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The effect of low temperature on cod, *Gadus morhua*

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The laboratory studies described in this paper involved over 200 cod. Fish held at controlled temperatures below 2°C for about 40 d in normal sea water (32–34°/$_{oo}$S) showed an increased ΔF (Ramsay-Brown method) and chloride content (coulometric titration) of their blood plasma. Muscle water content decreased suggesting that at least part of the blood plasma changes were due to dehydration. Cod kept in low salinity water (7–8°/$_{oo}$S) at low temperature showed a decreased ΔF and chloride content of their blood plasma. Muscle water content increased and the fish gained weight although they were not fed, suggesting that at least part of the blood plasma changes were due to hydration. There was no evidence to show that these changes were due to osmoregulatory failure. Cod survived for at least 80 d when held at 0°C and did not die when subjected to cold shock from 4°C to 0°C. The results suggest that the distribution of cod in arctic waters and the capture of dead cod in the North Sea during severe winters is unlikely to be related to a lethal effect of low temperature. Other experiments indicated that warm (10°C) low salinity (7–8°/$_{oo}$S) sea water may be lethal for cod, which suggested that their distribution in the Baltic Sea could be restricted during the summer. The effect of low temperature on cod is discussed in relation to condition factor, parasitism by *Lernaeocera*, photoperiod, freezing resistance, and the aggregation of cod at fronts between warm and cold water masses in the Svalbard area.

Introduction

Woodhead & Woodhead (1959, 1965) found that the blood plasma of Barents Sea cod caught in cold water (below 2°C) in winter had an increased salt content and an increased freezing point depression (ΔF). Further observations were made by Eliassen, Leivestad & Møller (1960) with cod caught in northern and Norwegian coastal waters and with others kept in the Bergen aquarium. Woodhead & Woodhead (1959) interpreted their results as indicating a disturbance, or partial failure, of the osmoregulatory system and assumed that cod would die if they remained in cold water. The physiological changes were thought to be significant in connection with the distribution of cod in relation to temperature, and in particular with the low catches of cod in water of less than 2°C during the autumn, winter, and spring trawl fisheries at Bear Island. But Leivestad (1965) suggested that the change in osmolarity could be regarded as a compensatory increase to reduce the metabolic load on the gills at low tempertures. Another possibility is that the increased osmolarity could be an adaptation to avoid freezing if the fish went into very cold water. The latter hypothesis recalls that put forward by Scholander et al. (1957) and Gordon, Amdur & Scholander (1962) to account for the low freezing points of the blood plasma of certain shallow-water fish found in Hebron Fjord, Labrador.

In view of the differences in the interpretation of the previous results, experiments with cod were carried out at the Fisheries Laboratory, Lowestoft.

North Sea cod were used because it was not practicable to bring back to Lowestoft regular supplies of live fish from the Barents Sea. North Sea cod rarely meet the low temperatures common in northern latitudes. If low temperatures have harmful effects on cod, they might be expected to show up more readily in fish from the North Sea. In the laboratory cod were held at controlled temperatures in water of different salinity. These experiments were carried out at different seasons (winter and summer) and under natural and controlled light regimes (short day and long night, or vice-versa). After being held (range 4h to 80 days) at the controlled temperature a fish was killed and measurements were made of the blood plasma freezing point, chloride, and in some cases, glucose. The changes in total body weight and the muscle water content were also determined. From November 1967 to December 1970 19 experiments were completed involving 219 fish. Table 1 gives some details of these experiments which have been grouped into five series according to the main objective.

Materials and methods

The methods were developed during a series of preliminary experiments involving 94 fish carried out from July 1966 to May 1967.

Table 1. Temperature experiments with cod (*Gadus morhua*) November 1967 to July 1970 inclusive grouped according to the main objective. Under the column headed light regime the letter *N* indicates natural illumination and day length for the months in which the experiments were carried out, and 16 h L indicates 16 hours artificial light, etc.

Series no.	Objective	Exp. no.	Dates within which the experiment was completed	Temperature, t°C		Number of cod		Period (h or d) held at experimental temperature	Light regime	Remarks
				Holding	Experimental	Start	Finish			
1	Mortality and plasma changes when cod were held at various temperatures in 32–34‰ S sea water. The change from the holding to the experimental temperature made slowly	1	Oct–Nov 1967	8–10	8–10	10	10	32 d	*N*	–
		2	Nov–Dec 1967	8–10	5	10	10	40–41 d	*N*	–
		3	Nov–Dec 1967	8–10	0	10	9	42 d	*N*	Aeration failure 1 fish died
		4	Nov–Dec 1967	8–10	13	10	10	38 d	*N*	Sampling failure, 1 fish; 1 fish not used
		5	Jan–Feb 1968	8–10	16	12	8	36–37 d	*N*	Aeration failure 3 fish died; 1 fish no explanation for death
		6	Jan–Feb 1968	8–10	2	10	10	41–42 d	*N*	–
		7	Jan–Feb 1968	8–10	−1·0 to −1·5	16	8	38–42 d	*N*	Cooling failure 5 fish died; 1 fish died of infection; 1 fish died with inflated swimbladder; 1 fish no explanation for death
2	Effect of low salinity sea water (7–8‰ S) on the changes observed in Series 1	8	Feb–Mar 1968	8–10	10	10	8	35 d	*N*	All fish very excitable. 2 died in convulsion
		9	Feb–Mar 1968	8–10	2	11	10	35 d	*N*	1 fish died with inflated swimbladder
		10	Feb–Apr 1968	8–10	0	10	10	32 d	*N*	–
3	Effect of temperature shock on the changes observed in Series 1	11	Dec 68–Mar 69	4	0	40	40	4 h–50 d	*N*	Sampling failure, 1 fish
		12	Mar–May 1969	4	0	10	9	42 d	*N*	1 fish died infected
		14	Dec 1969	4	0	10	10	6–10 d	*N*	–
4	"Summer" cod to compare with "winter" cod in Series 1 and 3	13	May–July 1969	9–10	0	10	9	42 d	*N*	1 fish died infected
5	Effect of photoperiod on the changes observed in Series 1, 3 and 4	15	Dec–Feb 1970	7	0	7	7	43 d	16 h L	–
		16	Dec–Feb 1970	7	0	8	8	40 d	8 h L	–
		17	Dec–Mar 1970	7	0	5	5	80 d	8 h L	–
		18	June–July 1970	7	0	10	9	40–43 d	8 h L	1 fish died of infection
		19	June–July 1970	9–7	0	10	5	40–43 d	16 h L	4 fish died of infection 1 fish no explanation for death

Cod

In most years cod are plentiful off Lowestoft from November to April. Two and three year-old cod, 30–45 cm in length, were caught by rod and line in depths of 4–6 m and held for 10–14 days in outside reception tanks (2·5 × 1·2 × 1 m deep). After a few days most fish were feeding on lugworm (*Arenicola marina*) and chopped squid (*Loligo forbesi*). The cod were considered to have recovered from any shock of capture and to be suitable for experimental work when they were feeding well, had normal colouration, clear eyes and quick alert movements. Some fish (summer experiments 13, 17, 18 and 19) had to be kept for 2–3 months before they were used and were transferred to indoor glass-fibre tanks (2·5 × 2·5 × 1 m deