although not shown in
figure 9, there is a great increase in QO_2 between
unanaesthetized early and late yolk-sac plaice larvae
having mean values of 3.772 and 4.704 respectively
(df = 60, Variance ratio = 12.575, $(0.005 < p < 0.0005)$.
This is probably due to increased searching for food
at the end of the yolk-sac stage. Differences in
uptake between newly metamorphosed and older plaice

Table 4.

Changes in uptake per larva with development in herring

Unanaesthetized.

Stage	Age (days)	Mean uptake (µl)	N	S.E.	Degrees of freedom	Variance Ratio	Significance
Eggs	8½	.2211	70	.0036			
					88	272.94	**
Yolk sac	7	.3937	20	0.016			
					47	56.865	**
II weeks fed	21	.5270	29	0.0099			
					60	560.99	**
V weeks fed	41	1.5532	33	.0409			
					59	25.916	**
Premetamorphic	62	9.6093	28	1.7199			

Anaesthetized.

Stage	Age (days)	Mean uptake (µl)	N	S.E.	Degrees of freedom	Variance Ratio	Significance
Yolk-sac	7	0.1842	24	.0106			
					42	32.65	**
II weeks fed	21	0.2555	20	.0054			
					41	317.49	**
V weeks fed	41	0.9111	23	.033			
					29	77.586	**
Premetamorphic	62	6.1168	6	1.3128			

** 1% level

Fig.9. Oxygen uptake of eggs and larvae of the plaice
        with age at 10°C. Upper line, unanaesthetized
        larvae; lower line, anaesthetized larvae. Vertical
        lines indicate $\pm$ 2 S.E. Larvae started to feed at
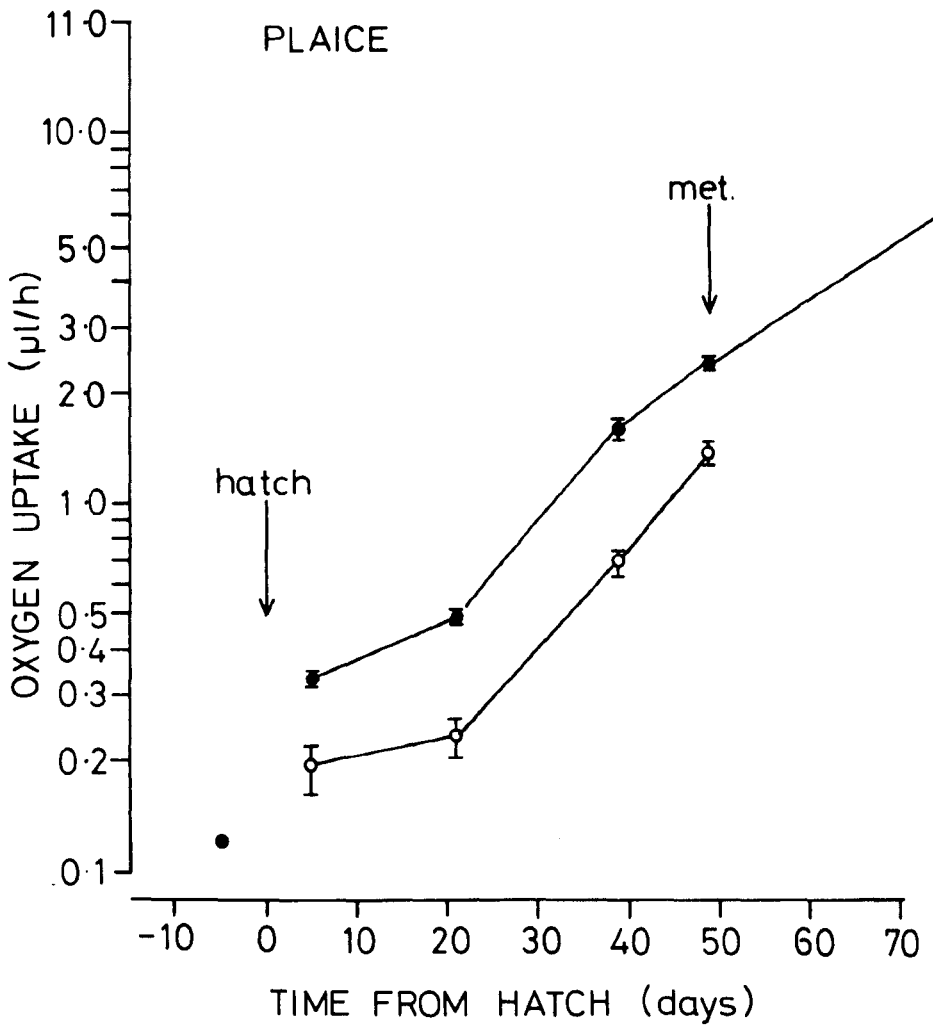   about 8 days post hatching.

Table 5.

Changes in uptake per larva with development in plaice

Unanaesthetized.

Stage	Age (days)	Mean uptake (µl)	N	S.E.	Degrees of freedom	Variance Ratio	Significance
Eggs	10	0.1217	29	0.0021			
					89	528.798	**
Yolk-sac	5	0.3395	62	0.0055			
					94	207.48	**
Two weeks fed - Stage 2	21	0.4920	34	0.0087			
					97	254.05	**
Stage 3	39	1.6236	65	0.0507		86.297	**
					116		
Stage V	49	2.3945	53	0.0676		76.509	**
					90		
Metamorphic	75	6.0183	39	0.4841			

Anaesthetized.

Stage	Age (days)	Mean uptake (µl)	N	S.E.	Degrees of freedom	Variance Ratio	Significance
Yolk-sac	5	0.1954	19	.0152			
					32	5.2916	*
Two weeks fed - stage 2	21	.2344	15	.0149			
					37	107.399	**
Stage 3	39	.6906	24	.0335			
					42	173.409	**
Stage 5	49	1.3731	20	.0400			

*   5% level

**   1% level

Fig.10. Change of QO_2 with age in herring. Mean $\pm$ 2 S.E.

for each stage. Upper line, unanaesthetized larvae;
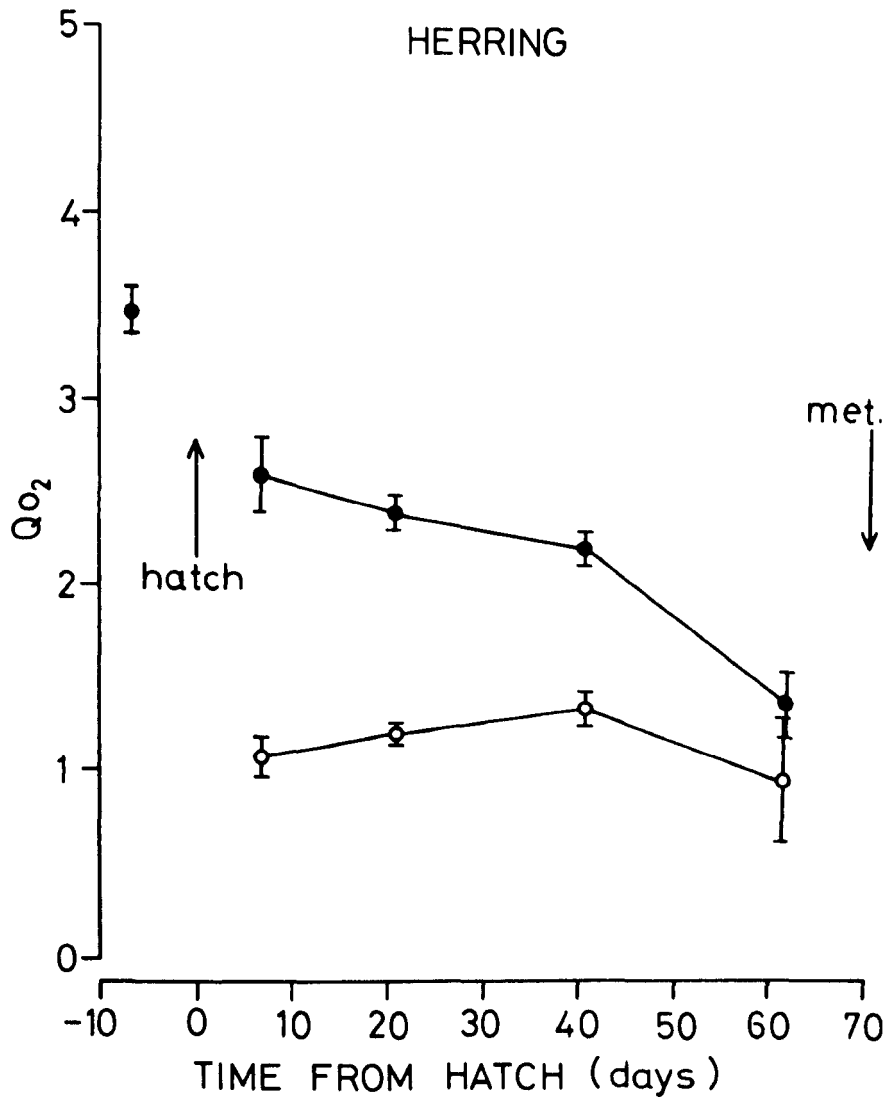
lower line, anaesthetized larvae.

Fig.11. Change of QO_2 with age in plaice. Mean $\pm$ 2 S.E.

for each stage. Upper line, unanaesthetized larvae;

lower line, anaesthetized larvae.

PLAICE
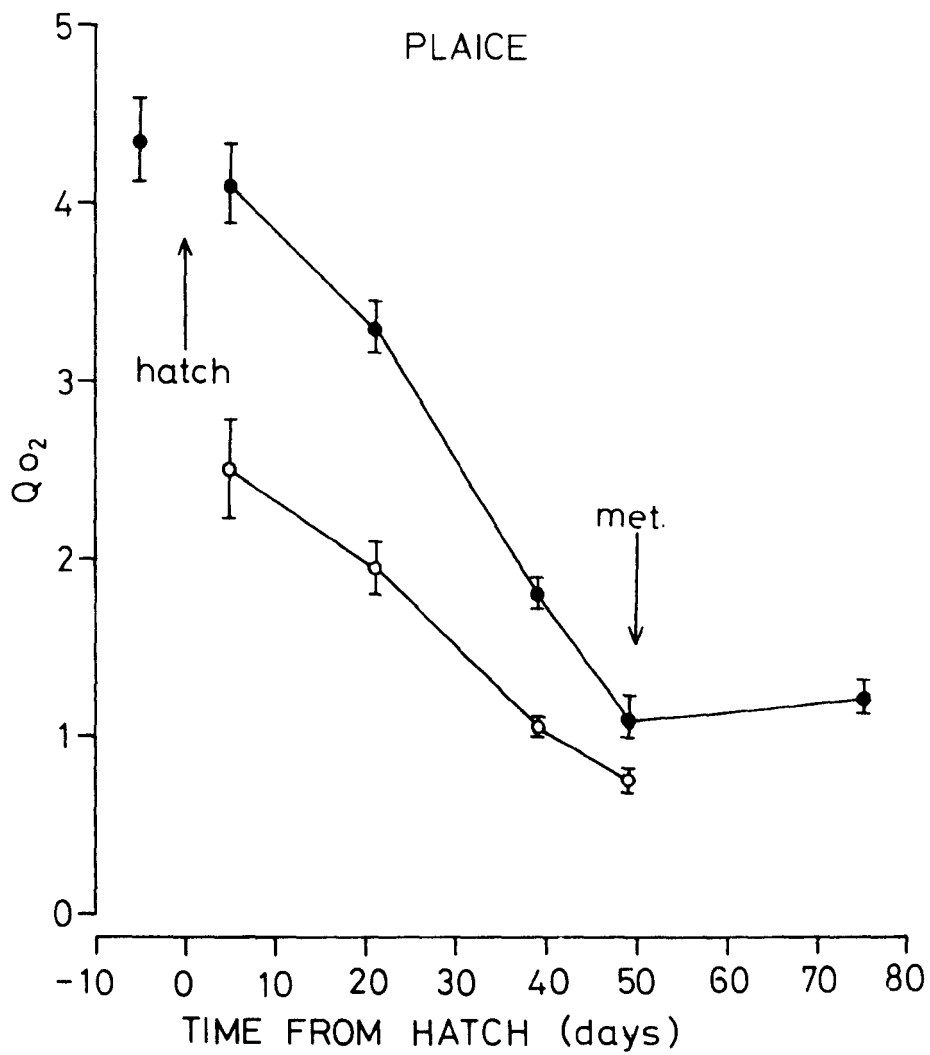
Table 6.   Change in QO_2 with development in herring.

Stage	Age (days)	Mean	N	S.E.	D.F.	V.Ratio	Signif.	Mean	N	S.E.	D.F.	V.Ratio	Signif.
						Unanaesthetized					Anaesthetized		
Eggs (-chorion)	8½	3.477	70	.066				–	–	–	–		
					88	41.344	**						
Yolk-sac	7	2.617	20	.100				1.085	24	.056			
					47	4.587	*				42	3.211	n.s.
2 weeks fed	21	2.394	29	.052				1.203	20	.026			
					60	8.181	**				41	5.092	*
5 weeks fed	41	2.211	33	.039				1.329	23	.047			
					59	79.988	**				27	8.988	**
Premetamorphic	62	1.364	28	.092				0.946	6	.178			

n.s. not significant

*   5%

**   highly significant

Table 7. Change in QO_2 with development in plaice.

Stage	Age (days)	Unanaesthetized						Anaesthetized					
		Mean	N	S.E.	D.F.	V.Ratio	Signif.	Mean	N	S.E.	D.F.	V.Ratio	Signif.
Eggs (-yolk & chorion)	10	4.33	25	.012				–	–	–			
					89	1.989	n.s.						
Yolk-sac	5	4.118	62	.120				2.520	19	.146			
					94	30.262	**				32	9.986	**
2 weeks fed	21	3.308	34	.081				1.952	15	.083			
					97	306.605	**				37	142.00	**
Stage 3	39	1.815	65	.045				1.048	24	.031			
					116	185.967	**				42	40.097	**
Stage 5-just metamorphosed	49	1.119	53	.013				0.746	20	.037			
					90	5.214	*						
Metamorphosed	75	1.227	39	.053									

n.s.   not significant

*   5%

**   highly significant

were significant only at the 5% level.

The relationship between uptake/animal/h and QO_2 with body weight for herring and plaice plotted on log-log coordinates are shown in Figs 12, 14 and 13 & 15. respectively. The equations and standard deviation of the slopes of the regression lines for both species expressed both as QO_2 and oxygen uptake/larva/h are given in Table 8. The differences between the slopes of the lines for anaesthetized and unanaesthetized herring are highly significant; this is not so for plaice larvae.

A limited number of experiments were done on herring larvae at the yolk-sac stage over a period of 24h. The experiment was made under indoor daylight conditions. Oxygen uptake varied greatly but there was a significant decrease at dusk (Fig.16) in unanaesthetized larvae when compared to the daylight readings. After dusk the oxygen uptake tended to increase. The minimum uptake for unanaesthetized larvae (at dusk) was not significantly different from anaesthetized larvae, suggesting that the larvae were metabolising at a basal level at dusk.

II.4. Discussion

Eggs of herring showed higher rates for QO_2 compared to those reported by Holliday et al. (1964). Stelzer et al.

Fig.12. Uptake per larva related to body weight in

herring. Lines of best fit for the oxygen

uptake of unanaesthetized (upper line) and

anaesthetized (lower line) larvae at $10^{O}C$.

Vertical lines at the ends and centre indicate

95% confidence limits.
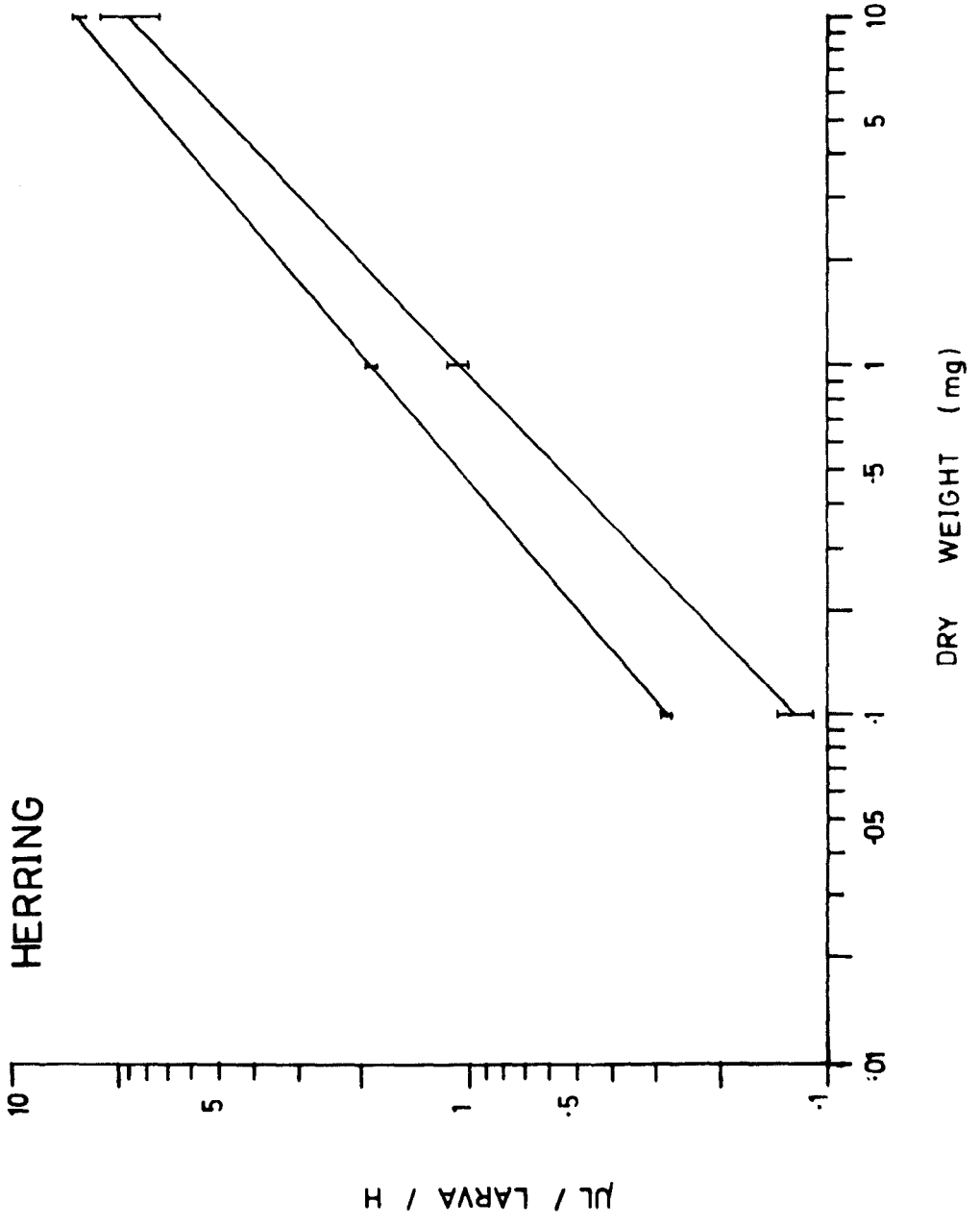
HERRING

DRY WEIGHT (mg)

μJ / LARVA / H

Fig.13. Uptake per larva related to body weight in plaice.
Lines of best fit for the oxygen uptake of
unanaesthetized (upper line) and anaesthetized
(lower line) larvae at $10^{o}C$. Vertical lines at
the ends and centre indicate 95% confidence limits.

PLAICE

μl / LARVA / H
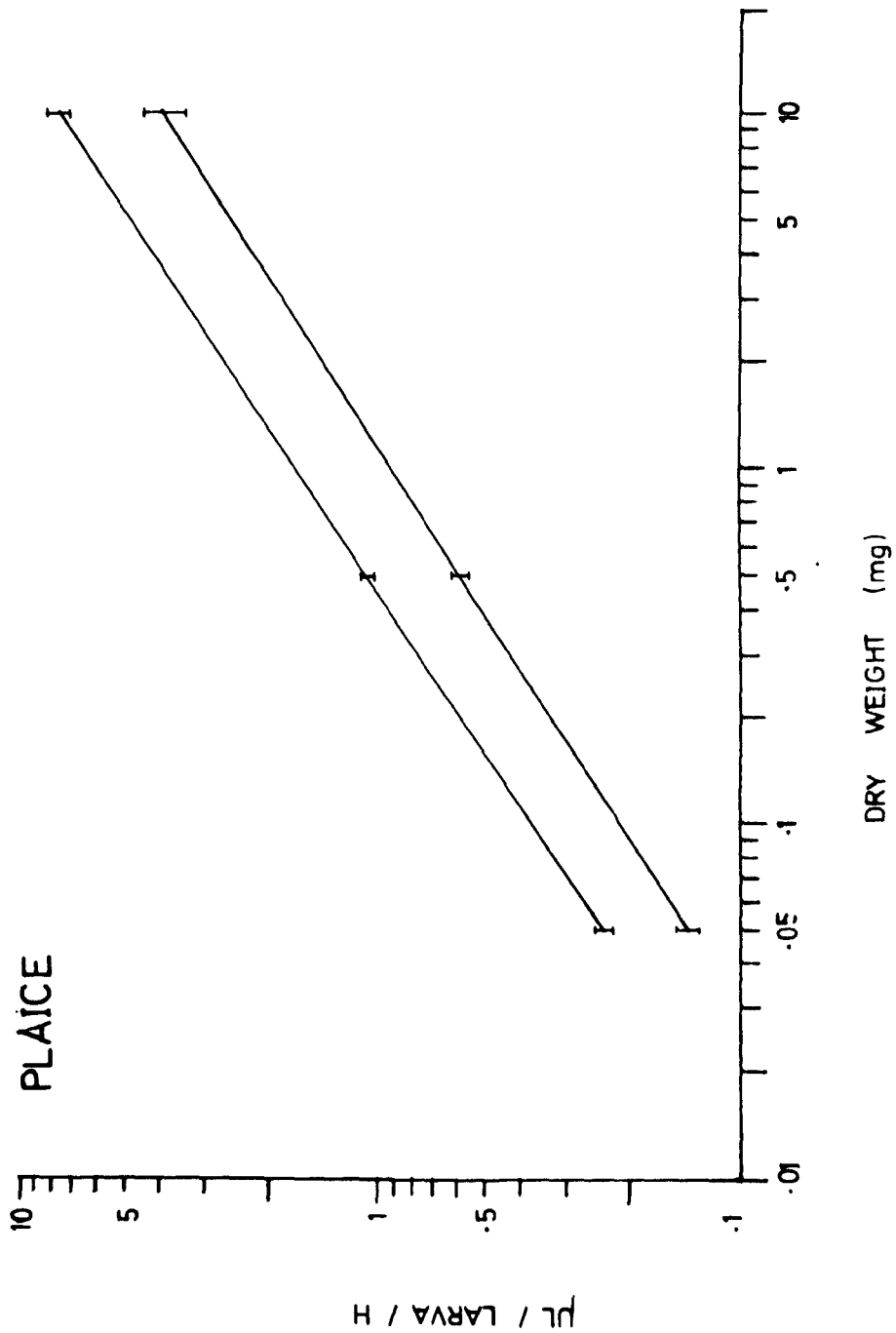
DRY WEIGHT (mg)

Fig.14. QO_2 related to body weight in herring. Lines
of best fit for the oxygen uptake of unanaesthetized
(upper line) and anaesthetized (lower line) larvae
at $10^{O}C$. Vertical lines at the ends and centre
indicate 95% confidence limits. n = number of
observations.

HERRING

n=110

n=73

Q

10.0
5.0
2.0
1.0
0.5
0.1

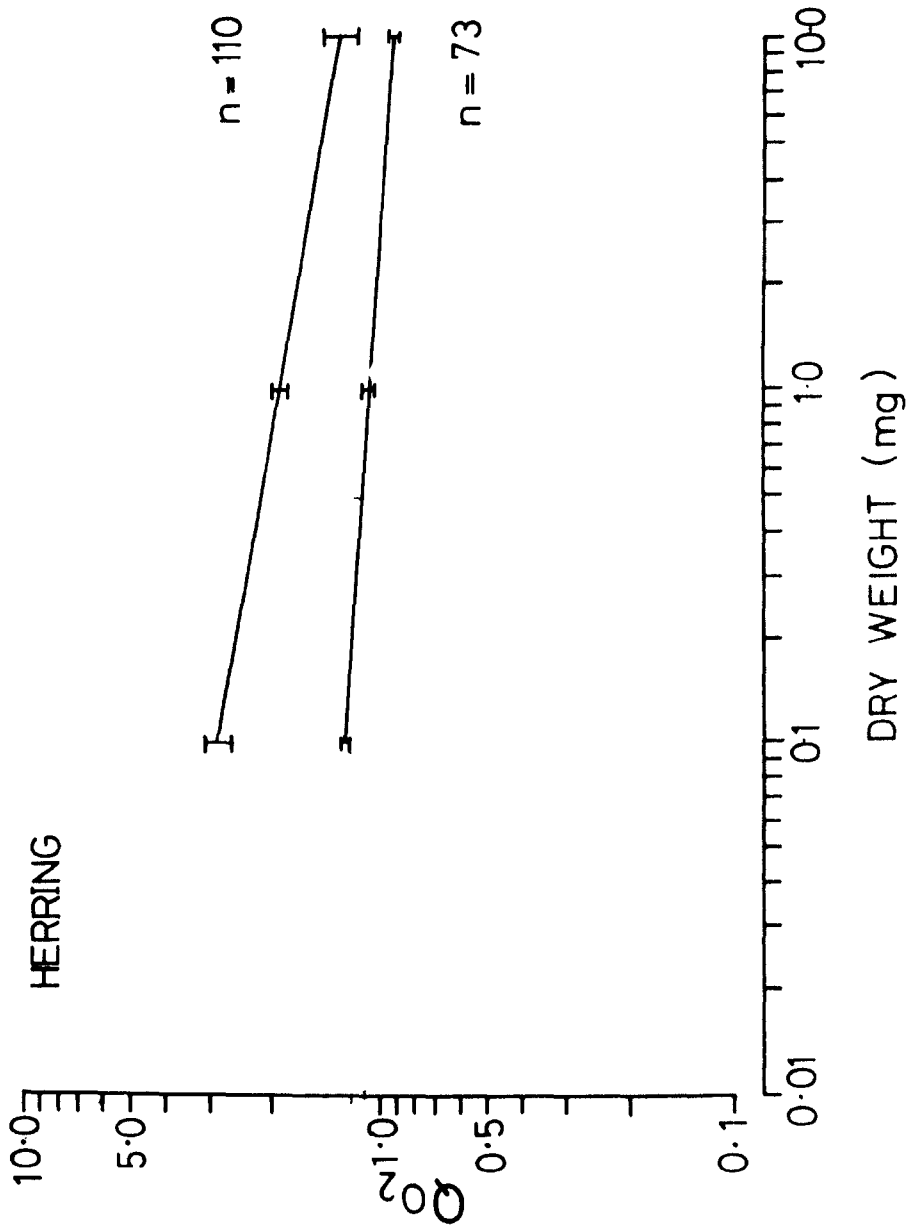0.01   0.1   1.0   100

DRY WEIGHT (mg)

Fig.1.5. QO_2 related to body weight in plaice. Lines
of best fit for the oxygen uptake of unanaesthetized
(upper line) and anaesthetized (lower line) larvae
at 10^OC. Vertical lines at the ends and centre
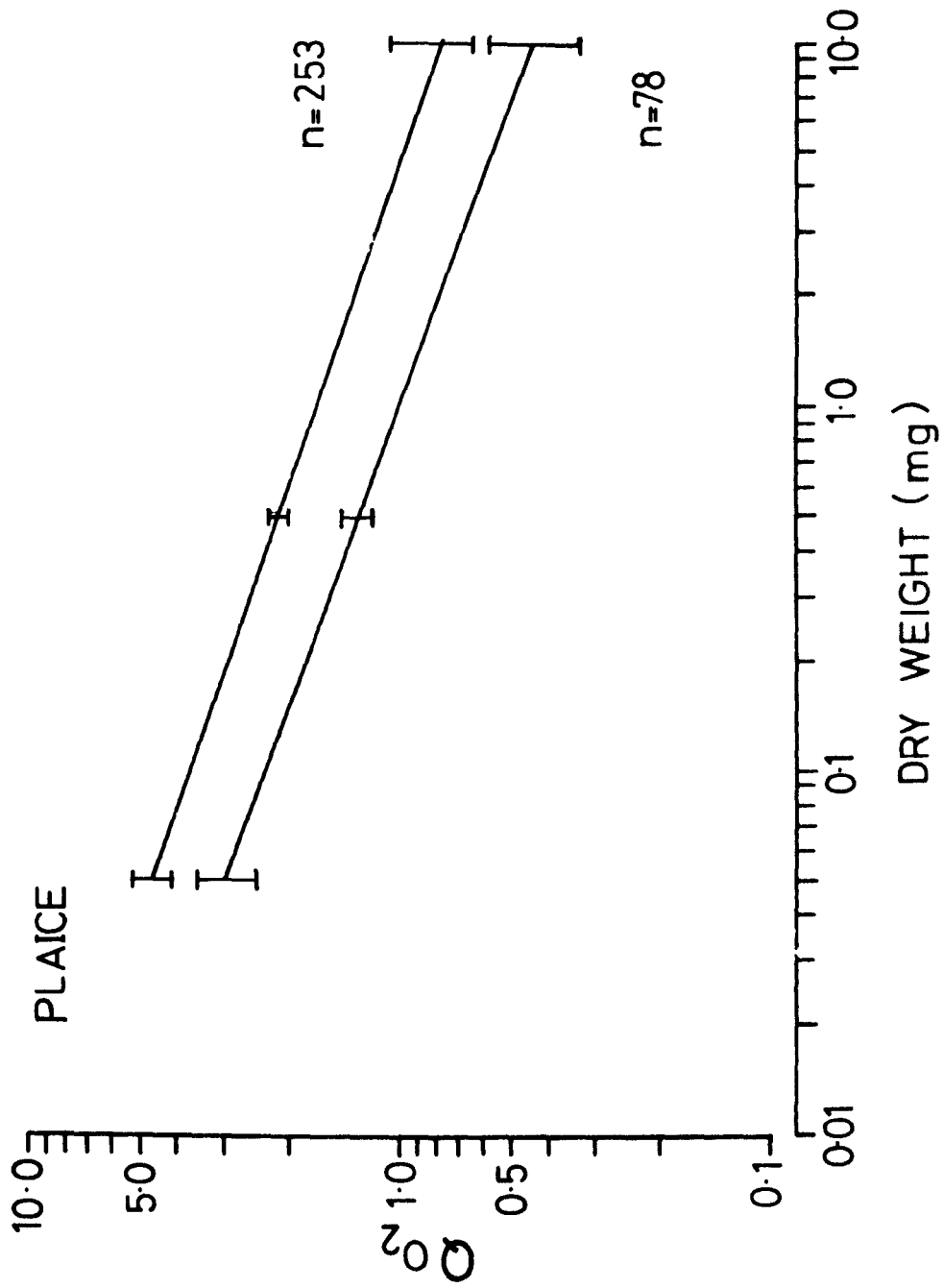indicate 95% confidence limits. n = number of
observations.

Table 8. Equations for the relation between oxygen uptake, QO_2 and weight in herring and plaice at $10^{O}C$.

		Herring	S.D. of Slope	N
$\mu lO_2/larva/h$	Unanaesthetized	Log Y = 1.880 + .822 Log X	.066	110
	Anaesthetized	Log Y = 1.063 + .934 Log X	.031	73
QO_2	Unanaesthetized	Log Y = 1.880 - .177 Log X	.013	110
	Anaethetized	Log Y = 1.063 - .065 Log X	.031	73

		Plaice	S.D. of Slope	N
$\mu lO_2/larva/h$	Unanaesthetized	Log Y = 1.67 + .65 Log X	.009	253
	Anaesthetized	Log Y = 1.01 + .63 Log X	.022	78
QO_2	Unanaesthetized	Log Y = 1.67 - .34 Log X	.008	253
	Anaesthetized	Log X = 1.01 - .36 Log X	.016	78

Fig.16. Effect of time of day on the oxygen uptake of
herring larvae 10 days old, at 10°C. ●, un-
anaesthetized, O anaesthetized. Arrows
correspond to sunrise and sunset.
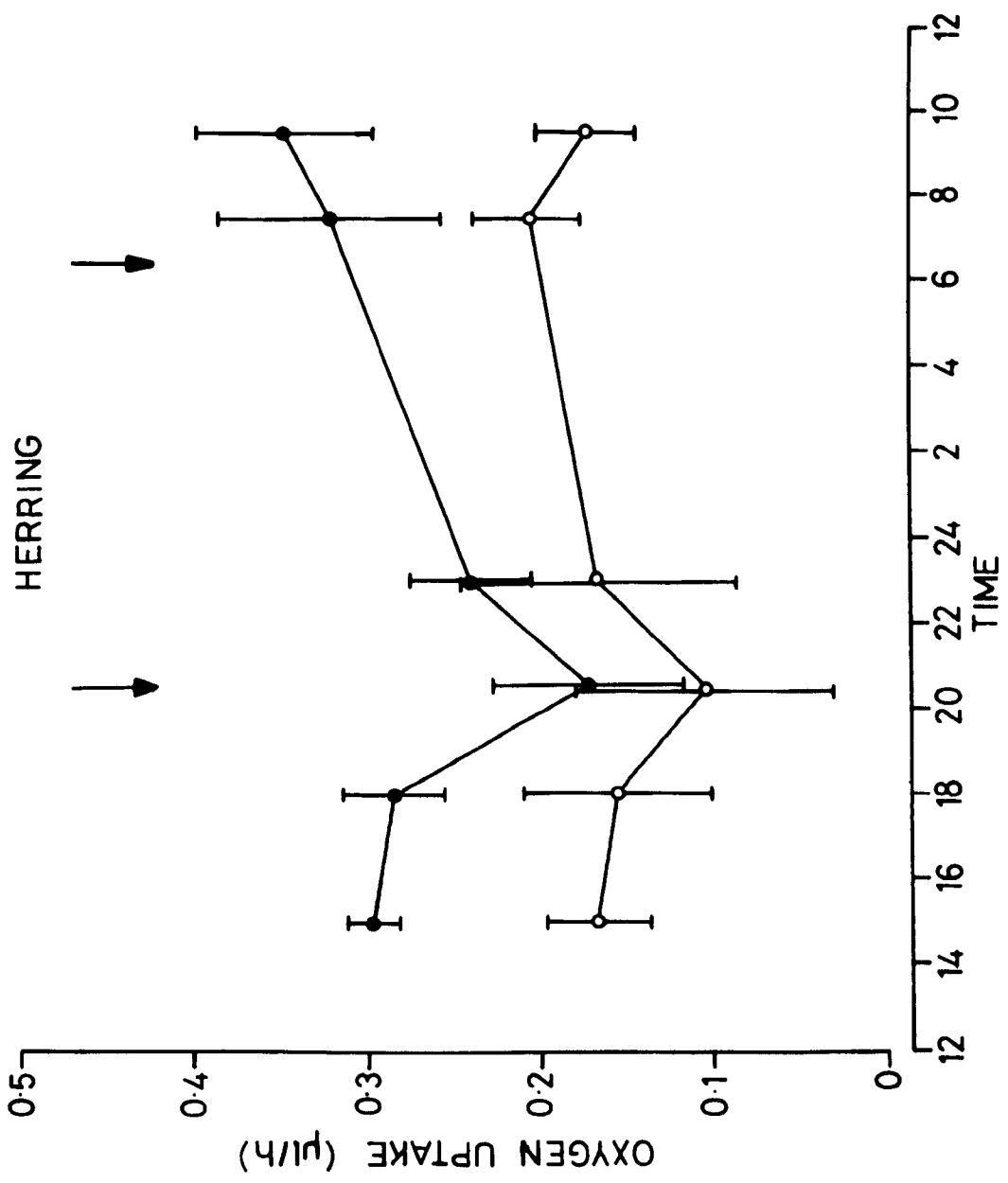
(1971) found that herring embryos showed respiratory bursts. Such bursts giving temporarily high QO_2's in the egg could also explain the disparity between egg and yolk-sac larval QO_2's.

The oxygen uptake of plaice eggs at 10 days post fertilization was about 0.12 µl/egg/h. Burfield's (1928) data recalculated give a value of 0.89 ul/egg/h for plaice eggs of unspecified age, which seems remarkably high, but his measurements were made on groups of 2000 eggs. Lasker et al. (1962) obtained values of 0.07 µl/egg/h for a 70 h sardine embryo. The equivalent QO_2 values obtained by these authors were low compared to the present data due to their expression per total weight of the egg. Alderdice et al. (1968) obtained a QO_2 of 0.56 mg/g wet weight for sole eggs. These data when recalculated assuming dry weight of the egg is approximately 8% wet weight (for plaice, Erhlich, 1972) give a value of 4.7 µl/mg dry wt/h which agrees with the present value of 4.35 (see Fig.9). Blaxter (1969) recalculating the data of Hayes et al. (1951) obtained a value of 0.9 for the QO_2 of a salmon embryo.

The values of QO_2 of unanaesthetized herring larvae in the present experiment overlap into the range obtained by Holliday et al. (1964) for anaesthetized larvae. This

could be due to a difference in the level of anaesthesia in the experiments or to lack of continuous shaking. Generally unanaesthetized larvae took up oxygen at about twice the rate of anaesthetized larvae. This may be compared with the data of Lasker et al. (1962) who found that swimming increased oxygen uptake up to 3 times the quiescent value, while Holliday et al. (1964) obtained active values of up to 10 times the resting value. Ivlev (1960) stated that oxygen uptake of Salmo salar could increase up to 14 times during bursts of activity. He obtained QO_2 values of 1.6 - 2.0 for salmon fry and Lasker et al. a value of 1.33 for sardine larvae. Plaice larvae at the end of the yolk-sac stage showed an expected higher uptake/larva, compared to the early yolk-sac stage. QO_2's were also higher, being significantly different at the 1% level. This is in agreement with Lasker et al. (1962) who found that swimming activity increased as the yolk-sac was consumed and the eyes pigmented in sardine larvae.

The QO_2's of the egg of both herring and plaice when compared to that of the yolk-sac larvae were remarkably high. This is unusual for Volodin (1956) and Fry (1957) suggested that the presence of the chorion might inhibit gaseous exchange. Nevertheless they could

be explained on the basis of the observations of Stelzer

et al. (1971) of respiratory bursts. Table 9 gives a

comparison of the present data with values obtained by

other authors for different species. Devillers (1965)

suggests that increases in oxygen uptake with age in

eggs may be expressed by the exponential equation

$$Q = ae^{kt}$$

when t is time and a and k are constants.

The differences in the slopes shown in Table 8 of

0.82 and .93 respectively for unanaesthetized and

anaesthetized herring larvae during development suggest

a relative decrease in routine metabolism at least up

to the pre-metamorphic stage. The differences in the

values of 'a' for unanaesthetized and anaesthetized

herring of 1.88 and 1.06 suggest a higher routine and

basal rate of metabolism when compared to the values

of 1.67 and 1.01 for plaice. Winberg (1960), reviewing

the earlier literature, concluded that in general for

resting metabolism the equation

$$QO_2 = 0.3 \ W^{0.8} \ at \ 20^{o}C$$

adequately expresses the results. From the present study

the weight exponents in the relationship between oxygen

Table 9.   Comparison of the present data for oxygen uptake with that of other authors.

Species	Stage	Temperature °C	QO_2 Standard	Active	Author
S. salar (salmon)	Egg	10°	0.9		Hayes, Wilmot & Livingstone (1951) cited by Blaxter, 1969.
S. salar (salmon)	Fry (no yolk)	20°	1.6	12.1	Ivlev (1960)
Sardinops Caerulea (California sardine)	Eggs larvae	14° 14°	0.8 1.33	1.79 2.68	Lasker et al. (1962)
Clupea harengus (herring)	Eggs larvae (minus yolk)	8° 14°	1.5 3.5	5.0	Holliday et al. (1964)
C. harengus (herring)	larvae (minus yolk)	10°	1.1	2.6	Present data
Pleuronectes platessa (plaice)	larvae (minus yolk)	10°	2.5	4.1	Present data

uptake and weight were found to be 0.82 and 0.65 for herring and plaice respectively. Paloheimo and Dickie (1966) conclude that although the value of b = 0.8 is adequate, variations in 'a' dependent on the environment and condition of the animal are too great for the prediction of a single value to be useful. The biological meaning of 'b' is still obscure in spite of the hypotheses of Zeuthen (1947, 1953) and von Bertallanfy (1951) among others. Edwards, Finlayson and Steele (1969) measuring the metabolic rate of 'O' group plaice obtained exponent values   of 0.72. Beamish and Mookherjii (1964) pointed out that in many of the earlier experiments spontaneous activity alon increases the respiration above a basal or resting value. In the present experiments 'routine' metabolism as measured by unanaesthetized larvae showed a considerable variation attributable to spontaneous activity and was approximately double the basal value as measured by anaesthetized larvae. Beamish (1964) defined the "scope for activity" as the difference between active and resting metabolism. In the present experiments the scope for activity in herring as judged by the difference between unanaesthetized and anaesthetized larvae is shown

in Fig.17. As evident from the figure the "scope for activity" appears to decrease at least up to the pre-metamorphic stage in herring.

The oxygen uptake of spontaneously active yolk-sac herring larvae at 10°C was about 0.60 μl for a larva of wet weight 1.53 mg (from Table 8). The uptake per day would then correspond to 0.014 ml/day. Using Winberg's (1960) oxycalorific coefficient of 4.77 the calorific requirements per day for routine metabolism would amount to 0.067 cals. This agrees reasonably well with the calculated values of 0.082 for active larvae at the same weight by Blaxter (1966). For routinely active plaice larvae of mean wet weight 0.49 mg at the yolk-sac stage, uptake per larva per h was about 0.34 μl at 10°C. Thus consumption per day would amount to 0.0082 ml per day and calorific requirements would amount to 0.039 cals a day, using the Winberg coefficient.

Fig.17. Scope for activity related to body weight in herring. The scope for activity is calculated by subtracting the QO_2 for anaesthetized larvae from the QO_2 for unanaesthetized larvae (see Fig.7).
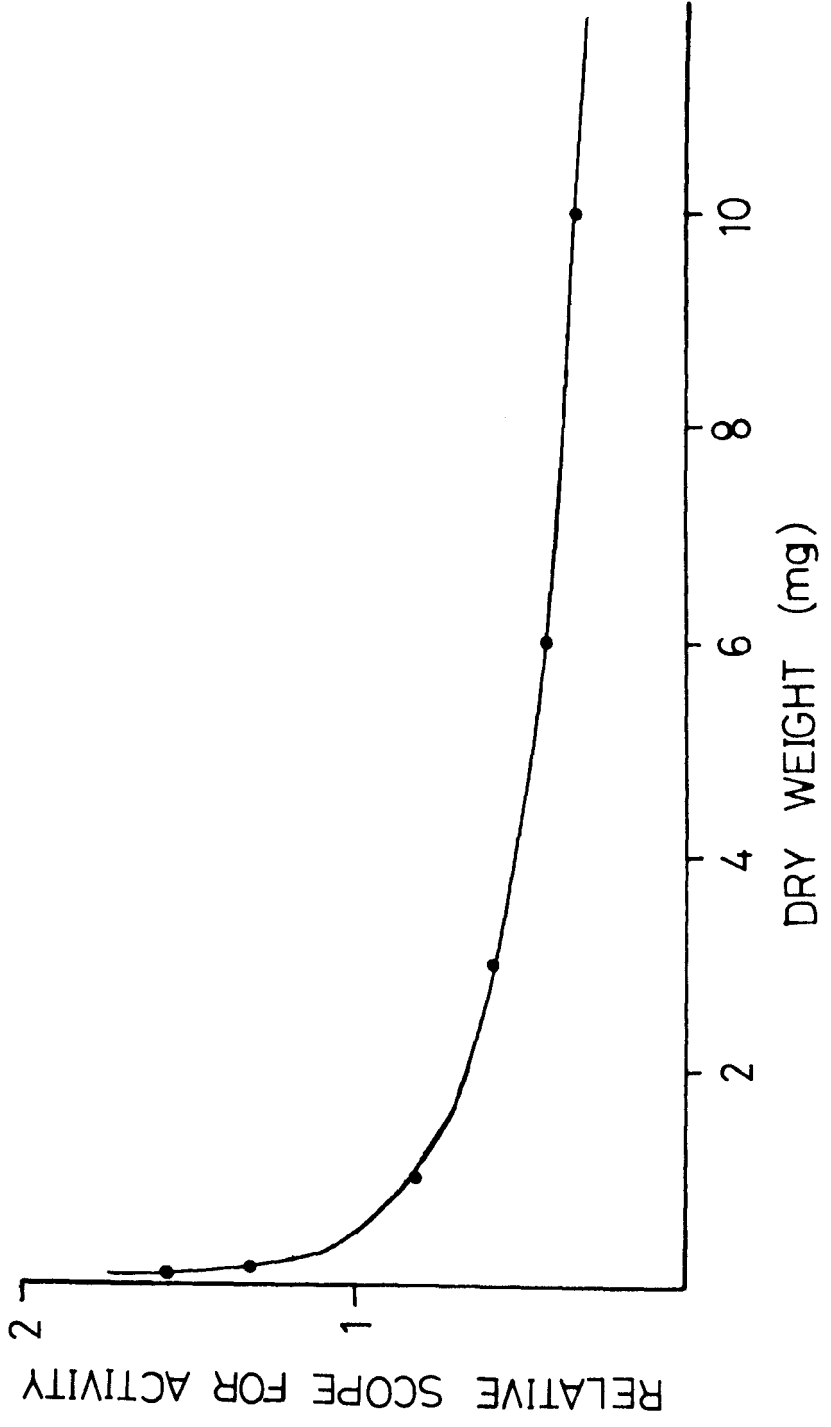
PART THREE. OXYGEN UPTAKE AT REDUCED OXYGEN CONCENTRATIONS.

III.1. Introduction

The ideal way to distinguish between regulation and conformity with respect to oxygen is to measure oxygen concentration (or pressure) in the tissues of an animal in different environmental oxygen concentrations (Prosser in Prosser & Brown, 1969). Technically this is difficult, therefore the alternative is to measure oxygen consumption as a function of environmental oxygen.

When the animal regulates its oxygen consumption down to some critical pressure below which its oxygen consumption declines rapidly, it is called a regulator, i.e. it shows a wide range of oxygen independence. Alternatively its oxygen consumption may increase as the environmental oxygen concentration goes up i.e. it is a conformer.

The only previously published information on the influence of oxygen concentration on the respiratory rate of fish larvae is the work of Hayes (1949) on salmonids.

In the present work three developmental stages of herring were used: 2 weeks of feeding, 7 weeks of feeding and post metamorphosis. Three stages of plaice

were studied as well: yolk-sac, 3 weeks of feeding
and post metamorphosis.

III.2. Methods

The syringe method of Ewer (1942) was used for
the young stages of herring and plaice. The analysis
of the oxygen content was made by the Fox and Wingfield
(1938) method. Two sizes of syringes were used, 10 ml
hypodermic and 100 ml veterinary. In the latter a
brass adaptor was fitted to the base of the syringe
to enable sampling. Individual syringes were placed
on a perspex rack and separated from one another by
black perspex partitions, the tops being covered by
black perspex to reduce outside interference. The
water bath in which the syringes were placed was
maintained at 10° ($\pm$ $0.1^{\circ}C$). The whole system was
covered with a black cloth to reduce outside interference.

The larvae used in the experiments were reared at
the experimental temperature, as in the experiments in
part two. They were transferred into filtered seawater
(filtered through Whatman glass filter GF/C) and left
overnight to defaecate, a second change being made prior
to transfer to the experimental syringe to reduce algal
respiration. Marshall and Orr (1961) observed that
addition of streptomycin did not have any harmful

effects on the feeding of <u>Calanus finmarchicus</u>. In an

earlier study (1958) the same authors found that the

oxygen consumption of female <u>C. finmarchicus</u> was unaffected

by antibiotics. Nevertheless antibiotics were not used

in the present study as ∧ effects on fish larvae are
                          their

unknown. Experimental times corresponded approximately

to those of the Gilson experiments to eliminate fluctuations

due to time of day and usually lasted 4 - 5 h. Groups of

5 - 6 larvae were used in the early stages. The syringe

operated as a closed vessel respirometer in which the

pO_2 was reduced by larval respiration. The pH was

checked at the end of the experiments. No appreciable

difference was obtained between 'control' and 'active'

syringes. Titrations for oxygen were carried out in

small glass vessels painted white on the outside as

recommended by Strickland & Parsons (1965). 'Control'

syringes showed no appreciable difference in oxygen

content over the experimental period.

For metamorphosed herring cylindrical perspex

chambers of 160 ml capacity were used, the water being

replaced by ∧deoxygenated filtered seawater after each
           partly

determination, to avoid any Bohr effect in the blood

due to high CO_2 levels. The same procedure was adopted

for plaice using the 100 ml syringes. Individual larvae were used in the case of metamorphosed herring and 1 - 2 metamorphosed plaice. Air saturation corresponded to 6.0 - 6.3 ml/l for the experiments.

III.3. Results.

These are expressed as μlO_2/mg dry weight/h to eliminate the influence of differences in size. Herring larvae at 2 and 7 weeks of feeding (Fig.18A & B) showed a great deal of variation in oxygen uptake at levels corresponding to air saturation. This variation decreased as the oxygen concentration fell, as did the oxygen uptake. The younger larvae appeared to regulate better than older ones, which seemed to be "conformers".

Plaice larvae at the yolk-sac stage also showed a wide variation in oxygen uptake at levels near air saturation (Fig.19A) and also 50% air saturation. A greater degree of "regulation" was observed when compared to the early feeding herring larvae. Plaice at 3 weeks of feeding seemed to have become complete "conformers" like the older herring (Fig.19B).

Metamorphosed herring and plaice showed an increased ability to regulate (Fig.20A & B) when compared to the earlier feeding stages though the experiments were not taken down to the incipient lethal level in the case of

Fig.18. Effect of oxygen concentration on oxygen uptake
for herring larvae at A. 2 weeks of feeding and
B. 7 weeks of feeding at $10^{O}C$. Arrow indicates
incipient lethal level (LD_{50}).

A   HERRING   2 wks feeding

B   7 wks feeding

OXYGEN UPTAKE (μl/mg/h)

OXYGEN CONCENTRATION (ml/l)
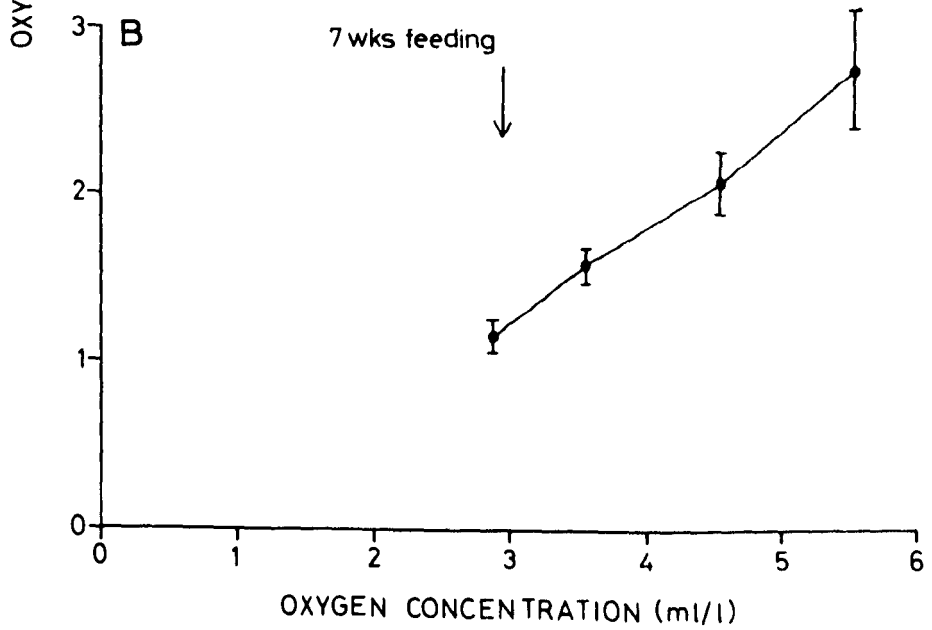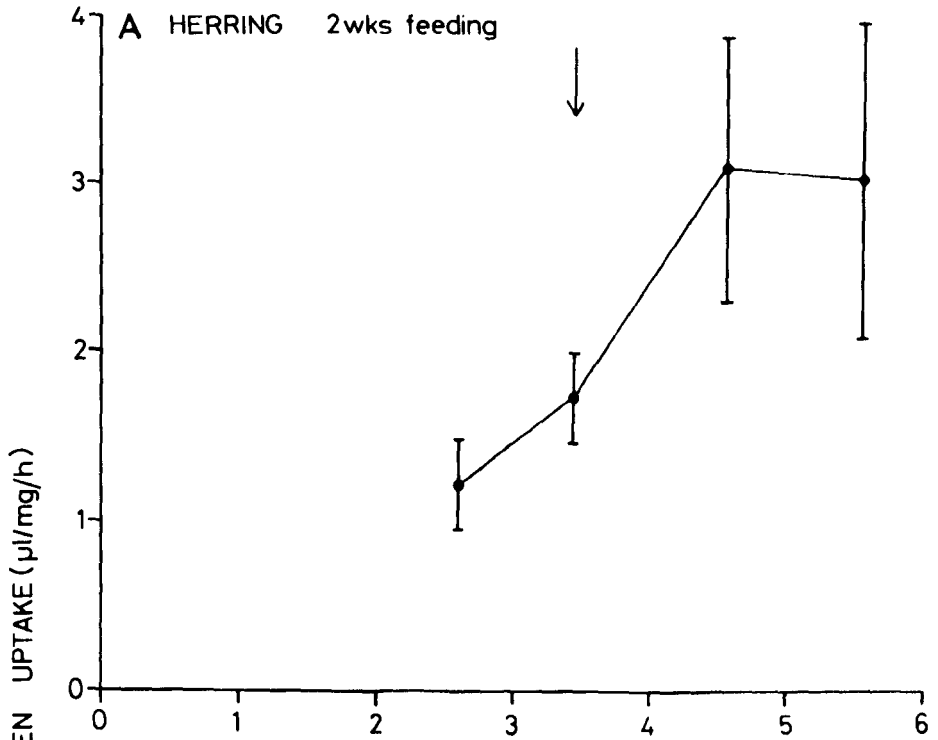
Fig.19. Effect of oxygen concentration on oxygen uptake

of plaice larvae at A. yolk-sac and B. 3 weeks

of feeding. Arrow indicates incipient lethal

level (LD_{50}).

A PLAICE yolk sac

B 3 wks feeding

OXYGEN UPTAKE (μl/mg/h)

OXYGEN CONCENTRATION (ml/l)
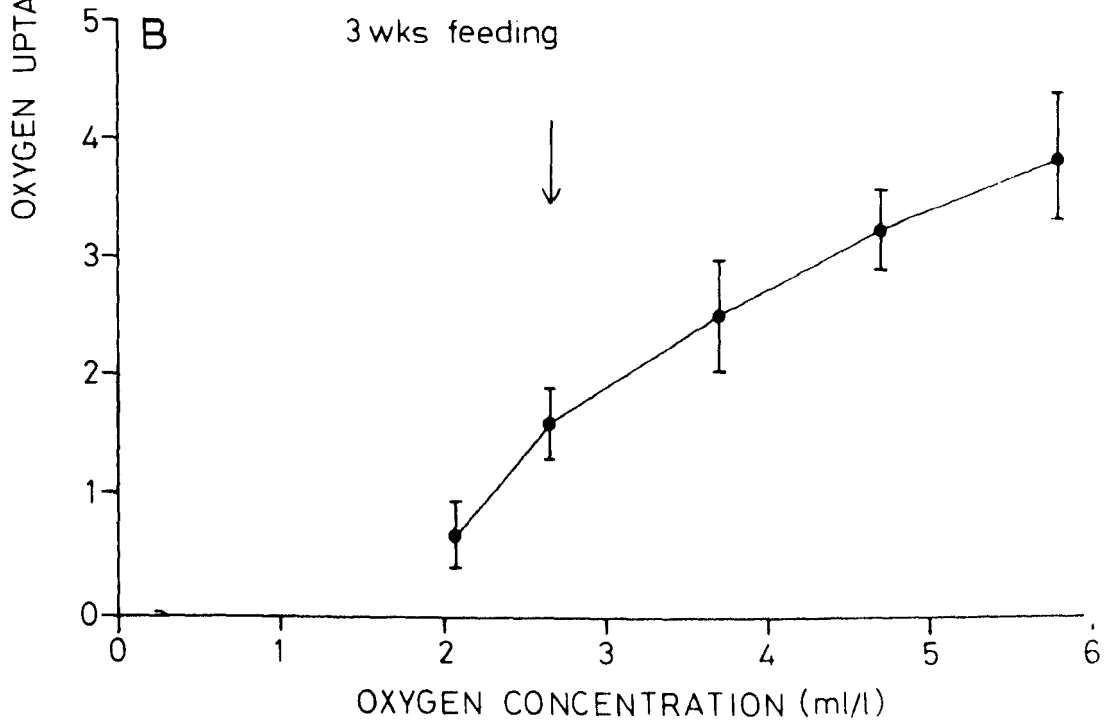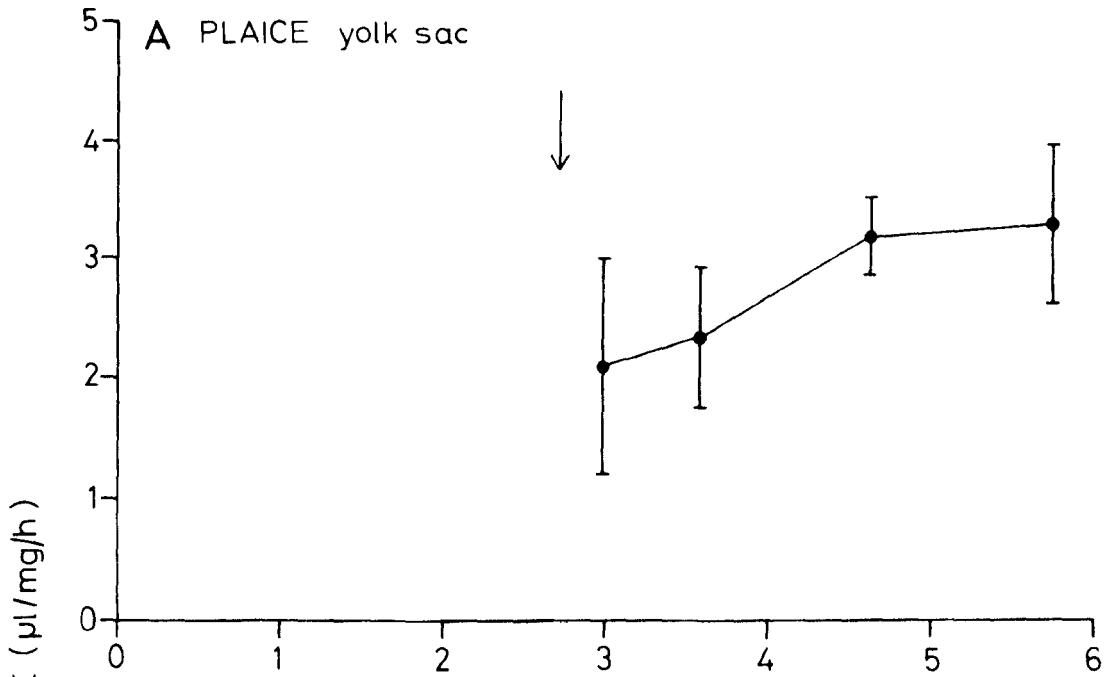
Fig.20. Effect of oxygen concentration on oxygen uptake

for A. metamorphosed herring ($1 = 50 \pm 5mm$)

  B. metamorphosed plaice ($1 = 18 \pm 3mm$).

Arrows indicate the incipent lethal level

(LD_{50}).

OXYGEN UPTAKE (µl/mg/h)

OXYGEN CONCENTRATION (ml/l)
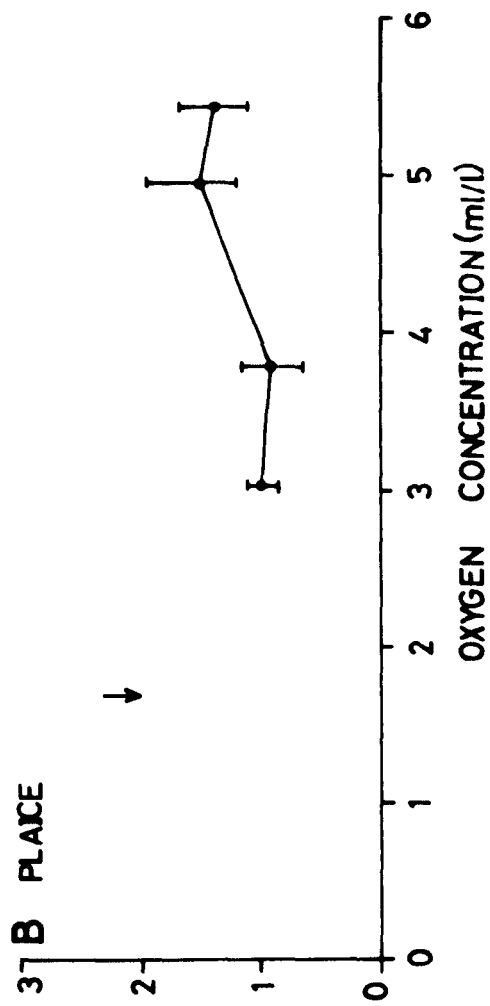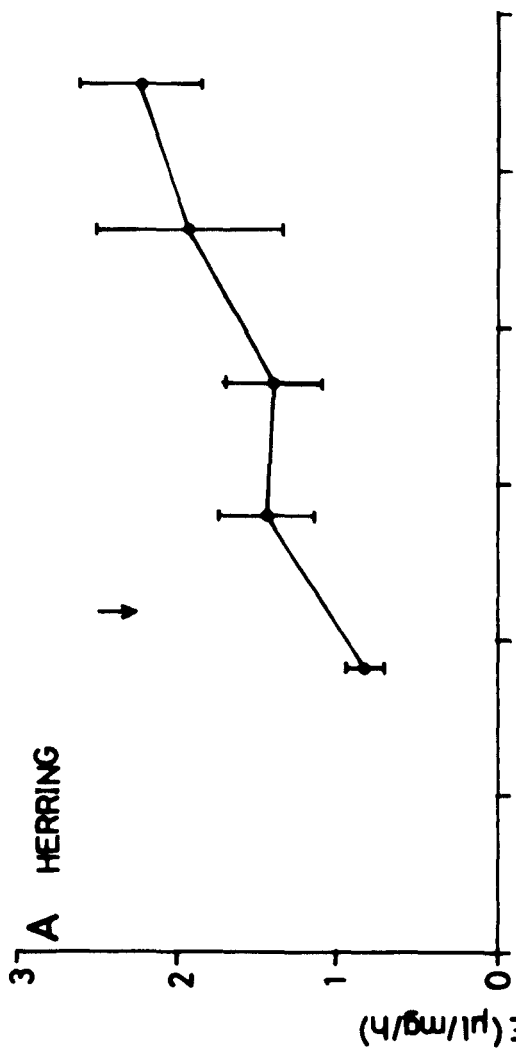
A HERRING

B PLAICE

the plaice. Nevertheless at levels near 50% air saturation
the plaice appeared to regulate better than the herring.

III.4. Discussion

Dependence on environmental oxygen tensions appears
to increase with age in both herring and plaice up to
metamorphosis. The oxygen uptake at the incipient lethal
level in the larval feeding stages of both species
corresponded approximately to the uptake of the
anaesthetized larvae as measured by the Gilson
experiments (see Figs 8 & 9; and Tables 4 & 5). This
shows that larvae survived at lower oxygen levels by
reducing their activity with no apparent oxygen debt
being incurred. Hayes (1949) also observed conformity
in relation to lowered oxygen tensions in salmonid
larvae. The apparent later independence (regulation)
of the metamorphosed fish could be explained on the
basis of the respiratory pigment which is present at
this stage.

Animals from high oxygen environments have higher
oxygen consumption at air levels and higher critical
pressures than animals from low oxygen environments
(Prosser in Prosser and Brown, 1969). This is observed
in some invertebrates (Fox, Wingfield and Simmonds, 1937)
and as shown here appears to hold for marine fish
larvae as well.

PART FOUR. MORPHOLOGICAL MEASUREMENTS OF THE SURFACE

AREA OF THE BODY AND GILLS IN RELATION TO

DEVELOPMENT.

IV.1. Introduction.

The present work was an attempt to determine the
respiratory surface available to the developing larva
of plaice and herring, which at hatching possess only
gill . bars   in the case of plaice, even these being
absent in the case of herring. Correlated with this,
the gill area was determined from its time of appearance
up to and beyond metamorphosis in both species. The
surface area of the body including the fins was also
measured as a means of determining the surface area
available for cutaneous respiration.

Many workers have attempted to measure the surface
area of the body in an attempt to relate it to metabolism.
The general relationship between surface area and weight,
is expressed by the equation

$$S = K W^{\frac{2}{3}}$$

where S equals the surface area, W the weight and K is a
constant for a particular species. Benedict (1938)
reviewing his own work and that of others, said that
with the exception of animals that become spherical or
greatly elongated, 'K' is in the neighbourhood of 10,

this being the best value for 21 species of a total of

30 birds and mammals. This was reviewed in turn by

Zeuthen (1947) and Kleiber (1947) who called attention

to variations and discrepancies in the literature.

Kleiber (1947) suggested the use of 0.75 power of the

weight as being representative of metabolic size.

Later Gray (1953) obtained lower values of K for round

fishes while depressed or compressed fishes gave

correspondingly higher values.

Measurements of gill dimensions have been made for

a large number of fish species by many authors. Reiss

(1881) was the first to measure accurately the gill

surface in pike. Putter (1909) observed that the

respiratory surface is proportional to body surface but

not to body weight, as maintained by Reiss. Price

(1939) studied the development of gills in small mouthed

bass Micropterus dolomieu. Schottle (1931) compared the

gills of terrestrial gobiiform fishes and strictly aquatic

species. More recently gill areas have been investigated

by Byckowska-Smyk, (1957, 1958, 1959) Gray (1954), who

tried to correlate it to body surface area, Hughes (1966)

Muir (1969) Saunders (1962) Hughes and Shelton

1962) Hughes and Gray (1972) Muir and Hughes (1969).

Several of these authors have tried to correlate gill

area with metabolic rate and activity. So far no
attempt has been made to study gill development in
young fish larvae, apart from Harder (1954) who
measured the development of elements of the branchial
region.

IV.2. Methods.

Larvae were anaesthetized with MS 222 SANDOZ
(I/ 15,000) and immediately the outline of the body
and fins was drawn on graph paper using a WILD M5
binocular microscope with a camera lucida attachment.
The width was not measured due to the problem of
handling delicate larvae with forceps which would
have damaged them and thus prevented a proper measurement
of the wet weight which was made immediately afterwards
on a Beckmann EMB 1 microbalance. Larvae were rinsed
in distilled water, placed on filter paper and immediately
a stopwatch was started. They were then transferred to
small boats made of foil and weighed at one minute
intervals. Later the weight was extrapolated back
to zero time. They were then frozen for ½ an hour,
freeze dried and stored till the dry weight was determined.

In herring the body surface area was calculated as

being equal to a cylinder, i.e. πdl where d is the average depth of five measurements taken at the following positions along the body.

      (a) Behind the eye.

      (b) Halfway between the eye and insertion of the dorsal fin.

      (c) At the insertion of the dorsal fin.

      (d) Just posterior to the anus.

      (e) Base of the caudal fin.

The average measurement thus obtained corresponded very closely to an average measurement of the depth measured at the insertion of the dorsal fin and the true width. A total of 89 herring larvae were examined, the observations being grouped into length classes to facilitate handling of the data.

Seventy fish were studied in the case of plaice. For the later stages of plaice the value obtained for the surface area of one side was doubled to give the total surface area. The method used for the herring larvae was used for the earlier stages of plaice. A point to be noted is that epithelial respiration at least in the very early stages may also be taking place through the intestinal and buccal epithelia. No attempt was made to determine these proportions with development. Nevertheless a measure of the relative change of the

available respiratory surface with development could be
obtained.

The material for the gill measurements was fixed in
10% seawater-formalin, after the length was measured.
The wet weight was computed for these lengths from the
surface area and weight relationships. This method
was adopted due to the time involved in weighing small
larvae, length being used as a indicator of body size
by extrapolation. This avoided the time consuming
weighing procedures. For the larger plaice and herring
fixed long after metamorphosis both the wet weight and
length was determined before fixation.

Prior to measurement the gills and pseudobranch of
the left side were dissected out in herring, and the
arches separated. In plaice since the left eye migrates,
the gills of the right side were dissected instead. All
the measurements were made under a WILD M5 binocular
microscope with a micrometer eyepiece. No allowance
was made for shrinkage but Gray (1954) and Hughes (1966)
measuring the gills of large fish observe that shrinkage
is slight. The total length of each arch was measured
by summing up the lengths of the epibranch, ceratobranch
and hypobranch. The total number of filaments were
counted on each side of the arch. In the earliest stages

examined, only those filaments with visible lamellae were counted. Since the gills were just starting to develop the methods used by Hughes (1966) and Muir and Hughes (1969) where detailed measurements on the filaments of the second arch were extrapolated to give the gill area of the whole fish could not be used.

Preliminary investigation showed a considerable variation not only in the filament length along the length of the arch, but also in the length of the inner and outer filaments.

As these were at the rapidly developing stage when measurements were being made at nearly the limit of the binocular light microscope the following procedure was adopted. The length of every fifth or sixth filament was measured on the epibranchial, ceratobranchial and hypobranchial (see Fig.21). This was done for both inner and outer filaments. The average of all these measurements was taken as the filament length for that particular arch. In addition the longest filaments on the epibranchial and ceratobranchial as well as the shortest filament at the junction of the epibranchial and ceratobranchial and the filament at the junction of the ceratobranchial and hypobranchial were used to measure the following: the height (l) of the secondary lamella,

Fig.21. A.(i) and (ii). Two views of a gill filament
        of herring (x 12). l, secondary lamella.

        B. Diagram of a gill arch of herring adapted
        from Harder (1954). c, ceratobranchial;
        e, epibranchial; f, gill filament;
        h, hypobranchial; r, gillraker (postmetamorphosis).

A

(i)

l →

(ii)

l →

1·67mm

B

e

r →

c

h

f →
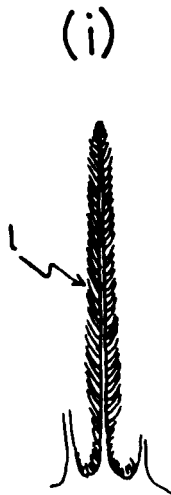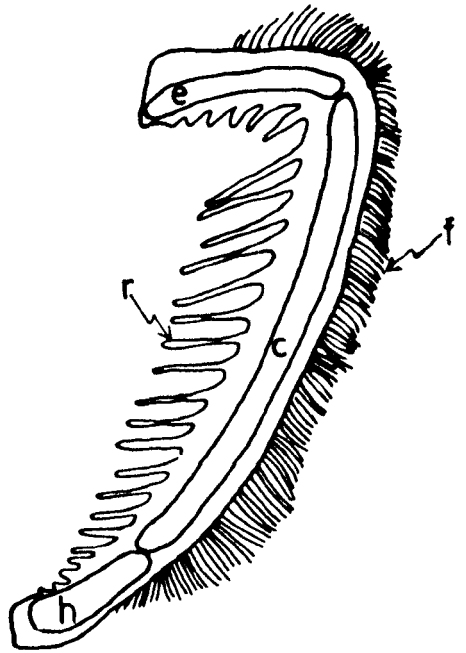
the number of lamellae falling into the field of part
of the micrometer eyepiece, so as to obtain the
spacing (d) at the middle region of the filament, and
after dissection of the filament from the arch, the
width (b) of the lamella was noted.

Since preliminary examination showed that the
pseudobranch was well developed in these early stages
and Harder (1954) believes it to be active in herring
respiration the lamellar area of the pseudobranch was
determined in both species. The average filament
length was obtained by measuring the lengths of the
filaments halfway between the longest filament (at
the middle of the arch) and the end of the arch. The
average of these two measurements was taken as the
average filament length for the pseudobranch. More
detailed checks have shown that this is satisfactory.
The height, depth and spacing of the secondary lamellae
on these two filaments were determined in the same manner
as for the gills.

The gill area was calculated from these measurements
as follows. The measurements made on each gill arch were
averaged to give values representative for that particular
arch. The measurements for each arch were then averaged
to obtain a value for all four arches. Since the secondary

lamellae are roughly triangular in shape the area of one side of an average secondary lamella would equal half the average height (l) multiplied by the average depth (b) i.e. $\frac{1}{2}$ l x b. As both sides of the lamella are used for gaseous exchange the total area would be twice the area of one side i.e. 2($\frac{1}{2}$ l x b) = l x b. For the whole arch the area of an average secondary lamella would equal the product of the weighted height ($\bar{l}$) and the weighted depth ($\bar{b}$).

The total no. of lamellae for the arches of one side which is equal to the average length (L) for the whole side multiplied by the product of the weighted spacing ($\bar{d}$) and the total number of filaments for the four arches (N). This is then multiplied by 2 due to the fact that lamellae are present on either side of the filament.

i.e. the total number of lamellae for the arches of one side = 2 (L x $\bar{d}$ x N)

The total number of lamellae for both sides of the arch = 4 (L x $\bar{d}$ x N)

Total gill area would be equal to the area of the secondary lamellae which is equal to the product of the total number of secondary lamellae and the weighted area of a secondary lamella. i.e. = 4 (L x $\bar{d}$ x N) ($\bar{b}$ x $\bar{l}$)

The secondary lamellar area of the pseudobranch was calculated in a similar manner.

Some material was also fixed for histological examination. The fixatives used were Bouins solution and 10% buffered formalin. After 12 hours the specimens were transferred to 70% alchohol and after several changes the material was subsequently stored in alcohol of the same concentration. For older larvae the duration of fixation was extended. The specimens were embedded in paraffin wax and sections cut at 4-6 μ on a rotary microtome. Sections were cut in two planes at right angles to one another, namely, parallel to the length of the larva and transversely to the length. These sections gave further confirmation of some of the measurements made on fixed material. Most of the sections were stained in Mayers haemalum. Some were stained in Mallory Aniline Blue and Heidenhain Iron Haematoxylin.

Computations were greatly aided by the use of a Hewlett Packard desk computor 9100B.

IV.3. Results.

IV.3.1. Relationships between surface area and length.
The relationships between surface area and length (viz.

total surface area, body area and fin area) for herring are
shown in Fig. 22A,B,C & D. and Table 10 The results were grouped into

length classes to facilitate handling of the data. The

computed curves correspond to a line of best fit obtained

by regression analysis. Since length is a good criterion

of size as well as weight, the relative surface areas were

computed against length as well as weight.

Total surface area (i.e. including fins) varied by a

'b' value of 2.13, while body surface area varied by a

value of 2.27 in herring (Fig. 22A & B). In plaice on the

other hand the body area to length relationship has a

slope value 2.09 while the total surface area increases
and Table 11
at a rate of 2.57 (Fig.23A & B). This is to be expected

because the plaice is changing shape throughout development

becoming increasingly flatter and broader as metamorphosis

approaches, before finally assuming the flattened shape of

the adult. The fin area as expected showed a slower rate

of increase with length in herring when compared to the

body and total surface area (slope value of 1.59). However

the power curve regression fitted to the data did not show

a good fit at the late premetamorphosed and metamorphosed

stage. Percentage fin area/body area ratio showed a rapid

decrease in the early stages but showed a levelling out

Fig.22. Relationship between surface area and length
         in herring.
         A. Total surface area and length.
         B. Body surface area and length.
         C. Fin surface area and length.
         D. Percentage fin area/body area ratio with
             length.

HERRING

A TOTAL SURFACE AREA ×10 (mm²)

B BODY SURFACE AREA ×10 (mm²)

C FIN AREA (mm²)

D % FIN AREA/BODY AREA
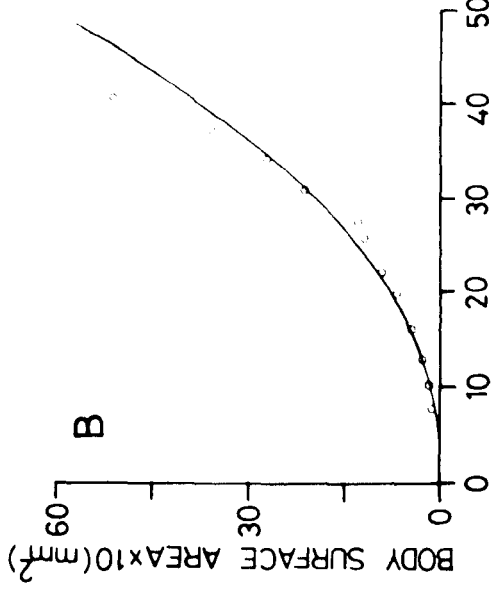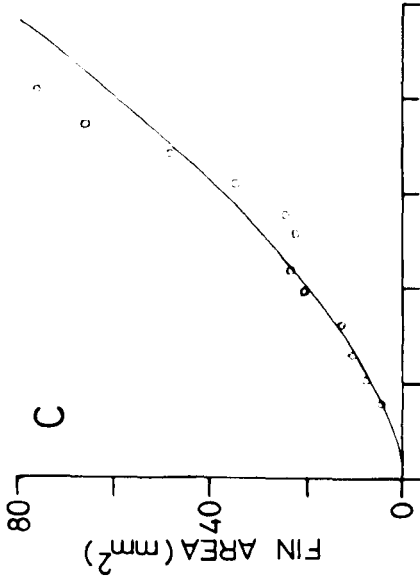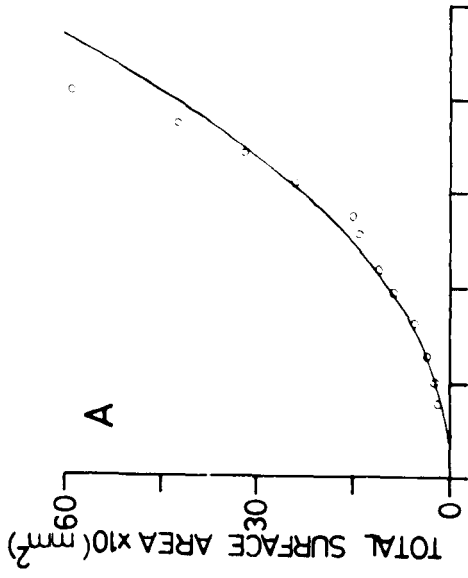
LENGTH (mm)

Table 10. Summary of surface area measurements in herring

Length class	Length (mm)	Body Surface Area (mm^2)	Total Surface Area (mm^2)	Fin Area (mm^2)	% Fin Area/Body Area	Wet Weight (mg)	Dry Weight (mg)	Total Surface Area/unit wet weight
6.00- 8.99	7.83	11.90	16.51	4.61	38.99	-	-	
9.00-11.99	10.26	16.73	24.26	7.53	43.29	1.138	.157	21.32
12.00-14.99	12.93	27.10	35.18	10.16	38.97	1.827	.249	19.26
15.00-17.99	16.18	44.31	57.33	13.01	29.22	4.582	.744	12.51
18.00-20.99	19.79	68.58	87.89	20.14	29.65	10.649	1.777	8.25 *
21.00-23.99	22.04	89.77	113.06	23.30	19.79	15.611	2.692	7.24
24.00-26.99	25.75	118.88	141.38	22.50	19.20	29.249	4.633	4.83
27.00-29.99	27.54	128.28	152.52	24.24	18.33	35.215	5.824	4.33
30.00-32.99	31.00	211.56	246.36	34.80	16.46	66.230	12.612	3.72
33.00-35.99	34.21	271.89	320.32	48.42	15.56	110.220	18.822	2.91
36.00-38.99	37.33	357.85	423.82	65.97	18.29	141.560	26.680	2.99
39.00-41.99	40.74	513.13	588.85	75.73	14.76	236.160	46.080	2.49

*gills appear

Fig.23. Relationship between surface area and length in plaice.

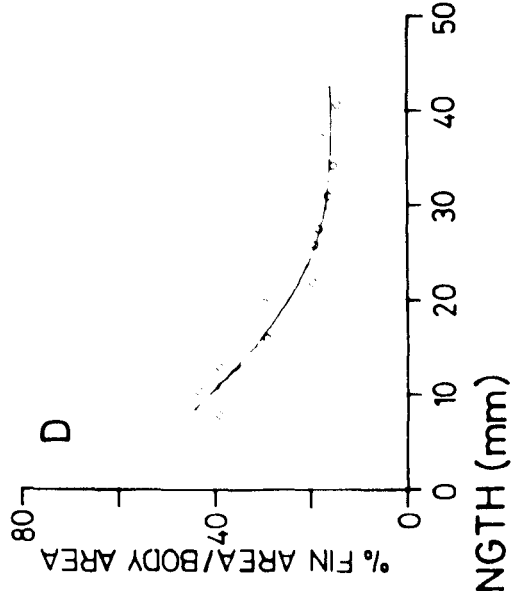A. Total surface area and length

B. Body surface area and length.

PLAICE
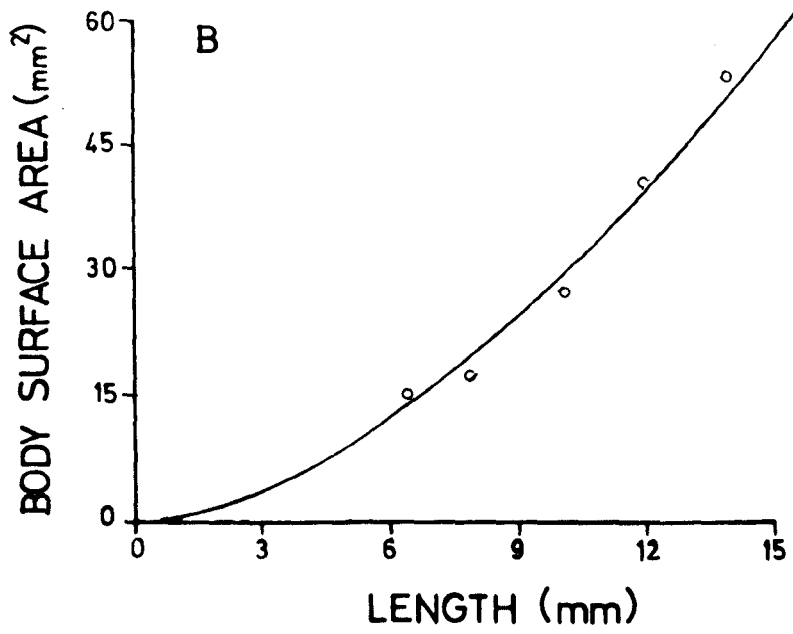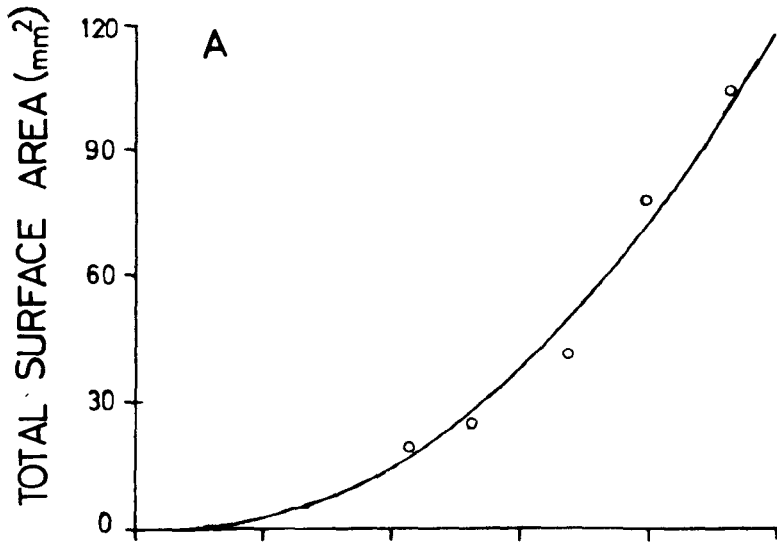
Table 11. Summary of surface area measurements in plaice

Length class	Length (mm)	Body Surface Area (mm^2)	Total Surface Area (mm^2)	Fin Area (mm^2)	% Fin Area/Body Area	Wet Weight (mg)	Dry Weight (mg)	Total Surface Area/unit wet weight
5.00- 6.99	6.413	10.440	14.570	3.79	40.09	.370	.079	39.38
7.00- 8.99	7.903	17.502	25.071	6.657	39.48	1.162	.218	21.58
9.00-10.99	10.122	27.237	41.506	14.27	52.77	3.512	.631	11.82 *
11.00-12.99	11.941	40.604	77.981	37.38	91.83	10.161	1.439	7.67
13.00-14.99	13.900	53.28	104.52	51.24	95.51	20.654	2.74	5.06

* gills appear

from about the 22 mm stage onwards (Fig.22D). Significantly
this is the time of appearance of the gills (Harder, 1954,
and the present study). Plaice did not show this pattern
probably due to its different body proportions. The
equations expressing the relationships with length on the
X axis in a log-log transformation of the data are as
follows:

For herring

   Log Y = 0.162 + 2.135 log X where Y = total surface area

   Log Y = 0.0869 + 2.267 log X where Y = body surface area

   Log Y = 0.167 + 1.589 log X where Y = fin surface area

For plaice

   Log Y = .1188 + 2.579 log X where Y = total surface area

   Log Y = .2201 + 2.093 log X where Y = body surface area

   IV.3.2. Relationships between surface area and weight.

   The general relationship is given by the equation

$$Y = aW^b$$

or log Y = log a + b log W,

where Y = surface area, a, the surface area of a 1 mg
larva , W, the weight in mg, 'b' the regression coefficient.

   The relationships between total surface area and weight,
body surface area and fin area with weight in herring are

given by equations i, ii and iii. These are based on a
least squares regression analysis.

For herring,

		S.D. of slope	

$$\log Y = 22.93 + .572 \log W \qquad 0.015 \qquad \text{(where } Y = \text{total surface area)} \quad \text{(i)}$$

$$\log Y = 16.59 + .608 \log W \qquad 0.015 \qquad (Y = \text{body surface area)} \quad \text{(ii)}$$

$$\log Y = 7.079 + .409 \log W \qquad 0.026 \qquad (Y = \text{fin area)} \quad \text{(iii)}$$

For plaice,

$$\log Y = 23.420 + 0.497 \log W \qquad 0.067 \qquad \text{(where } Y = \text{total surface area)} \quad \text{(iv)}$$

$$\log Y = 16.03 + 0.402 \log W \qquad 0.050 \qquad \text{(where } Y = \text{body surface area)} \quad \text{(v)}$$

$$\log Y = 6.728 + 0.679 \log W \qquad 0.011 \qquad \text{(where } Y = \text{fin area)} \quad \text{(vi)}$$

From the equations given above it is evident that the
total surface area increases at a higher rate compared to the
body surface area. The reverse is observed in plaice
(equations iv & v). This is probably a reflection of the
changing shape of the plaice as it approaches metamorphosis.

Similarly the fin area increases at a higher rate in plaice compared to herring (equations vi & iii, respectively).

Length-weight relationships calculated on a log-log basis for herring and plaice are given by equations vii, viii and iv & x. These relationships expressed as a power curve regression are illustrated in Fig.24 (A,B,C,D). The general equation is

$$Y = a W^b$$

or $\log Y = \log a + b \log W$,

where Y = wet weight in mg, a = total weight of a 1 mg larva, b = the regression coefficient, W = the length in mm.

For herring with length,

Log Y =   0.0001 + 3.96 log X (vii) where Y = wet weight

Log Y =   0.00001 + 4.19 log X (viii) where Y = dry weight

For plaice with length,

Log Y =   0.000024 + 5.18 log X (ix) where Y = wet weight

Log Y =   0.000016 + 4.58 log X (x) where Y = dry weight.

As mentioned earlier no attempt was made to evaluate the extent of respiration through the gut with development.

Fig.24. Length-weight relationships in herring and
plaice.

     A.   Length - wet weight relationship in herring.

     B.   Length - dry weight relationship in herring.

     C.   Length - wet weight relationship in plaice.

     D.   Length - dry weight relationship in plaice.

HERRING

PLAICE

A   WET WEIGHT (mg)   240   120   0

B   DRY WEIGHT (mg)   44   22   0

C   WET WEIGHT(mg)   24   12   0

D   DRY WEIGHT(mg)   3·2   1·6   0

LENGTH(mm)
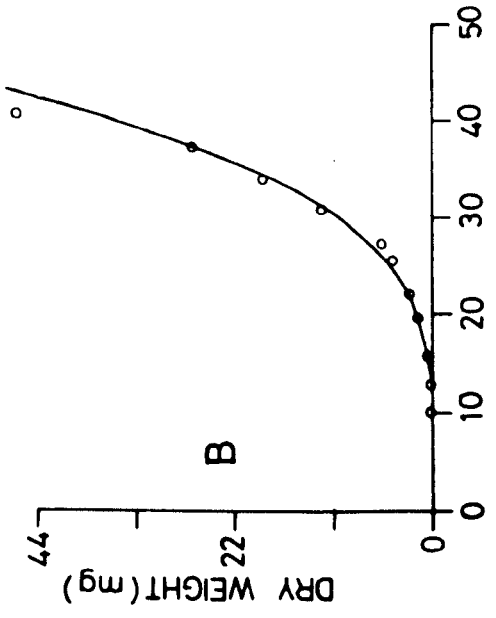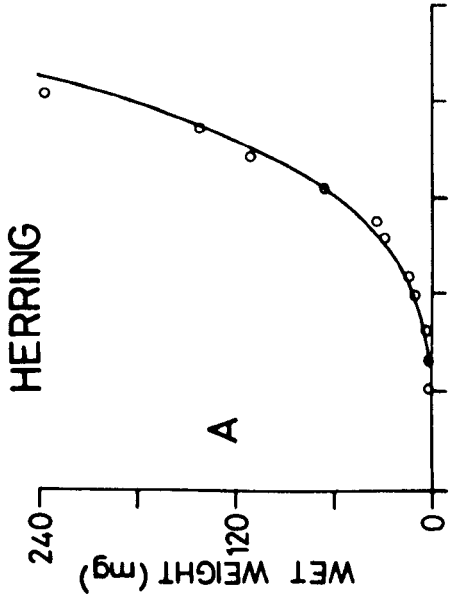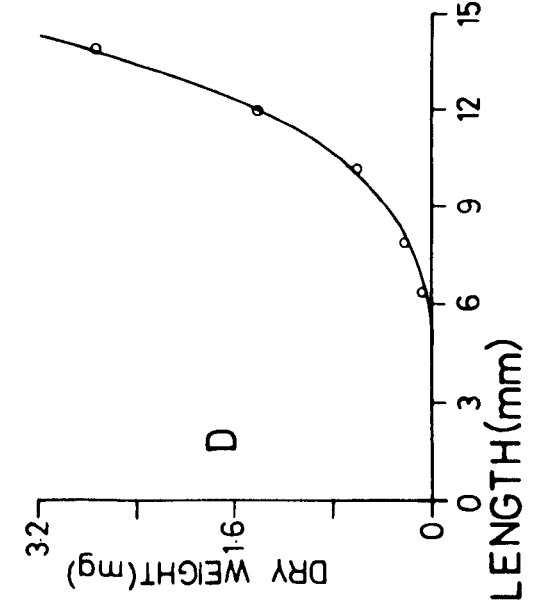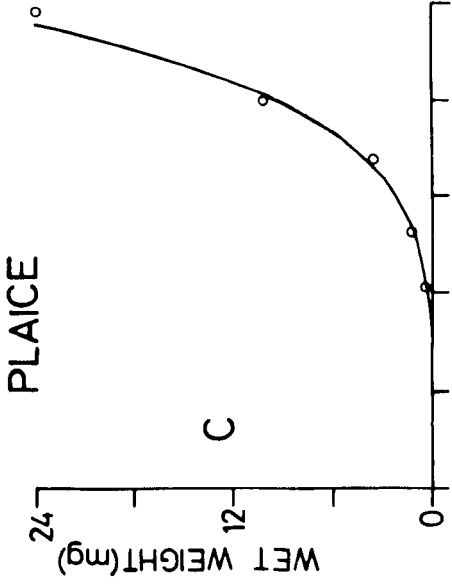
Nevertheless some measurements on yolk-sac larvae of herring and plaice show that this is about 25% of the body surface area in herring and 11% in plaice. Expressed as a % of the total surface area in herring and plaice this becomes equal to 17% and 8% respectively.

IV.3.3. Relationships between body weight and total gill area.

A plot of gill area and weight on log-log co-ordinates showed a curve at the upper ends of the graph in both herring and plaice. Closer visual examination revealed what appeared to be a curve with a continuously changing slope, the maximum change occuring at a length roughly corresponding to metamorphosis in both species. A power curve regression of the same data also revealed a poor fit at the upper ends of the curve. Hence it was decided to extend the curve in the upper range. This was done in 1972 and the data were replotted with separate lines fitted to the data up to metamorphosis and beyond it. The results are summarised in Tables 12 & 13. Lines were fitted by least squares regression to the points. (Figs 25 & 26).

The general equation for this relationship is

$$Y = a \, W^b$$

or $\log Y = \log a + b \log W,$

where Y = total secondary lamellar area in mm^2, a = total

Fig.25.   Relationship between gill area and weight in
          herring.

Fig.26. Relationship between gill area and weight in

plaice.

Table 12. Summary of gill measurements in herring.

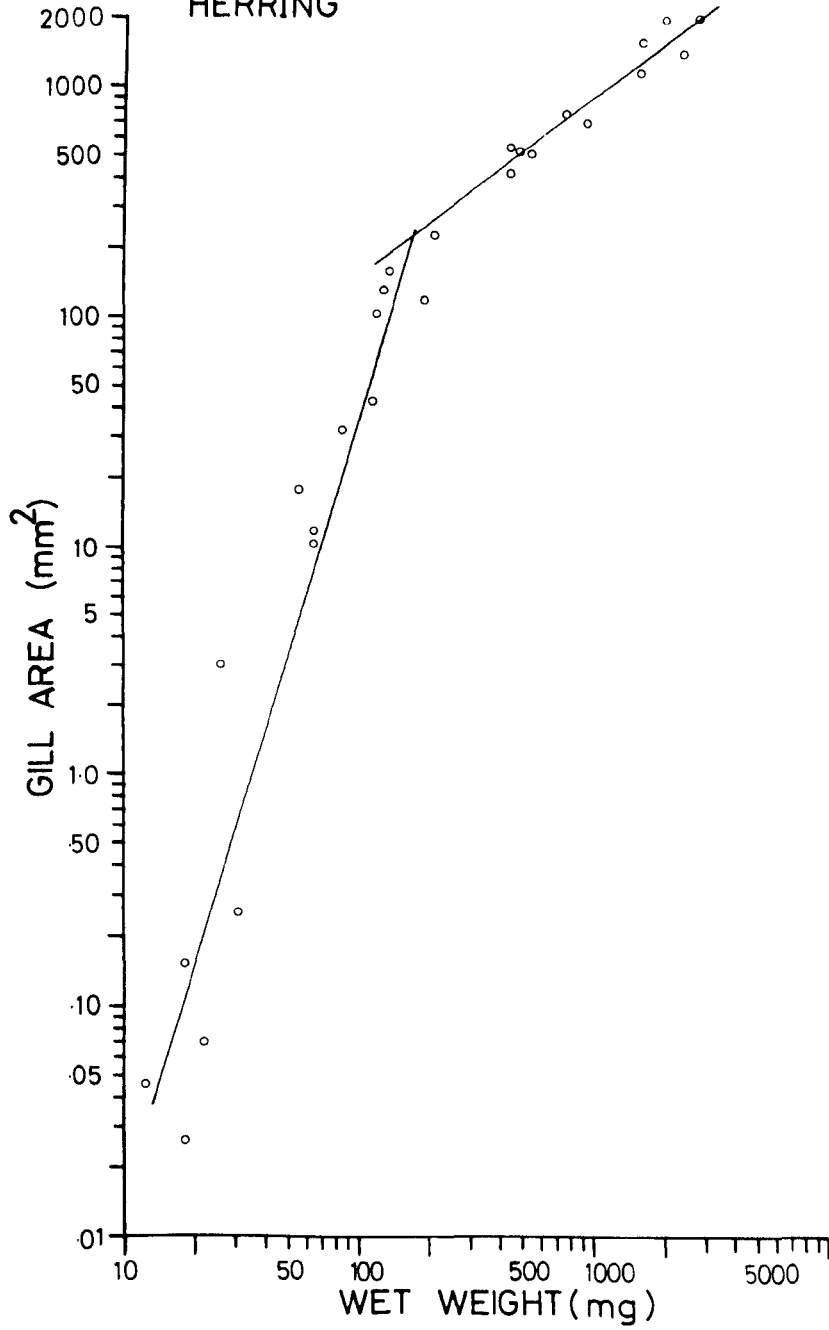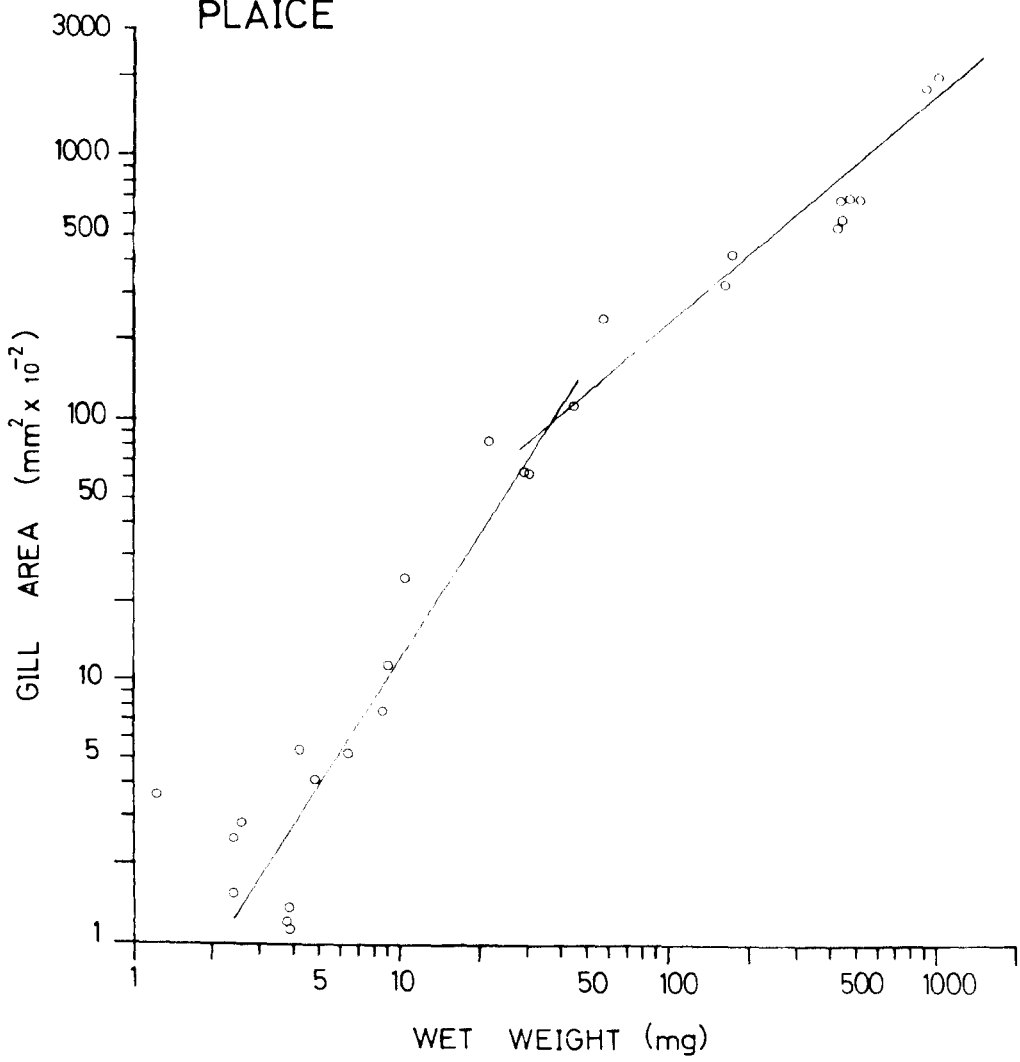| Length (mm)	Weight (mg)	Total No. of filaments	Average filament length (mm)	Total filament length (mm)	Spacing/mm of filament	Total No. of secondary lamellae	Average width (b) of secondary lamellae (mm)	Average height (h) of secondary lamellae (mm)	Average secondary lamellae area (mm^2)	Gill Area (mm^2)	Gill Area/mg (mm^2)	Pseudobranch area (mm^2)	Gill Area/ Pseudobranch Area
20	12.548	82	0.0629	4.5904	19.90	91.3	0.0101	0.0251	0.00025	0.0462	0.0037	–	
22	18.392	86	0.1407	7.697	19.90	153.2	0.0101	0.0503	0.00051	0.1546	0.0084	*	
23	22.056	70	0.0787	4.900	19.90	97.5	0.0075	0.0511	0.00038	0.0752	0.0034	*	
22	18.392	56	0.0663	3.304	19.90	65.7	0.0101	0.0201	0.00020	0.0266	0.0015	*	
24	26.247	200	0.1722	38.630	23.63	912.8	0.0344	0.0603	0.00207	3.0010	0.1143	0.733	4.09
25	31.012	138	0.1284	15.773	14.93	235.5	0.0094	0.0581	0.00055	0.2584	0.0083	*	
29	56.881	484	0.2364	101.834	25.29	2575.4	0.0336	0.1065	0.00358	18.4209	0.3239	3.298	5.58
30	65.334	442	0.2018	79.383	28.19	2237.8	0.0201	0.1156	0.00230	10.3995	0.1592	1.854	5.61
30	65.334	490	0.2203	96.089	25.87	2485.8	0.0201	0.1206	0.00242	12.0530	0.1845	1.289	9.35
32	85.054	548	0.3367	164.181	26.43	4339.3	0.0408	0.0942	0.00384	33.3870	0.3925	5.803	5.75
35	122.675	662	0.4515	265.990	30.47	8104.7	0.0577	0.1124	0.00650	105.075	0.8565	8.533	12.31
34	115.669	698	0.3794	235.15	33.32	7835.2	0.0324	0.0854	0.00280	43.425	0.3754	5.213	8.33
35	129.997	698	0.4685	290.99	28.92	8415.4	0.0665	0.1226	0.00820	137.338	1.0565	12.971	10.59
36	137.645	834	0.4834	358.79	29.85	10709.8	0.0529	0.1419	0.00750	160.704	1.1675	11.427	14.06
39	190.912	830	0.4698	347.02	33.58	11652.9	0.0358	0.1438	0.00520	120.033	0.6287	11.026	10.88
40	211.720	806	0.6532	468.53	28.92	13549.9	0.0679	0.1244	0.00850	229.118	1.0822	19.626	11.67
48	446.057	956	0.8719	741.76	30.78	22831.4	0.0792	0.1519	0.01200	549.729	1.2324	27.694	19.85
49	485.269	974	0.9186	796.34	30.47	24264.5	0.0623	0.1777	0.01110	537.315	1.1073	18.082	29.71
46	449.00	860	0.6194	532.68	30.63	16315.9	0.0547	0.0968	0.00529	418.794	0.9327	*	
51	549.00	884	0.5914	680.95	31.04	21136.7	0.0544	0.1067	0.00580	526.27	0.9585	18.536	28.39
55	778.06	968	1.0113	871.29	33.12	28857.1	0.0667	0.2010	0.01340	774.19	0.9950	64.750	11.95
59	948.00	970	0.9313	903.36	31.09	28085.5	0.0616	0.1269	0.00782	711.10	0.7501	56.725	12.53
73	1628.01	1208	1.4259	1533.07	31.56	48383.7	0.0882	0.1909	0.01680	1630.58	1.0016	135.041	12.08
74	2068.03	1252	1.5990	1786.59	32.88	58743.1	0.0886	0.2016	0.01790	2094.15	1.0126	157.98	13.26
75	2415.30	1200	1.5630	1669.32	33.42	55788.6	0.0901	0.1439	0.01290	1445.93	0.5987	119.85	12.06
72	1712.02	1100	1.4196	1398.74	32.34	44944.2	0.0842	0.1357	0.01140	1777.71	0.6879	124.72	9.44
77	2780.08	1252	1.2589	1576.14	32.18	50720.2	0.0830	0.1734	0.01439	2033.03	0.7313	88.38	22.89

– no lamellae
* lost.

Table 13.   Summary of gill measurements on plaice.

Length (mm)	Weight mg	Total no. of filaments	Average filament length (mm)	Total filament length (mm)	Spacing/mm of filament	Total No. of secondary lamellae	Average width of secondary lamellae (mm)	Average height of secondary lamellae (mm)	Average secondary lamellae area (mm^2)	Gill area (mm^2)	Gill area /mg (mm^2)	Pseudobranch area	Gill area/ Pseudobranch area
8.03	1.243	40	0.0787	3.1480	19.900	62.6	0.0067	0.0435	0.00029	0.0365	0.0294	0.012	3.01
9.03	2.414	48	0.1061	5.0928	24.875	126.7	0.0100	0.0402	0.00040	0.1535	0.0636	*	
9.03	2.414	60	0.1224	7.3470	24.875	182.8	0.0167	0.0405	0.00068	0.2481	0.1028	*	
9.81	3.800	58	0.1189	6.8962	19.900	137.2	0.0101	0.0436	0.00044	0.1202	0.0316	0.006	20.72
9.12	2.548	74	0.1346	9.9604	23.034	229.4	0.0138	0.0448	0.00062	0.2845	0.1117	0.013	25.18
9.86	3.908	56	0.1206	6.7536	19.900	134.4	0.0101	0.0502	0.00051	0.1358	0.0347	*	
9.90	3.995	70	0.1259	8.8130	19.900	175.4	0.0075	0.0430	0.00032	0.1138	0.0285	*	
10.00	4.222	78	0.1469	11.4582	25.705	294.5	0.0182	0.0503	0.00092	0.5391	0.1277	0.053	10.44
10.24	4.8078	82	0.1482	12.1524	22.387	272.0	0.0150	0.0501	0.00076	0.4107	0.0854	0.031	13.25
10.80	6.436	112	0.1127	12.6224	25.490	321.7	0.0176	0.0461	0.00081	0.5215	0.0810	*	
11.40	8.656	120	0.1323	15.8760	23.631	375.2	0.0197	0.0519	0.00102	0.7683	0.0888	0.109	7.07
11.50	9.080	118	0.1591	18.7738	26.516	497.8	0.0234	0.0486	0.00114	1.2344	0.1249	0.186	6.09
11.80	10.456	134	0.2211	29.6274	21.347	632.5	0.0345	0.0568	0.00196	2.4791	0.2312	*	
13.46	21.507	170	0.2792	47.4640	23.630	1121.5	0.0487	0.0762	0.00371	8.3425	0.3879	0.204	40.98
14.24	29.285	176	0.2639	46.4464	23.009	1068.7	0.0421	0.0708	0.00298	6.3696	0.2175	0.465	13.69
14.32	30.198	168	0.2371	39.8328	23.631	941.3	0.0452	0.0735	0.00332	6.2406	0.2067	0.555	11.25
15.97	44.850	206	0.2492	51.3352	24.875	1276.9	0.0454	0.0871	0.00395	10.1150	0.2255	0.879	11.50
19.00	57.50	266	0.3420	90.9720	26.703	2429.2	0.0544	0.0909	0.00495	24.0530	0.4183	1.832	13.12
25.00	176	374	0.3747	140.138	28.049	3930.7	0.0546	0.0987	0.00540	42.3540	0.7366	*	
26.00	166	380	0.3650	138.70	29.230	4054.2	0.0460	0.0850	0.00390	32.1270	0.1936	3.801	8.45
28.47	270	432	0.4167	180.014	28.347	5102.9	0.0509	0.1039	0.00529	53.9340	0.1998	4.887	11.04
28.65	400	440	0.4484	197.296	27.830	5490.7	0.0591	0.1059	0.00626	68.7506	0.1719	3.982	17.26
30.03	390	524	0.5063	265.301	26.740	7094.1	0.0471	0.1018	0.00479	68.0319	0.1744	5.236	12.99
32.29	500	484	0.5523	267.313	25.808	6898.8	0.0474	0.1064	0.00504	69.5713	0.1391	10.333	6.73
33.50	450	452	0.4965	224.418	28.55	6406.8	0.0473	0.0942	0.00446	57.1652	0.1270	*	
49.00	925.6	622	0.5954	370.339	26.895	9960.3	0.0697	0.1319	0.00919	183.201	0.1979	11.485	15.95
50.00	1026.8	608	0.5893	358.294	26.895	9636.3	0.0734	0.1432	0.01051	202.519	0.2568	*	

*pseudobranch lost

secondary lamellar area for a 1 mg fish in mm^2, W = weight of fish in mg, b = regression coefficient or slope. The equations obtained with the 95% confidence intervals for the regression are,

Herring (up to 40 mm, n = 16 points)

Log Y = : 0.000006918 + 3.36 log W; $(2.8 < b < 3.82)$ - (I)

Plaice (up to 12 mm, n = 13 points)

Log Y = 0.03122 + 1.59 log W; $(1.0 < b < 2.18)$ - (2)

Beyond metamorphosis,

Herring (n = 11 points)

Log Y = 3.764 + 0.79 log W; $(0.62 < b < 0.97)$ - (3)

Plaice (n = 16 points)

Log Y = 0.465 + 0.85 log W; $(0.79 < b < 0.90)$ - (4)

The wide confidence limits are to great extent a reflection of the small number of points, as well as the restricted size range examined. From equations (1) and (3) there appears to be a sharp change in the value of the slope at metamorphosis in herring but examination of Fig. 25 shows that this is not so. There is a gradual change in the slope at metamorphosis but for convenience

the two stages are treated separately. The same explanation holds for the plaice data.

IV.3.4. Factors influencing the changes in gill area during development.

The effect of size on a number of gill dimensions can be determined from the data summarised in Tables 12 & 13. By reference to the formula on page 42 it can be observed that the values for gill area were computed from 3 sets of measurements viz. i) the total filament length which is equal to 'L' x N x 4; ii) the average secondary lamellar area which is equal to b x 1; iii) and the average spacing/mm of filament 'd'. Logarithmic transformations gave the best approximations to a straight line, wet weight again being used as a convenient expression of body size.

(i) Total filament length.

The total filament length was plotted against weight on a log-log scale for both species (Figs 27A & 28A). The lines were obtained by regression analysis. The general equation is

$$\text{Log } 1 = \log a + b \log W$$

The equations obtained together with the 95% confidence limits are as follows:

Herring (up to 40 mm, n=16)

Fig.27. Relationship between gill dimensions and

weight in herring.

A. Total filament length and weight.

B. Area of an average secondary lamella and
weight.

C. Secondary lamellae/mm of filament and
weight.

HERRING
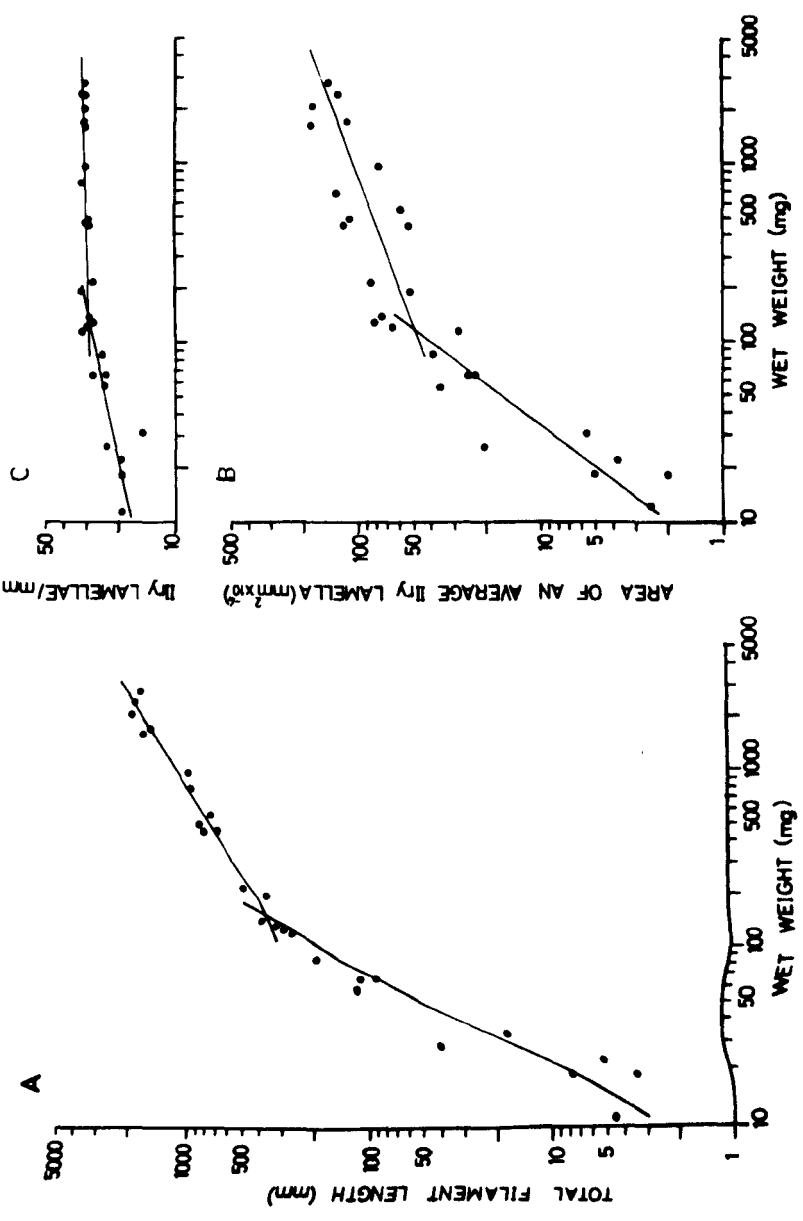
Fig.28. Relationship between gill dimensions and
weight in plaice.

A. Total filament length and weight.

B. Area of an average secondary lamella
and weight.

C. Secondary lamellae/mm filament and
weight.
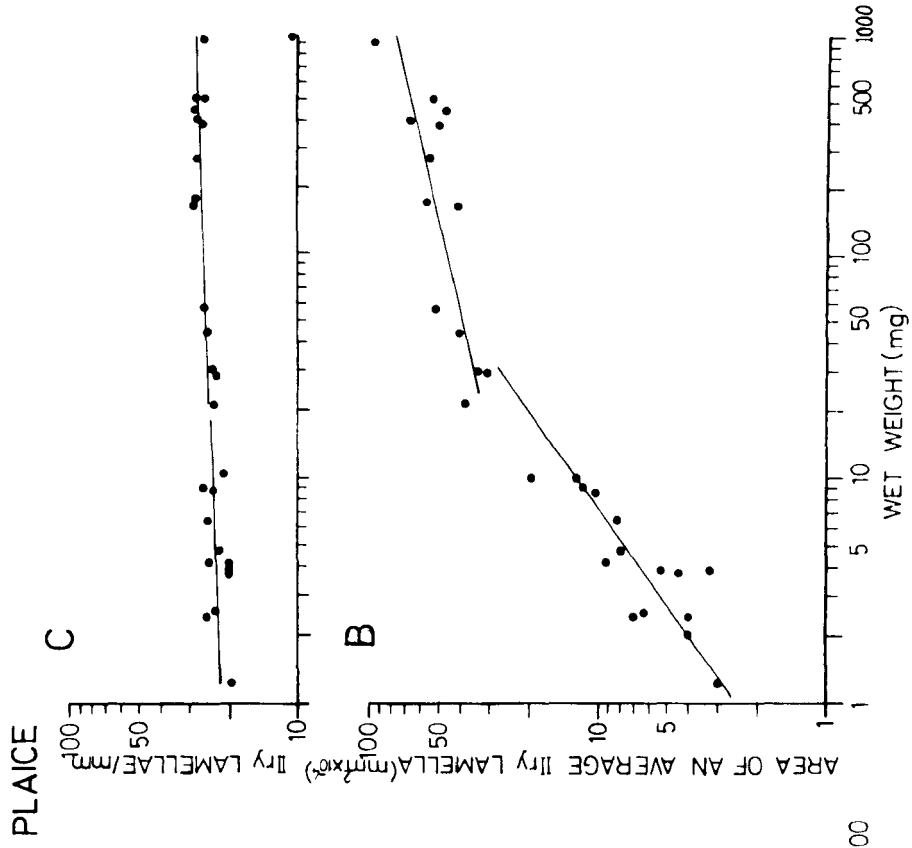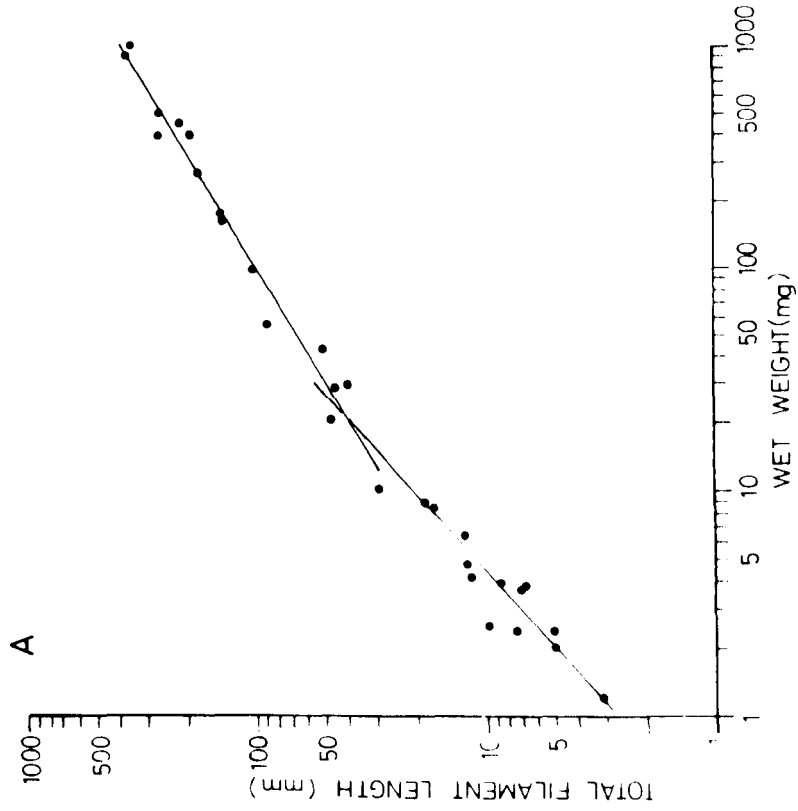
PLAICE

Log L =   0.0342 + 1.84 log W (1.56< b< 2.12) - (5)

Plaice (up to 12 mm, n=13)

Log L =   0.3586 + 0.88 log W (0.64< b< 1.12) - (6)

Beyond metamorphosis,

Herring (n=11)

Log L = .   20.89 + 0.57 log W (0.44< b< 0.70) - (7)

Plaice (n=16)

Log L =   6.609 + 0.59 log W (0.55< b< 0.61) - (8)

The wide confidence intervals are to some extent due to the smaller number of points and probably due to the variation observed in the population as well.

Filaments with lamellae first appear on arch III and IV in herring. This is followed soon after by their appearance on arch II and lastly on arch I. Although the filaments appear last on arch I the number of filaments increase rapidly until after the 35 mm stage, arch I has the greatest number of filaments.
The detailed patterns are given in Appendices III A & B.

In plaice the patterns are rather different. Filaments are present on   arch I, II and III at hatching. Later (about 9 mm) the filaments appear on arch IV, and secondary

lamellae on arch III. Lamellae appear first on arch II
followed by arch I. It is evident from Appendix III A
that from the start arch II has the largest number of
filaments in contrast to herring. Detailed patterns
of development are given in Appendix III A & B.

(b) <u>Area of a single secondary lamella.</u>

The weighted average lamellar area was plotted
against weight on log-log coordinates for all the
individuals studied in both species (Fig. 27B & 28B).
Each point represents the total area of a single average
lamella i.e. for both sides of the lamella obtained as
described in section IV.2., for that particular larva.
As in the previous section all the points were found to
fall on a curve whether they are plotted against length
or weight, and therefore as in the previous section the
two groups were separated out and the points plotted on
log-log coordinates as before.
The general equation is

$$\text{Log } Y = \log a + b \log W$$

where Y = area of an average secondary lamella (mm^2), $a=$
area of an average secondary lamella for a 1 mg fish, b =
the slope and W = weight of fish in mg.

The equation and 95% confidence intervals for the slopes are as follows;

For herring (up to metamorphosis), n=16)

$$\log Y = 0.0000098 + 1.3014 \log W \quad (1.01 < b < 1.59) - (9)$$

For plaice (up to 12 mm, n=13)

$$\log Y = 0.00024 + 0.71 \log W \quad (0.43 < b < 1.85) - (10)$$

Beyond metamorphosis,

Herring,

$$\log Y = 0.00088 + 0.36 \log W \quad (0.04 < b < 0.68) - (11)$$

Plaice,

$$\log Y = 0.00163 + 0.21 \log W \quad (0.17 < b < 0.26) - (12)$$

From the values for 'a' in the above equations it is evident that a 1 mg plaice has a higher average secondary lamellar area than a herring of equal weight. In contrast the rate of development of the secondary lamellar area is higher in herring than in plaice.

(c) <u>Spacing of the secondary lamellae.</u>

The average spacing per mm of filament was plotted against weight on log-log coordinates as before. Over the size ranges examined the spacing did not increase significantly in both species. The lines obtained by regression analysis are shown in figs. (27C & 28C).

The general equation is as follows;

$$\log Y = \log a + b \log W$$

where Y = spacing/mm of filament, a = spacing for a 1 mg
fish, b = the weight exponent and W the weight in mg.
The equations obtained with the 95% confidence intervals
for the slopes are as follows:

Up to metamorphosis,

Herring (up to 40 mm, n = 16 points),

$$\log Y = 10.65 + 0.201 \log W \quad (0.13 < b < 0.27) - (13)$$

Plaice (up to 12 mm, n = 13 points)

$$\log Y = 21.21 + 0.045 \log W \quad (-0.07 < b < 0.11) - (14)$$

Beyond metamorphosis,

Herring (n = 11 points),

$$\log Y = 24.89 + 0.035 \log W \quad (0.02 < b < 0.04) - (15)$$

Plaice (n = 16 points),

$$\log Y = 21.45 + 0.040 \log W \quad (0.05 < b < 0.03) - (16)$$

From the above equations it is evident that in the early
stages the spacing for 1 mg of wet weight in herring is
about half that of plaice. Beyond metamorphosis however
the spacing per mm of filament for 1 mg of weight as
denoted by the 'a' values are approximately equal.
Comparison of the slopes gives a different pattern. In
herring there is an initial high rate of development in

the spacing per mm filament when compared to plaice,
(b values of 0.20 & 0.05 respectively). After
metamorphosis the rate slows down in herring to
approximately the same rate as in plaice.

The pseudobranch is rather difficult to dissect out
especially in the early stages of its development and
hence some were lost in the process of dissection. The
results for both species are given in Tables 12 & 13.
It is evident from these values that the pseudobranch
does not keep pace with gill development and its
contribution towards uptake of oxygen as measured by
its surface area in the pre-metamorphic stages of both
species is very small.

From histological sections it was evident that the
skin of newly hatched larvae of both species was extremely
thin confirming the observations of other workers on these
two species. At the pre-metamorphic stage in herring
around 31 mm the skin is slightly thickened being about
1 - 2 cells in thickness. In newly metamorphosed fish
it is about 3 - 5 cells in thickness. Plaice also show
a thickening of the skin at metamorphosis (Roberts et al.

1973, and the present study).

Newly hatched herring larvae show no evidence
of corpuscles in the blood even though the heart starts
to beat prior to hatching and a circulatory system is
present in the larvae. From histological sections it
was evident that at about 14mm there was still no
evidence of corpuscles; however, in the cavity of
the ventricle and around the gut region a matrix
was present which stained light green with Mallorys
trichrome stain. Later (16mm) definite corpuscles
were present but it was not possible to distinguish
between erythrocytes and leucocytes. At about 29mm
both erythrocytes and leucocytes could be distinguished.
The number of corpuscles were present in greater
concentration in metamorphosed fish and the different
types of leucocytes were also easily distinguished.

Plaice larvae show no corpuscles in the blood at
hatching as in herring, nor at the start of feeding.
Corpuscles of both types are observed at about 10mm as
the larvae metamorphose.

IV. 4. Discussion.

The general surface area to length relationships in
herring and plaice show isometric growth (page 44 & 45)
according to Huxley and Teissiers (1936) terminology
(cited by von Bertallanfy, 1938).

Dry weight-length relationships for herring are
similar to that of Marshall, Nicholls and Orr (1937), who
obtained a slope value of 4.52 for spring spawned larvae
in the Clyde. Ehrlich (1972) obtained a value of 4.54 for
the slope in larvae reared up to the pre-metamorphic stage.
These are comparable to the value of 4.19 in the present
study which included metamorphosed fish as well.

Ryland (1966) assumed a slope value of 3 for the dry
weight length relationship for plaice larvae based on the
data of Meek (1903). Ehrlich (1972) obtained a slope value
of 3.92 for plaice up to 25 mm, as compared to 4.58 in the
present study which also included only newly metamorphosed fish.

The fins appear to play an important role in cutaneous
respiration in the early stages as is evident from the
slowing down of the fin area proportional to body weight
and the subsequent gill development in herring. An inverse
relationship is observed between fin area and gill area in
the pre-metamorphic stages in both species. Holeton (1971a)

found that in young trout larvae the rhythmic movement of the pectoral fins tended to create considerable currents of water around them. The pectoral fins probably act as accessory ventilatory organs in the early stages.

In both species, the slope of the gill area body weight relationship is greater in the early stages of its development than that obtained several weeks long after metamorphosis. In herring it decreases from a value of 3.36 to 0.79 while in plaice it decreases from 1.59 to 0.85. The values for the slope after metamorphosis are similar to those of the oxygen consumption to weight relationships. For the herring and plaice larvae the values are 0.82 and 0.65 respectively, but included only newly metamorphosed fish (see Part Two), unlike the gill area work.

Winberg (1960) derived 0.8 as a value representative of the O_2 consumption/body weight relationship of a large number of species, while Paloheimo and Dickie (1966) observed that 0.80 characterised most species. Nevertheless a great deal of variation from this value has been observed and Muir and Hughes (1969) hypothesize that species variation in the gill area to weight relationship could be expected as well.

However it must be pointed out that the anatomical areas of the gill measured need not necessarily be the functional area as well. It is only a measure of the maximum possible functional area (Randall, 1970b). Hughes

(1966) estimated that the respiratory surface was between 60-70% of the total lamellar surface, based on the area of blood channels in the secondary lamellae. Shunt mechanisms too, may come into operation and therefore the functional area may be smaller than 60-70% of the lamellar surface area (Randall, 1970b), since alternate non respiratory pathways that shunt blood past the secondary lamellae can be used to decrease lamellar blood flow (Steen and Kruysse, 1964).

Price (1931) obtained values of 0.78 and 2.38 for the slope in the gill area to weight and gill area to length relationship in fish of a size range of 2-40 cm. This compares favourably with the values of 2.76 and 2.61 for the gill area to length relationship in herring and plaice respectively. Ursin (1967) analysing Gray's (1954) data reported values of 0.82 for the slope of the gill area to weight relationship. Muir and Hughes (1969) obtained values of 0.85 for three species of tuna and 0.90 for the blue fin tuna alone. Comparison of equations (3) and (4) shows that a 1mg herring has a correspondingly higher gill area than a plaice of equal weight. This is to be expected as a herring is comparatively more active than plaice, also borne out by the high proportion of red to white muscle in herring, which is correlated with the activity of the fish (Love, 1970). Red muscle has not been recorded for plaice. Nevertheless it may be

significant that the slope value for 'O' group plaice
is higher than that of herring though not significantly
different (f = 0.23, P < 0.05). It is therefore
tentatively concluded that the high rate of gill
development may be of some adaptive value, young plaice
being known to migrate up to the shallower parts of
bays (Macer, 1967; R. Gibson, personal communication).

Table 14 gives a comparison of the present data with
that of other authors for fish of the same weight. It is
evident that a 1g herring and bass have approximately the
same gill area while that of a plaice is five times less.
Tunas on the other hand have gill areas about five times
that of a herring or bass. The roach has a gill area about
half that of a herring or bass. Many authors have
commented on the relationship between gill area and the
activity of a fish. Gray (1954) found that the gill area
per g of weight of an active fish <u>Brevoortia</u> <u>tyranus</u> was
10 times greater than that of a slow moving fish (<u>Opsanus</u>
<u>tau</u>). In herring after metamorphosis the gill area per
unit weight is about 3.5 times that of plaice.

Price's (1931) observation that in the early stages
an increase in the gill area is brought about mainly by
an increase in the total filament length, is confirmed in
the present data. From equations (7) and (8) the total

Table 14. Total gill area of herring and plaice (postmetamorphosis) compared with values of other species based on a log-log relationship of gill area to body weight.

Species	1g	100g	Author
Herring	882.36 mm^2	33,546 mm^2	Present data
Plaice	165.02 "	8,269 "	" "
Bass	865 "	33,000 "	Price, 1931 – based on Muir & Hughes calculations of Price's data
Skipjacktuna	5,218 "	262,000 "	Muir & Hughes 1969
Yellowfin & blue fin tuna	4,025 "	200,000 "	" "
Roach	398 "	19,000 "	" "

filament length for a lg herring and plaice was found to
be 1071 mm, and 389 mm respectively giving a ratio of 2.75. Table
15 gives a comparison of the present data with that of
Price who studied some fish in the size range investigated
here.

One interesting observation is that the spacing per mm
of filament increases with size, in the size range examined
for both species. In other species of fish studied the
spacing decreases (Muir and Hughes, 1969; Muir, 1969). Price
(1931) who has made the only other developmental study on bass has
not observed this either, probably due to his grouping of
the data. From the present data lg m herring and plaice
possess spacings of 31 and 28 lamellae per mm of filament
respectively as compared to Price's value of 40 per mm,
but since the slopes are positive it is possible that an
initial increase in the spacing per mm of filament is
followed by a decrease later on. The spacing of the
secondary lamellae varies greatly in different species
of fish. Hughes, (1966) and Hughes & Shelton (1962) have
considered its significance from an ecological viewpoint.
They state that in general, the more active fish possess
a larger number of filaments of greater length, (borne
out by the results in Tables 12 & 13) and therefore a
greater number of secondary lamellae. Further the secondary

lamellae are smaller and more closely packed than those

of a more sluggish fish. The smaller pore size in the

active species is believed to be advantageous in that

the diffusion distance is decreased although the gill

resistance will be increased. Thus more sluggish fish

have widely spaced lamellae and a reduced total filament

length, the resistance to flow relative to area being

less in these species. From Table 15 it is evident that

larval herring and plaice show their similarity to the

'active' and 'sluggish' category from a comparative

viewpoint as regards spacing of lamellae & the pore size of is

metamorphosed herring and plaice which 0.058 and 0.77 mm

respectively, (determined from histological sections).

Harder (1954) believes that the pseudobranch

contributes towards gas exchange in herring. However,

in the early stages of its development in herring and

plaice the surface area is too small to make a

significant contribution towards gas exchange. It may

be doing so in the post-metamorphic stages, but one

cannot make definitive conclusions about it. Unlike the

gill area to weight relationship where a change was

observed in the slope around metamorphosis, no such

change was observed in the case of the pseudobranch.

As the pseudobranch is believed to have an osmoregulatory

Table 15. Gill dimensions for herring and plaice compared with that of Price's (1931) data on bass for the same weight range.

Species	Weight mg	Total filament length (mm)	Spacing/mm of filament	Total number of pores (N) x 10^4 *
Herring	300	539.38	30.39	1.745
Plaice	300	191.28	26.95	0.709
Bass	332	252	28.08	0.706

* calculated using Hughes (1966) method.

function its relationship with size is bound to be a reflection of this parameter as well.

It is between the time of appearance of the gills and metamorphosis that the gills must develop in structure and function to take on the role of the primary gas exchange organs. Correlated with this there is a rapid development of gills initially in both species, with a slowing down in the rate after metamorphosis. The gill area of newly hatched trout is small and gas exchange after hatching probably takes place through the vascularised surface of the yolk sac (Holeton, 1971a). Here too, a rapid growth and development of the respiratory pumps was observed during the first 18 days after hatching, similar to the gill development in the present study. The skin is extremely thin in newly hatched plaice (Roberts et al. 1973) and herring (Jones et al. 1966). At metamorphosis the skin is thickened in herring and in plaice (Roberts et al. 1973) to take on the protective function of the adult.

PART FIVE.   THE DEVELOPMENT OF HAEMOGLOBIN.

V.1. Introduction.

Although the haematology of adult fish is well
documented (see Hunn, 1959; Mawdesley-Thomas, 1969;
Hawkins and Mawdesley - Thomas, 1972;) in recent
years its importance has increased due to its value
as a tool in the diagnosis of fish diseases (Larsen
& Snieszko, 1961; Snieszko, 1960; Summerfelt, 1967)
particularly in view of fish farming. In addition
fish are increasingly being used as a test species
in toxicological screening particularly in pollution
studies (Mawdesley - Thomas 1971). Thus many workers
have stressed the need for establishing normal
haematological values for fish. In contrast to this
haematological parameters in young fish larvae have
been sorely neglected. Radzinskaya (1960) attempted
to determine the first appearance of haemoglobin in
embryonic sturgeon, while Ostroumova (1962) determined
its appearance in embryo's of rainbow trout. Later
Radzinskaya (1966, 1968) measured its concentration in
embryonic and juvenile salmon (<u>Salmo salar</u> L.).
The present investigation was an attempt $\overset{(a)}{\underset{\wedge}{}}$ to determine
(a) the time of appearance of haemoglobin using the method
of Slonimski (1927) and (b) measure it quantitatively using

the pyridine-resorcin micromethod of Korzhuev and Radzinskaya (1957). The above methods involve the use of the peroxidase properties of haemoglobin. These properties of haemoglobin have been used by many authors for its determination, both qualitatively and quantitatively when it is present in very low concentrations (Crosby and Furth, 1956) and forms the cited by Radzinskaya 1960 basis of the benzidine and pyridine-resorcin method. Other derivatives of haemoglobin such as methemoglobin, oxyhaemo-globin and carboxyhaemoglobin also give a peroxidase reaction, while other iron containing haemin compounds such as cytochrome, cytochrome oxidase, catalase and myoglobin also possess peroxidase properties (Radzinskaya, 1960).

V.2. Methods.

(a) Histochemical identification.

The benzidine method of Slonimski and Lapinski (1932) as cited by Radzinskaya (1960) was used with slight modification (refer Appendix IV). Drawings were made of the stained larvae and some photographs were also taken.

(b) Spectrophotometric determination.

The method of Korzhuev and Radzinskaya (1957) which involved a visual matching of colours was used with modification for the spectrophotometric methods of analysis. Haemoglobin acts as a catalyst in the oxidation process where hydrogen peroxide oxidises the pyramidon giving a violet colour. During the

reaction the haem group is believed to form an intermediate compound which is coloured violet by the hydrogen peroxide. Details of the standards used and the analytical method are given in Appendix IV & V.

V.3. Results.

V.3.1. Histochemical identification.

The results are summarised in Table 16 and are illustrated by Fig.29A & B. According to Radzinskaya (1960) the slight bluish colour observed in the early stages is not a true peroxidase reaction, the bluish green colour being the true one. Peroxidase synthesis appears to take place very early in development in both species (Table 16). Plaice at stage 4b (Shelbourne; 1957) show no visible pink colouration of the body and no peroxidase activity is observed. There is however a light pink colouration of the cardiac region particularly above (dorsal to) the ventricle which is thought to arise from haemoglobin or a precursor in the pericardial fluid. The fluid when released gives a deep blue colour with the benzidine reagent. In stage 5 plaice (i.e. just metamorphosed) the blood corpuscles in the vessels to the gills turn a visible pink colour. In addition the paired dorsal aortae are also pink at this stage. The fluid from

Fig.29. Sites of peroxidase synthesis in larvae as
determined by the benzidine test.

A. Herring at (i) 1 week of feeding.

   (ii) 5 weeks of feeding.

B. Plaice at (i) 2 weeks of feeding (ventral
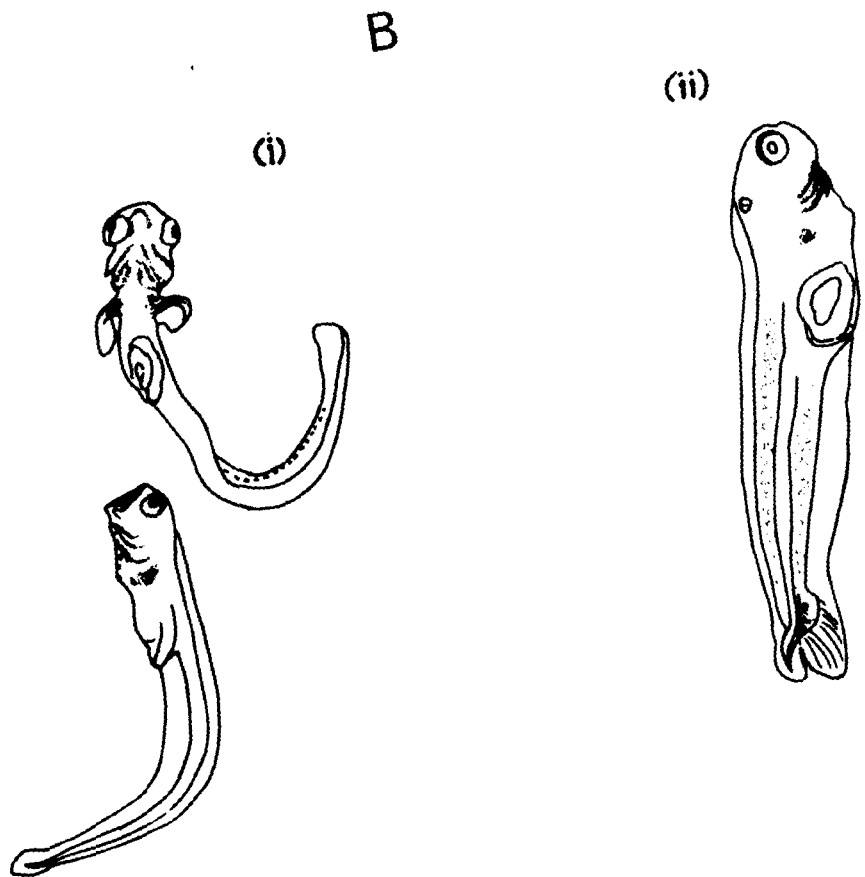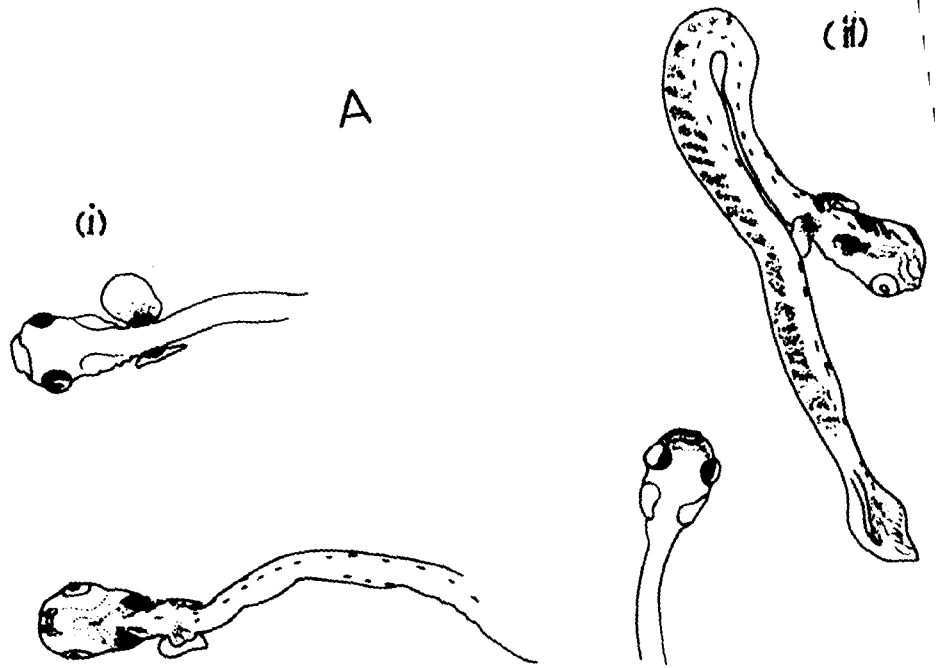   and lateral view). (ii) 3 weeks of feeding.

A

(i)

(ii)

B

(i)

(ii)

Table 16. Summary of the staining characteristics of the two species using the benzidine test of Slonimski (1927).

Stage of development	Species	Staining characteristics
Eggs	Herring	48-68 h eggs show a slight bluish reaction at the region of the blastopore, and at the attachment discs. 4 day old eggs show a more intensive staining of membrane but not of larvae. Later (10 days) membranes stained very rapidly (2-3 mins) and after about 5 mins the larva took on the stain. Separation of the larva from the egg membrane showed that the region of the body just behind the head and until about the last quarter of the body gave a slightly bluish stain. No stain was observed in the heart region or connection to the yolk sac, indicating the absence of peroxidase in these areas.
	Plaice	Membranes do not give a reaction in 3 day old eggs, but the larva gives a slight reaction. Later stage eggs (10 days old) give a more intense stain in the larva.
Yolk-sac	Herring	Gill region and heart give a bluish green colour, within 1-2 mins rest of the body took on a light green stain.
	Plaice	" " " " " " " " " " " " " " " " " "
1 week of feeding	Herring	Similar to the yolk-sac stage. In addition branches of the mandibular arterial arch also gave a stain, and at the base of the pectoral fins an intense stain appears in the form of granules (Fig.29 A(i)).
2 weeks feeding	Plaice	Similar to yolk-sac stage except that gill and heart region stain more intensely, and blood vessels to gill and ventral head region take on the stain as well (Fig.29 B(i)).
3 weeks feeding	Plaice	The ventricle does not stain probably due to a thickening of its wall, but sinus venosus stains intensely, as well as the blood vessels of gill and ventral part of the head. In addition the base of the caudal fins also give an intense stain. (Fig.29 B(ii)).
4 weeks feeding	Herring	Similar to 1 week of feeding. Gills staining more intensely than heart, probably due to increased muscle development in or around the heart.
5 weeks feeding	Herring	Similar to 4 weeks feeding. In addition an intense staining was observed at the base of the caudal fin (Fig.29 B(ii)).

these vessels give a deep blue colour when released. It
is probable that both corpuscles and fluid give the reaction
although no attempt was made to distinguish between them.

In herring the following changes are observed around
metamorphosis.

(a) At about 32 mm there is no visible pink colour in the
heart or gills and the larva is still transparent.

(b) Later (35-36 mm) the heart is light pink in colour,
the gills being lighter. Slight pigmentation was observed
dorsally and there was no silvery colour on the body (i.e.
no scales are present).

(c) At 37-38 mm the heart and gills are bright pink in
colour. The belly region is silvery at this stage. The
pericardial fluid when released gives a dark blue colour
with the benzidine reagent.

(d) About the 44 mm stage the heart and gills are bright
red in colour and the vessels along the length of the body
are a deep reddish pink. The dorsal part of the body is
deeply pigmented at this stage and the branchiostegal
apparatus is silvery in colour.

V.3.2. Quantitative measurements.

The results are expressed graphically as total
peroxidase concentration/larva and peroxidase concentration/
unit weight against weight on a log-log scale (Figs 30, 31 &
32 and Table 17). In herring the concentration per

Fig.30. Relationship between peroxidase concentrations per

larva and weight in herring.

∇ - I97I

▼ - I972
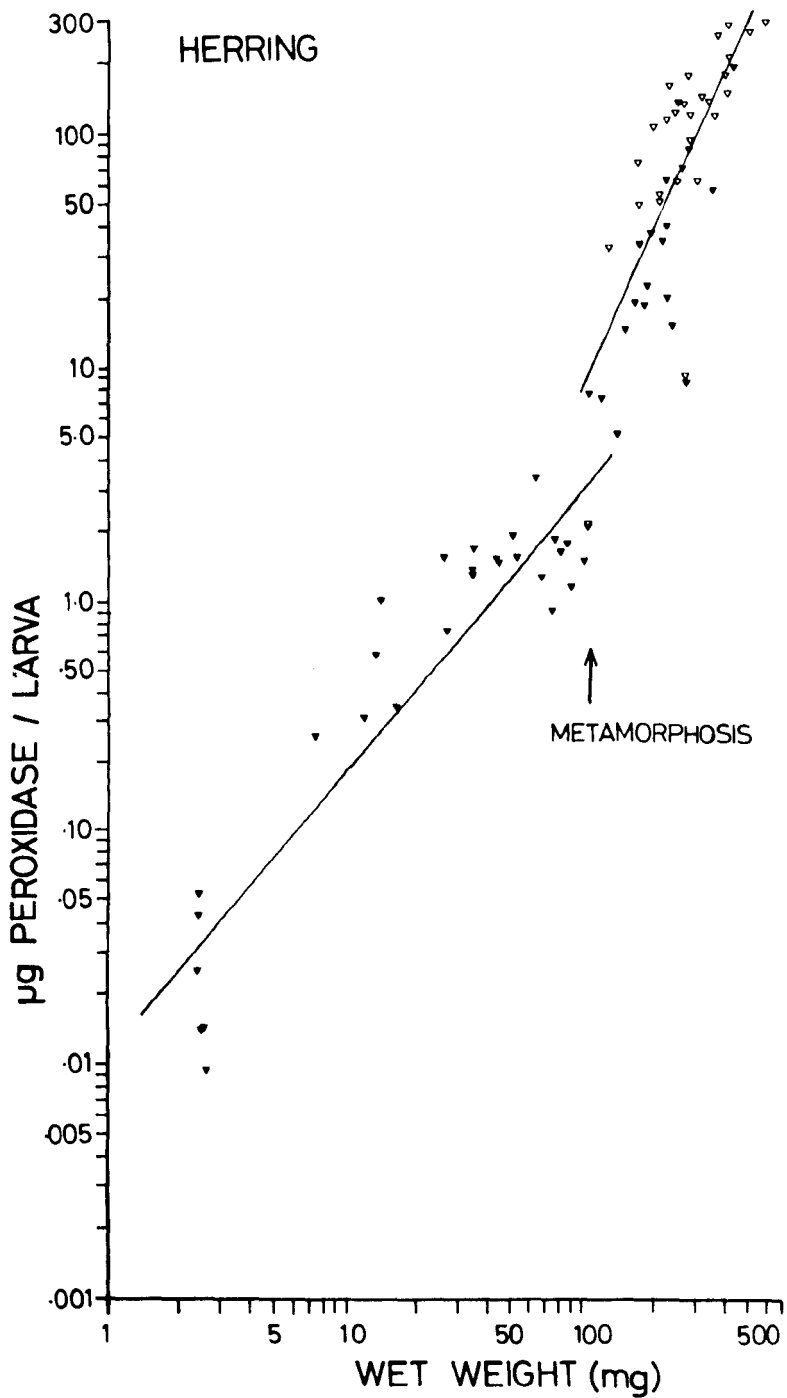
Fig.31. Relationship between peroxidase concentration per larva and weight in plaice.

PLAICE

REARED   WILD
▽–1971   ○–1971
▼–1972   ●–1972
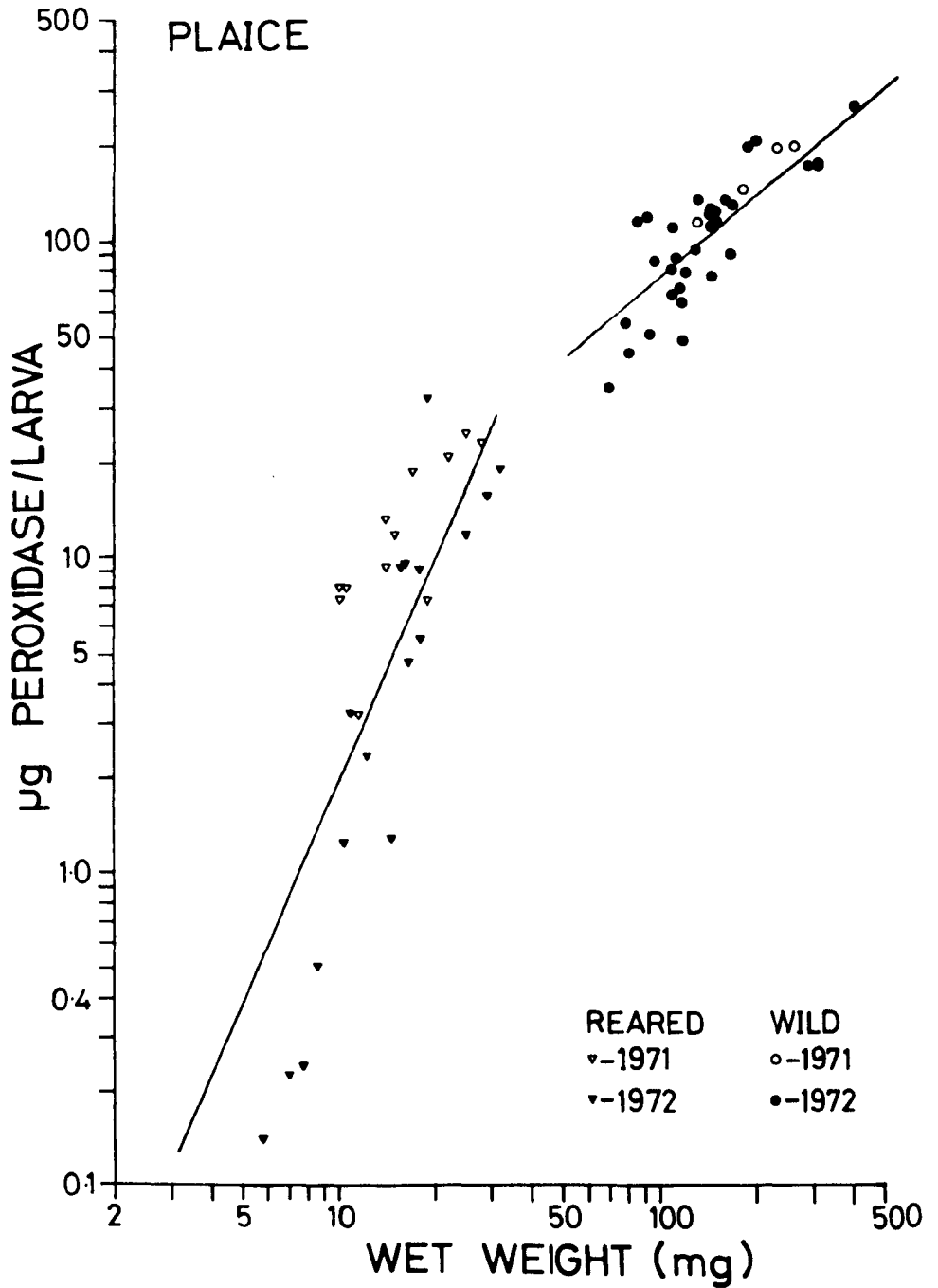
Fig.32. Relationship of peroxidase concentration per

unit weight, to weight in A. Herring.

B. Plaice.
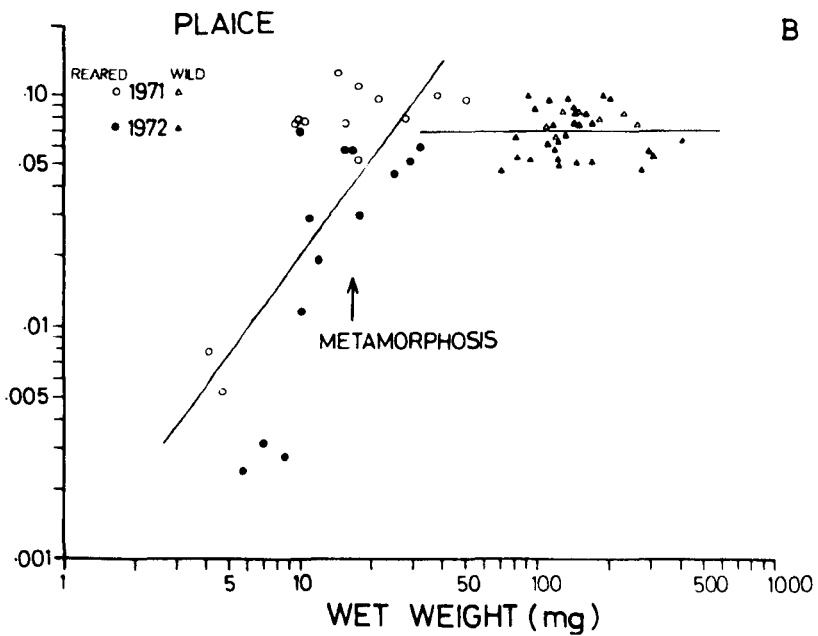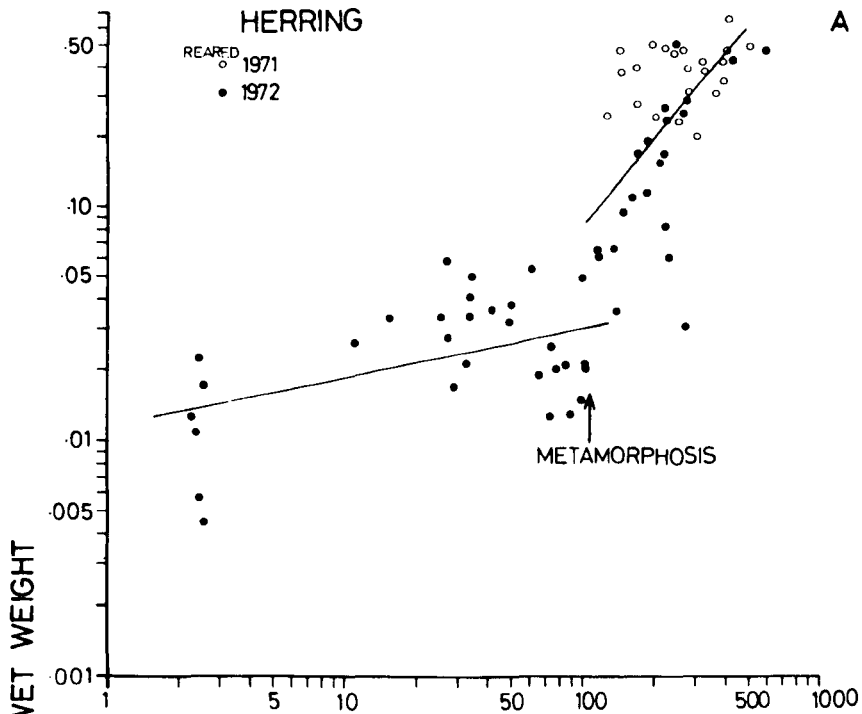
Table 17. Equations for the relationship between concentration of peroxidase and wet weight in herring and plaice.

Herring		Equation	S.D. of Slope	N
μg Peroxidase/Larvae	Reared – up to met.	Log Y = 0.110 + 1.222 Log X	0.08	31
	metamorphosed	Log Y = 0.002 + 2.267 Log X	0.22	49
μg/mg Wet Wt.	Reared – up to met.	Log Y = 0.110 + 0.214 Log X	0.08	31
	metamorphosed	Log Y = 0.002 + 1.262 Log X	0.22	49
Plaice				
μg Peroxidase/Larvae	Reared	Log Y = 0.089 + 2.353 Log X	0.28	27
μg/mg Wet Wt.	Reared	Log Y = 0.090 + 1.356 Log X	0.28	27
μg Peroxidase/Larvae	Wild	Log Y = 1.521 + 0.848 Log X	0.10	37
μg/mg Wet Wt.	Wild	Log Y = 0.662 + 0.009 Log X	0.10	37

larva increases rapidly from its time of appearance up
to metamorphosis, followed by an apparent further increase
in the slope in newly metamorphosed fish. This difference
is significant (p〉0.0005).

In plaice the slope decreases after metamorphosis as

reared

compared with 'wild' plaice caught locally. The difference
is highly significant (p〉0.0005).

Peroxidase expressed per unit weight shows an increase
in the slope at metamorphosis in herring (Fig. 32A and
Table 17) which is highly significant. (p〉0.0005). Plaice
on the other hand, show a decrease in the slope after
metamorphosis (p〉0.0005) (Fig.32B and Table 17). Comparison
of the relative amounts of peroxidase shows these values to
be higher in plaice than in herring.

V.4. Discussion.

The sites of peroxidase activity in herring and plaice
larvae **are** similar to the observations of Radzinskaya (1960,
1962) on embryonic sturgeon and salmon, and Ostroumova (1962)
on embryonic rainbow trout. The slight reaction observed in
the egg membranes are probably due to other respiratory enzymes
such as cytochrome, cytochrome oxidases and catalases, since
they are the sites of gas exchange at these stages
(Radzinskaya, 1960). The staining of the body musculature
as well as the intensive staining at the base of the pectoral

and caudal fins is probably indicative of myoglobin or
its precursors in the feeding stages. This may probably
play a respiratory role in the life of the larva.

Quantitative measurements show a higher rate of
haemoglobin synthesis per individual at the pre-metamorphic
stage in plaice when compared to herring, but not at
metamorphosis. It is evident that even in the early
stages of its appearance both absolute and relative
values of peroxidase are higher in plaice than in herring.

The change in the slope at the post-metamorphosis
stage in plaice (Fig.32 and Table 17) is indicative of
a slowing down of its rate, initially from 3.00 to 0.95.
The high rate of haemoglobin synthesis around metamorphosis
in herring as compared to the pre-metamorphic stages
(Fig.30 and Table 17) is probably a reflection of the
increasing activity of the fish at these stages. It may
also be possible that the role of haemoglobin in the
inflation of the swim bladder acts as a contributory
factor in the post metamorphosis stages.
        for its increase

A comparison of the present data with that of
Radzinskaya (1966) on salmon larvae is shown in Table 18.
Although the total concentration of haemoglobin in
Radzinskaya's data is low compared with the present
data, expressed as a ratio of weight it appears to be

Table 18.   Comparison of values of total and relative amounts of peroxidase of the present data with that of other workers.

Stage of development	Species	Weight Range (mg)	µg peroxidase/larvae			µg peroxidase/unit weight			Author
			mean	N	S.E.	Mean	N	S.E.	
Newly hatched	Salmo salar L.	-	0.106	18	-	0.60	-	-	Radzinskaya (1966)
20-25 days old	Salmo salar L.	-	0.120	36	-	0.65	-	-	"
1 month old	Salmo salar L.	-	0.140	12	-	0.74	-	-	"
6 weeks old	Clupea harengus	1.93 - 2.75	0.028	7	0.006	0.011	7	0.003	Present data
6 weeks old	Pleuronectes platesa L.	5.80 -15.00	0.238	5	0.030	0.042	5	0.010	"
10-11 weeks old Pre metamorphic	C. harengus	12 - 105	1.340	23	0.120	0.031	23	0.003	Present data
17 months old	S. salar	38,000	49.000	-	-	1.28	-	-	Radzinskaya (1966)
Newly metamor-phosed	C. harengus	141 - 438	93.99	49	11.84	1.548	49	1.238	Present data
" "	P. platessa	6 - 32	13.41	22	2.556	0.648	22	0.068	" "
'Wild'	P. platessa	92 - 410	115.19	37	8.585	0.716	37	0.031	" "

high at least in comparison to the values for herring.
Houston and DeWilde (1972) observe that in the adult
carp no significant correlation was obtained between
haemoglobin concentration (in the blood) and weight.
Nevertheless statistically significant correlations
were obtained between these two parameters for these
young larvae. Unfortunately Radzinskaya's data does
not give the weights of the larvae so that graphical
relationship cannot established.

    Haemoglobin increases the affinity for oxygen and oxygen
carrying capacity of the blood even at low pO_2's
(Wittenburg, 1959, 1963; Hemmingsen, 1963; Moll, 1966).
Fox (1954) concluded that many fish must use their
haemoglobin only in emergencies while Holeton (1971b)
observes that active fish need the use of their
haemoglobin even for resting respiration. Nevertheless
there is an increasing body of evidence that fish,
especially those from temperate environments are not
as dependent on their quantity of functioning haemoglobin as much
as a homeotherm. The most striking observation is the
discovery by Ruud (1954) of the haemoglobin-free fish
of the antarctic (the chaenichthyids) whose blood has
an oxygen capacity no higher than that of sea water.
Further most species of fish appear to have the ability
to lose most of their haemoglobin without suffering any

great damage. Steen and Berg (1966) and Ryback (1960)
give reports of fish with severe anaemia but otherwise
appearing normal. Many authors have used carbon monoxide
to block more than 90% of the oxygen carrying capacity of
the haemoglobin in a variety of fish without any harmful
effects (Nicloux, 1923; Anthony, 1961; Holeton, 1971b)
and also fish larvae (Holeton, 1971a). Fish from higher
latitudes appear to have lower levels of haemoglobin
(Everson and Ralph, 1967). Holeton (1972) suggests that
in the red blooded fish of the antarctic the haemoglobin
may serve primarily for the uptake of oxygen from a
hypoxic environment, emphasising the qualitative rather
than the quantitative aspects of haemoglobin.

Thus in fish larvae, the lack of a respiratory pigment
in the early stages of development in the plankton where no
oxygen depletion is likely to occur would not greatly affect
the metabolic requirements of the animal, this being offset
by the high body surface area to weight ratio (see Part Four).
Around metamorphosis when activity increases in an inverse
proportion to diminishing body surface area the appearance
of gills followed by the appearance of respiratory pigment
offsets any disadvantages that might otherwise accrue,
especially under hypoxic conditions (de Silva and Tytler,
1972).

GENERAL DISCUSSION.

Although the study of larvae is a very rewarding one in that it is a relatively new field, considerable difficulties lie in the seasonal nature of the material and the rearing of larvae which is very time-consuming. From the present study it appears that the early feeding stages of both species are sensitive to low oxygen from the point of view of their oxygen requirements as shown by the high LD_{50} values at these stages. Survival at low oxygen tensions was achieved in the early feeding stages of both species by a fair degree of "regulation" that is , as pointed out earlier they regulate their oxygen consumption down to some critical pressure, followed by "conformity" in the later feeding stages where the oxygen consumption increases with increasing environmental oxygen concentration. A certain degree of switching back to regulation was observed in the metamorphosed fish due to the appearance of haemoglobin at metamorphosis. This results in the metamorphosed fish being more resistant to lowered oxygen concentrations due to the increased oxygen-carrying capacity of the blood. The development of both gill area and haemoglobin concentration (measured as peroxidase) show similar patterns in that the relationships of these parameters with size show a change in the slope around metamorphosis. The peroxidase concentration per unit weight is higher in plaice than in herring but the gill area to body weight ratio is lower, probably

offsetting the haemoglobin concentration in metamorphosed fish.

One cannot say whether the gills are functional after the appearance of the secondary lamellae at 3 weeks and 5 weeks of feeding in plaice and herring respectively, but it is highly probable that they play a role in respiration however slight as the external surface area is decreasing considerably in relation to body size. Further, the skin thickens as metamorphosis takes place in herring and plaice as shown in the present study. This has also been reported for plaice by Roberts et al. (1973). The gills will presumably have to take over the major respiratory role at this time. It may also be significant that even in the early stages of gill development the longest filaments are found on that part of the gill arch where maximum water flow occurs.

Gill area to body weight relationships for both species up to metamorphosis have high slope values of 3.36 and 1.59 for herring and plaice respectively. When the total respiratory surface area, including that of the gills when they appear (Table 19 & 20), is plotted against body weight on a log-log basis, slope values of 0.59 and 0.51 are obtained for herring and plaice. Since the oxygen uptake to body weight relationship has been found to be similar to that of the gill area to body weight relationship for adult fish the total respiratory surface

Table 19. Total respiratory surface with development in herring and plaice.

Herring

Total Surface Area (mm^2)	Gill Area (mm^2)	Total Surface Area + Gill Area (mm^2)	Wet Weight (mg)
24.268	-	24.268	1.138
35.199	-	35.199	1.827
57.330	-	57.330	4.583
87.899	-	87.899	10.649
113.065	0.071	113.136	15.612
141.38	0.591	141.97	29.249
152.52	1.102	153.60	35.215
246.36	9.201	255.56	66.230
320.32	50.944	371.26	110.220
423.82	118.102	541.92	141.560
(588.85)	659.282	659.282	236.160

Plaice

Total Surface Area (mm^2)	Gill Area (mm^2)	Total Surface Area + Gill Area (mm^2)	Wet Weight (mg)
14.57	-	14.57	0.370
25.07	-	25.07	1.163
41.51	.229	41.74	3.512
77.98	1.245	79.23	10.161
104.52	3.846	108.37	20.654

Table 20.   Equations for the relationship between the total respiratory

surface and weight in herring and plaice.

Correlation Coefficient

Herring   Log Y = 1.3476 + 0.594 log X   r = 0.9924

Plaice   Log Y = 1.3705 + 0.505 log X   r = 0.9988

where Y = Total respiratory surface (mm^2)

X = Wet weight (mg)

to body weight relationship should also be similar. The lower values obtained in the present instance is therefore surprising but other information such as the diffusion rates and the rate of ventilation needs to be known, as well as the thickness of the water - blood pathway is required before definitive conclusions are reached.

Vaznetsov (1953, quoted by Tesch, 1968) says that during their development fish typically pass through several phases each of which has its own length-weight relationship, that is with a change in the 'b' value (slope). Tesch suggests that these may be "crises" in the life history, metamorphosis being an example. Such a change in the slope is evident from the work of Marshall, Nicholls & Orr (1937 & 1939) on herring larvae and juveniles, and Le Cren (1951) on perch. The present study reveals that such changes were also evident in other relationships, for example that of gill area to body weight and haemoglobin concentration to body weight. There may well be other similar developmental events.

The lack of well developed respiratory structures would not be a disadvantage immediately after hatching as the external surface area is high compared to size, but as development proceeds and the larvae begin to feed and generally increase in activity it may become a critical factor. As the larvae increase in size the demand for oxygen increases and therefore the larvae are more susceptible to decreasing oxygen

concentration. This is illustrated by the high LD_{50} values in the early stages. Further, the absence of blood corpuscles in these stages would mean a decreased blood viscosity and improved blood flow, but the lack of haemoglobin means that the oxygen-carrying capacity is low. The present study has not revealed whether stages prior to haemoglobin formation are at a disadvantage due to its absence. The appearance of haemoglobin around metamorphosis may be due to the normal course of events, taking place at a particular length, or it may also be due to a functional need due to increasing activity at this time. Herring larvae start to shoal about 25-28 mm (Rosenthal, 1968) and plaice begin to move into inshore waters at metamorphosis (Gibson, personal communication). To summarise the above:

Herring

Young	Intermediate	Metamorphosed
Advantages		
(1) High surface area /body weight ratio.	Gills starting to develop	Gills increase area for gas exchange.
(2) Oxygen transport over short distance to site of requirement.	Low blood viscosity.	Hb - increases oxygen carrying capacity.
(3) Low blood viscosity.		
Disadvantages		
(1) Poor oxygen carrying capacity.	Poor oxygen carrying capacity.	High blood viscosity.
(2) Active.	Increasingly active.	Highly active, pelagic, shoaling.
	Surface area/ body weight ratio decreasing.	Low surface area/body weight ratio

In plaice the stages are similar to herring except in the metamorphosed fish where at metamorphosis they settle down on the bottom and lead a benthic existence.

The tolerance levels of herring and plaice larvae to water of low oxygen concentration can be correlated with their respective ecological niches. Herring lay demersal eggs and the low LD_{50} values for yolk-sac herring is probably advantageous when the larvae find themselves in water of low oxygen concentration in or around the layers of eggs on the spawning beds. Parrish et al.(1959) observed that eggs were 4-8 layers thick on the spawning bed off the Firth of Clyde while Bowers (1969) observed that it was 2-9 eggs deep in one patch off the Isle of Man. Plaice on the other hand lay pelagic eggs (Simpson,1951; Shelbourne, 1957) and therefore the larvae when hatched are more likely to find themselves in regions of high oxygen content.

Adult fish respond to hypoxia by both circulatory and respiratory adjustments. Some of these responses are observed in fish larvae. Holeton (1971a) observed a bradycardia in trout larvae at oxygen levels below the lethal limit reported for these species by other workers. In addition, ventilation rates may also increase as shown by the present study and by Holeton(1971a). In the early feeding stages there is a certain amount of regulation in the two species followed by conformity at the later feeding stages.

It has been pointed out by some workers (see Satchell,

1971) that the response of independent and dependent species
of adult fish (regulators and conformers respectively) may
each offer specific advantages under certain enviroⁿmental
conditions. Independent species (regulators) with their ability
to increase ventilation to offset lowered oxygen content in
inspired water can maintain the supply of oxygen to their
tissues, and as such are well suited to survive hypoxia of
short duration. On the other hand dependent species (conformers)
may be better able to survive longer term hypoxia. The
reduced rate of oxygen consumption by the older larvae may be
one of the courses open to them for survival during a time of
switching over either partially of completely, to gill respiration
especially if the energy involved in extracting oxygen from
water of low oxygen content. is too great to be maintained.

 One interesting aspect that must be discussed at this stage is
the behavioural response to water of low oxygen content. It
is well known for large fish that activity is reduced by a
reduction of swimming speed in hypoxic water. Davis et al.
(1963) observed a decrease in the swimming performance of
Pacific salmon in response to hypoxia as did Kutty (1968)
working on goldfish and rainbow trout. From oxygen consumption
studies at low oxygen concentration it was evident that when
~~the oxygen concentration of the environment is lowered~~ herring
~~and plaice larvae reduce their activity~~ . by
reducing their oxygen consumption. It might seem important
for organisms to increase their activity in progressively

hypoxic conditions to increase the chance of escape before the oxygen concentration reached a lethal level.

Rosenthal & Hempel (1970) estimated that herring larvae at the yolk-sac stage move about 12-36 m/h and larger larvae (18-20 mm) move 36-72 m/h. Further, the yolk-sac larvae show more intermittent movement followed by periods of rest and slow sinking, whereas larger larvae swam more continuously. Larvae also swam much faster before feeding. Blaxter (1962) observed that the maximum swimming velocities in herring larvae increased from 3 cm/sec at 8 mm to 30 cm/sec in 20 mm larvae, Rosenthal & Hempel(1970) observed that the velocity of darting increases with increasing length of the larvae from about 6 cm /sec in yolk-sac larvae of the herring to 25 cm/sec in larvae of 14 mm length. Plaice larvae were found to have darting speeds of 4-12 cm/sec (Ryland, 1963). This darting ability would be of great advantage to larvae in escaping from hypoxic conditions.

Some authors have estimated the ability to search for prey by using the cruising speeds of the larvae. Blaxter & Staines (1971) calculated that the volume searched by herring larvae ranged from 0.3 to 2.0 l/h at lengths of 8 to 16 mm and in plaice larvae from 0.1 to 1.8 l/h at lengths of 6 to 10 mm. Rosenthal & Hempel (1970) obtained higher values of 1.5 l to 10 l for the volumes searched, from the early stages of herring larvae up to 4 weeks post hatching. In contrast to this plaice larvae showed a 90% reduction in the volume searched at

metamorphosis. (Blaxter & Staines,1971). Under conditions of hypoxia herring which are more active, especially at metamorphosis would be able to escape more easily than plaice which are considerably less active.

It would be appropriate to consider the relationships between fecundity, egg size and the type of young produced among the fishes. The most primitive condition is observed in some marine fishes where the eggs and sperms are released into the open sea, fertilization being external and large numbers of eggs are produced to make up for predation and mortality that takes place. On the other hand where the eggs are laid in protected situations and there is parental care, the number of eggs produced is reduced as in some littoral species and most fresh water fish. Parental care reaches its highest expression in viviparous forms where the young are protected and nourished within the body of the female parent. Here the young that are born are at a well developed stage able to fend for themselves.

In pelagic food fish such as the herring fecundity ranges from 20,000 to 80,000 in Clyde herring (Baxter,1959) and in gadoids such as cod it ranges from 500,000 to 9,000,000 (May, 1967) while in plaice it ranges from 50,000 to 300,000 in the Clyde (Bagenal,1958). Further, the fecundity also changes with latitude, area and season (Baxter,1959). In these fish, the larvae are small at hatching ranging from 4-8 mm (see Blaxter, 1969). Respiration takes place mainly through the

skin (Fry, 1957). The surface area to body weight ratio is extremely high and thus oxygen is transferred rapidly by diffusion to the tissues. Further, the larvae are transparent and this serves for camouflage from predators.

On the other hand in most fresh water and littoral fish fewer eggs are produced. In salmonids fecundity ranges from about 2,000 to 15,000 per female (Mills,1971). A high fecundity is not essential as in herring or plaice since the eggs are laid in nests under several inches of gravel. The young which hatch out are large in comparison to marine fish larvae, and also possess functional gills and haemoglobin absent in the latter.

Gibson (1969) who has reviewed the biology of littoral fish observes that littoral fish in general lay demersal eggs. These are attached to stones where they are guarded by the male parent. Many adaptations are seen for retaining the eggs in one place such as the constructing of nests among weeds, in burrows and in crevices. In the sea-horse Hippocampus the eggs are attached to the male parent enclosed in a brood pouch. The low fecundity of littoral fish ranging from a few hundred to several thousand (Gibson, 1969) can be associated with the care given to laying eggs in protected places and the consequent guarding of the eggs to prevent predation.

Parental care reaches its highest stage of development

among ovoviviparous and viviparous forms. In the former
the female parent serves only for protection, while in the
latter respiratory and excretory demands are also met. In
general viviparous forms produce   a   small   number of
large young, relatively independent at birth. If the young
are more developed and larger at birth, their surface to volume
ratio will be low and the distance between the respiring tissues
and the source of oxygen would be great.   Under such conditions
the development of a respiratory pigment would be of selective
advantage.   Among the fishes, only the elasmobranchs and
teleosts have achieved true viviparity (Hoar, 1969).   In the
skates and rays ovoviviparity is the rule.   In Pteroplatea,
the "trophonemata" serve to connect the maternal tissue
with that of the embryo (Amoroso, 1960). Viviparity reaches
its highest expression in some selachians with the development
of a yolk-sac placenta.   In the teleosts viviparity is
confined to two orders and all grades of maternal
dependence are observed as in the elasmobranchs.

Associated with viviparity a foetal-maternal shift
is observed in the oxygen affinity of the haemoglobin.
During the gestation period of 22 to 23 months of the spiny
dogfish Squalus suckleyi the foetal haemoglobin was
found to have a higher oxygen affinity than the adult

pigment (Manwell, 1963). The oviparous ray has a transient embryonic haemoglobin of high oxygen affinity during the first few months of development (see Manwell, 1960). Apparently foetal haemoglobin has appeared several times in vertebrate evolution and hence it is believed to be significant for the success of viviparity (Prosser, 1969). In viviparous forms the transfer of oxygen takes place from air to maternal blood to foetal blood to foetal tissue. Instances of marine fish larvae possessing haemoglobin at hatching are rare. One example is the grunion _Leuresthes tenuis_. It spawns just after the turn of the high tide at certain times of the year, literally out of water (Lagler, _et al._, 1967). Eggs and sperms are deposited in pockets in the wet sand high up on the shore so that they are washed away by the next high tide two weeks later. When the waves come up the eggs hatch almost immediately and go out with the waves before the tide recedes. The blood corpuscles are coloured red, long before hatching (David, 1939) and at hatching the gill lamellae are visible.

Among the amphibia similar trends are observed in forms with direct development. In species that accord no parental care , large numbers of eggs are produced as in the common frog _Rana_, with a progressive decrease in the numbers of young produced with increasing parental care and tendencies

towards viviparity. Parental care takes many forms such

as the eggs being wrapped round the legs of the male in <u>Alytes</u>,

or carried in pits on the back of the female in <u>Pipa</u>, or

in a sac on the back in <u>Gastrotheca</u> (Young, 1962). In one

species of the latter calcareous plates give extra protection.

Vivipary is common among the Apoda. Mouthbreeding which

is observed in several fishes , is present only in one

amphibian species, <u>Hylambates brevirostris</u> (Cochran,1961;

cited by Etkin & Gilbert, 1968). Where there is no direct

development as in some Urodeles, many adults retain a

number of larval characters such as external gills and

partial or complete absence of limbs. In this group

the dramatic changes observed at metamorphosis in the

Anurans are lacking. Among the fishes too, metamorphosis

is not a dramatic change, comparatively speaking except

among a few groups such as the Pleuronectidae.

It would be interesting to find out whether

haemoglobin in newly metamorphosed herring and plaice

is functional at levels corresponding to air saturation,

using carbon monoxide which serves to incapacitate the

haemoglobin and therefore alter the oxygen-carrying capacity

of the blood. Further it would be useful to find out

if the appearance of haemoglobin could be accelerated

by rearing larvae in water of low oxygen concentration.

In many invertebrates, decrease of environmental oxygen
concentration has been found to increase the haemoglobin
concentration in the animal. In _Daphnia_ the haemoglobin
concentration increases in oxygen-deficient water. Similar
observations have been made on insect larvae such as
Anatopynia and _Chironomus_ and also _Artemia_ and _Planorbis_
(Fox, 1954, 1955). In _Chironomus plumosus_ larvae Weber
(1965; cited by Weber,1971) has shown that increased
concentrations of respiratory pigment may be of vital
significance since larvae that are rich in haemoglobin
survive gradual depletion of oxygen more successfully
than their paler counterparts. It is evident that more
work needs to be done on fish larvae so as to obtain a
deeper insight into their respiratory mechanisms.

SYNOPSIS

1. The development of respiratory mechanisms in herring and plaice larvae were investigated. The aspects studied were, tolerance and resistance to low oxygen levels, dependence or independence with respect to low oxygen concentrations, changes in oxygen uptake with development and development of gills and haemoglobin.

2. Incipient lethal levels (LD_{50}) varied with the stage of development of the two species. In herring and plaice at the yolk-sac stage it corresponded to 1.93 and 2.73 ml/l respectively. At two weeks of feeding the level changed to 3.08 and 2.66 ml/l. After the gills begin to appear the LD_{50} levels in both species fell to 2.91 and 2.52 ml/l and at metamorphosis to 2.17 and 1.69 ml/l for herring and plaice respectively.

3. Oxygen uptake/animal increased with development and the QO_2 showed an expected decrease for both anaesthetized and unanaesthetized larvae. The QO_2 of anaesthetized herring larvae ranged from 0.95 to 1.33 and of plaice 0.75 to 2.52. The QO_2 of unanaesthetized herring larvae ranged from 1.36 to 2.62 and of plaice 1.12 to 4.12. The basal metabolic

rate, as assessed from anaesthetized larvae, was approximately half the routine rate as determined from the oxygen uptake of unanaesthetized larvae.

4. Routine metabolism could be represented by the equation $Y = 1.88 \ X^{0.82}$ and $Y = 1.67 \ X^{0.65}$ for herring and plaice respectively where $Y = \mu l/larva/h$ and $X =$ dry weight in mg. Basal metabolism could be represented by the equations $Y = 1.063 \ X^{0.93}$ and $Y = 1.01 \ X^{0.63}$ for herring and plaice respectively.

5. The early stages of larvae of both species showed some degree of "regulation" of oxygen uptake as the oxygen concentration fell, i.e. they maintained their uptake to that at the air saturation level. Later feeding larval stages showed "conformity", i.e. the oxygen uptake decreased with the oxygen concentration of the surrounding water. After metamorphosis and the appearance of respiratory pigment a degree of "regulation" re-appeared.

6. Relationships of surface area to length in herring and plaice show isometric growth, and are represented by the equations $Y = 0.16 \ X^{2.14}$ and $Y_1 = 0.09 \ X^{2.27}$ for herring and $Y = 0.12 \ X^{2.58}$ and $Y_1 = 0.22 \ X^{2.09}$

for plaice where Y and Y_1 are the total surface area and body surface area respectively in mm^2, x = length in mm.

7. Relationships between surface area and weight in herring are expressed by the following equations $Y = 22.93 \ W^{0.57}$, $Y_1 = 16.59 \ W^{0.61}$, $Y_2 = 7.08 \ W^{0.41}$. For plaice relationships between surface area and weight are expressed by the following equations, $Y = 23.42 \ W^{0.50}$, $Y_1 = 16.03 \ W^{0.40}$ and $Y_2 = 6.73 \ W^{0.68}$ where Y, Y_1 and Y_2 are total surface area, body surface area and fin area respectively in mm^2, W being the wet weight in mg.

8. Length-weight relationships could be expressed by the following equations, $Y = 0.00001 \ X^{4.19}$ and $Y = 0.000016 \ X^{4.58}$ for herring and plaice respectively where Y = dry weight in mg and X = length in mm.

9. Secondary lamellae begin to appear on the gill filaments at about 9 mm and 22 mm in plaice and herring respectively.

10. Gill area to weight relationships in both species
    show a change in the slope around metamorphosis.
    In herring up to 40 mm, $Y = 0.000007 \ W^{3.36}$;
    beyond 40 mm, $Y = 3.76 \ W^{0.79}$, where Y = gill
    area in mm^2 and W = wet weight in mg. In plaice,
    the gill area to weight relationships, up to and
    beyond metamorphosis are represented by the equations,
    $Y = 0.03 \ W^{1.59}$ and $Y = 0.47 \ W^{0.85}$ respectively where
    Y = gill area in mm^2 and W = wet weight in mg. It
    is evident that gill development slows down after
    metamorphosis in both species.

11. Similar changes in the slope at metamorphosis are
    observed with gill dimensions such as the total
    filament length and area of an average secondary
    lamella with size in both species. Such a change
    was also evident in the spacing of the secondary
    lamellae with increasing size in herring but not in
    plaice.

12. Histological sections revealed a thickening of the
    skin in the two species at metamorphosis.

13. There was no evidence of corpuscles in the blood
    of newly hatched herring and plaice larvae.

Corpuscles of both types viz. lencocytes and erythrocytes were observed at about 10 mm and 29 mm in plaice and herring respectively.

14. Peroxidase precursors were present in the early feeding stages of both species as demonstrated by the benzidine reaction. Total peroxidase concentration in both species showed a change in the slope at the post metamorphosis stages. Relationship of total peroxidase concentration per larva with increasing size in herring is given by the following equations, $Y = 0.11 W^{1.22}$ and $Y_1 = 0.002 W^{2.27}$ up to and beyond metamorphosis respectively where Y = peroxidase concentration in μg and W = wet weight in mg.

15. In reared plaice the total peroxidase concentration per larva is given by the equation $Y = 0.09 W^{2.35}$. For 'wild' plaice $Y_1 = 1.52 W^{0.848}$ where Y = peroxidase concentration in μg and W = wet weight in mg.

16. Peroxidase expressed per unit weight in herring show an increase in the slope against weight on a log-log basis at metamorphosis from 0.21 to 1.26. Plaice, on the other hand, show a decrease in the slope from

1.36 to 0.01. These differences are highly

significant. Comparison of the relative amounts

of peroxidase show these values to be higher in

plaice than in herring.

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Appendix I. Resistance times in minutes in samples of herring larvae at the yolk-sac stage exposed to various lethal oxygen concentrations. Asterisks indicate incomplete mortality.

			Oxygen	levels	(ml/l)	
Number	0.06	0.55	0.76	1.38	1.65	2.07
1	7	12	45	60	125	120
2	8	18	50	70	140	200
3	8.5	20	54	76	160	255
4	9	21	70	100	170	270
5	9.5	23	90	110	200	290
6	10	25	115	130	250	350
7	10.5	27	140	155	400	400
8	11	29	180	175	450	*
9	11.5	35	230	200	480	*
10	12	42	435	230	*	*
11	13	52	-	300	*	*
12	13.5	64	-	400	*	*
13	14	95	-	670	*	*
14	15	225	-	800	-	*
15	15.5	-	-	-	-	*
16	16	-	-	-	-	*
17	16.5	-	-	-	-	*
18	17	-	-	-	-	*
19	18	-	-	-	-	*
20	20	-	-	-	-	-

Appendix II. Resistance times in minutes in samples of
             plaice larvae at 2-3 weeks of feeding exposed
             to various lethal oxygen concentrations.
             Asterisks indicate incomplete mortality.

Oxygen levels (ml/l)

Number	0.34	0.67	1.36	2.02	2.35	2.68
1	10	9	11	62	20	130
2	14	14	23	96	68	260
3	16	18	26	108	76	400
4	22	22	32	118	86	510
5	24	26	33	130	90	*
6	26	31	35	140	100	*
7	27	34	37	150	110	*
8	28	38	39	160	130	*
9	29	41	43	195	150	*
10	30	44	48	210	*	*
11	32	46	58	230	*	*
12	-	50	78	240	*	-
13	-	65	100	260	*	-
14	-	-	135	290	-	-
15	-	-	233	300	-	-
16	-	-	-	-	-	-

Appendix III A. Number of filaments on the arches of one side with increasing length in herring and plaice.

	Herring					Plaice			
Length mm	Arch I	Arch II	Arch III	Arch IV	Length mm	Arch I	Arch II	Arch III	Arch IV
20	–	–	26	15	8.03	4	10	6	–
22	17*	33*	22	21	9.03	10	14	12*	–
22	–	26	28	20	9.81	4	13	12	4*
22	–	27*	28	17*	9.12	8	13	13	3*
23	–	29*	34	21	9.86	4	14	10	4*
24	55	45	36	43	9.90	7	15	13	2*
25	–	25	27	17	9.03	8	17	17	4*
29	60	63	63	53	10.00	12	17	15	8
30	56	58	65	42	10.24	10	13	16	4
30	64	53	70	58	10.80	8	22	19	7
30	76	74	74	50	11.40	15	18	20	7
34	95	99	70	67	11.50	12	23	18	6
34.5	92	109	89	59	11.80	17	23	20	7
35.5	105	98	85	61	13.46	23	25	22	15
36	144	126	85	62	14.24	23	26	24	15
39	129	122	98	66	14.32	16	33	24	15
40	121	112	96	74	15.97	28	30	30	15
46	141	116	55	77	19.00	33	46	37	17
48	162	141	92	83	25.00	57	58	44	28
49	163	132	104	88	26.00	48	65	47	30
51.5	132	120	96	74	28.47	76	68	51	38
55	158	131	104	88	28.65	69	62	53	36
59.25	168	134	103	83	30.03	96	75	56	35
67	164	113	137	77	31.29	85	73	61	23
72	160	149	124	117	33.50	80	70	46	30
73.5	194	164	128	118	49	114	90	66	41
74.5	188	168	150	120	50	99	88	73	34
75.5	178	160	134	125					
77	166	142	114	104					

Appendix IIIB. Pattern of development of filaments in herring and plaice.

HERRING

Length mm	Arch I	Arch II	Arch III	Arch IV.
22 - 29	Filaments of anterior hemibranch only.	Filaments of anterior hemibranch only.	Filaments of anterior and posterior hemibranchs present but those of the anterior filaments are longer.	Filaments of anterior hemibranch only.
35 - 40	Both sets of filaments present but filaments of posterior hemi-branch are very short all along the arch except at the end of the hypobranch.	Similar pattern to arch I.	Both sets of filaments present but the filaments of the posterior hemibranch are increasing in length along the ceratobranchial region as well as on the hypobranch.	Both sets of filaments present, but the filaments of the posterior hemibranch are longer than those of the anterior filaments in the region of the hypobranch.
45 - 50	Both sets of filaments present. Filaments of the posterior hemibranch are shorter than those of the anterior hemibranch except on the hypobranch where they are longer and also greater in number. Further on the epibranch 1 - 2 extra filaments are present on the posterior hemibranch, except in arch IV where 5 - 6 extra filaments are observed.			
70 - 75	In all four arches the filaments on the posterior hemibranch are shorter than those of the anterior hemibranch, except at the origin of the epibranch and the end of the hypobranch where they are longer. The total number of filaments on the posterior hemibranch exceeds those of the anterior hemibranch.			

PLAICE

Length	Arch I	Arch II	Arch III	Arch IV.
9	Both sets of filaments present though some are found on epibranch, only on ceratobranch and hypobranch.	Similar to arch I	Similar to arch I	Only filaments on the anterior hemibranch are present.
14 - 18	"	"	Similar to above, in addition a few filaments of the anterior hemibranch are present in the epibranchial region.	In addition to the filaments of the anterior hemibranch a few filaments are found on the posterior hemibranch in the ceratobranchial region.
25 - 28	Both sets of filaments present, but still no filaments on epibranch.	Similar to arch I. In addition filaments of the anterior hemibranch are present in the epibranchial region	"	Both sets of filaments present on all parts of the gill arch except the epibranch where only filaments of the anterior hemibranch are present.
50	Similar to above, in addition filaments of the anterior hemibranch are present on the epibranch	Similar to above but both sets of filaments are present on the epibranch	"	Similar to above.

Appendix IV   Method of qualitative and quantitative

determination of peroxidase.

(a)   The haemoglobin was identified histochemically
using the benzidine method of Slonimski & Lapinski (1932)
as adopted by Radzinskaya (1960).   The reagent was
prepared as reported by Radzinskaya (1960).

0.1 - 0.2 g of benzidine were dissolved in 2-3 ml of
                                      96%
96% alchohol, after which 10 ml of ˄alchohol, 2 ml of 4%
hydrogen peroxide & 1 ml of a freshly prepared saturated
solution of benzine in glacial acetic acid.   Since
benzidine was only slightly soluble in cold alchohol,
after its addition it was heated gently on a hot-plate
for 5-8 mins.

The eggs and larvae were gently rinsed in distilled
water (after being anaesthetised in M.S.222 in the case
of the larvae) and transferred to a petri dish using a
glass capillary pipette.   A few drops of the reagent were
added the specimens being observed under the microscope.
The site of appearance of the blue stain was observed and
the time noted.   In the later stages of development the
egg membranes were teased out using glass needles prior
to the addition of reagents.   Drawings were made of the
stained larvae using a camera lucida attachment.

(b) Standards.

Initially the preparation of standard solution was performed using human haemoglobin in the form of a powder (William Hopkins Ltd). Matsaura & Hashimoto (1958) quoted by Blaxhall, 1972) found that fish blood haemoglobin resembled that of horse haemoglobin producing identical results with the same method of spectrophotometric analysis, confirming earlier findings that the haemoglobin spectrum appears uniform in all vertebrates. (Blaxhall, 1972).

A known weight of this haemoglobin was dissolved in the equivalent amount of diluent and read against standard cyanmethemoglobin (B.D.H. Ltd.). A similar sample was read against standard haemin solution using the alkaline haematin method (Hawk, Oser & Summerson, 1954; Wintrobe, 1967). It was found that about 75% of the haemoglobin was converted to the corresponding derivative in the first method while in the second method only about 53% was converted. Snieszko (1960) found that the cyanmethaemoglobin method was the most satisfactory one for haemoglobin estimation in trout blood the acid haematin method giving more inaccurate readings. Hence the cyanmethaemoglobin method was used as the standard, especially as it gave a higher conversion to the derivative.

One of the problems encountered with the powdered haemoglobin was that it tended to produce a foam, therefore after a preliminary shaking in distilled water the solution was allowed to stand until the foam had partially subsided. Then the excess foam was removed with a pipette and the solution made up to the mark with distilled water. A solution made up in this manner was then treated with the reagents as given by Korzhuev & Radzinskaya (1957). The reagents are as follows;

    Reagent I - Pyridine 9ml, Rescorcin 1 g, Pyramidon 5 g,
               96% ethanol 91ml.

    Reagent II - Glacial acetic acid 8ml,
               96% ethanol 92ml.

    Reagent III - 0.6% hydrogen peroxide from a 3% solution
               (freshly prepared).

The sensitivity of the method according to these authors was 5 ug peroxidase in 0.5 cc i.e. 1 mg/100ml of blood using a visual matching of colours. Calibration curves were made at two dilutions, the blank being distilled water and the colours obtained were read in a Unicam S.P. 500 spectrophotometer. The strength of the original solution was obtained by reading it against standard cyanmethaemoglobin. A great deal of variation was observed in the pyridine method, a fact which has been commented on

by the authors. Using the spectrophotometric method of analysis a sensitivity of 1 μg in 1ml of solution could be detected. The time interval between the addition of reagents and the time of reading the sample was also found to be critical. A decrease was observed with time. The authors recommended reading of the samples after 40-45 mins. Light was believed to have no effect. Therefore a set of samples was run to test the effect of 4 parameters, viz. darkness, light, time; and the effect of an acetate buffer in an attempt to stabilize the reaction. only one concentration of haemoglobin was used. The experiment was conducted in quadruplicate. Results showed that samples could be read within 25-45 mins, and more stability in the readings obtained in samples kept under dark conditions. The blank too gave a higher reading after being exposed to light, this may or may not be significant as replicates were not made. Nevertheless all samples were kept in the dark prior to centrifugation in the later analyses.

In later determinations the haemoglobin solution was filtered under pressure using a Millipore filtration apparatus with a cooked filter to remove particles which remained in solution. 1ml of this solution was diluted

to 10ml with diluent and read against standard
cyanmethaemoglobin (International Standard) at 540 mμ.
Another 10ml of the solution was diluted to 1000ml
(solution A) with distilled water and calibration
curves prepared. From this second solution 10ml was
pipetted out and diluted to 500ml (solution B).
1ml samples of these solutions were treated with the
reagents and used as the standards.

The calculations were made as follows.
Reading of the standard cyanmethaemoglobin (Rs) is
equivalent to 57.2 mg of haemoglobin/100ml of blood.
Therefore the reading of the unknown solution (Rx)
is equivalent to $\frac{57.2 \times Rx}{Rs}$ mg = X mg of haemoglobin.

Therefore 10ml of haemoglobin diluted to 1 litre also
contains X mg of haemoglobin i.e. solution A. i.e. 1ml
of solution A contains X μg of haemoglobin. Solution B
is 10ml of solution A diluted to 100ml. i.e. 1ml of
solution B contains 0.1 x X μg of haemoglobin. Solution
A was used as the standard for metamorphosed and
premetamorphic larvae with 1 cm cells, corrections
being made for the reagent blank. Solution B was
used as the standard for the younger larval stages,
and read in 2 cm cells with a narrower slit.

In 1971 the strength of the unknown solution was read off the calibration curves prepared, as preparation of calibration curves with each run was too time consuming. In 1972 a solution of haemoglobin made up from sheep haemoglobin crystals (B.D.H. Ltd.) was standardised against standard cyanmethaemoglobin as before and 1ml of the appropriately diluted solution used as a standard with each run. One problem that arose with the sheep haemoglobin was that cloudiness was observed after addition of diluent, and therefore a correction for turbidity was made.

The method used by Radzinskaya for the larvae is as follows: A homogenate of one larvae was made in 1ml of water.

0.5ml of the homogenate was transferred to a centrifuge tube and the following reagents added.

    a) 1ml of amidopyrine pyridine solution - Reagent I.

    b) 0.8ml of 8% acetic acid.   - Reagent II.

    c) 0.2ml of 3% hydrogen peroxide freshly diluted -

                                    Reagent III.

In the present determinations the eggs or larvae were homogenized in 3ml of water and the reagents added to 1ml samples in duplicate. Samples of about 30 eggs were used in both species and after hatching groups of about 20 larvae. At the premetamorphic stage in herring individuals were used while in plaice about 3-4 larvae were used per sample.

Appendix V. Decrease in the optical density of the
            haemoglobin solution with time.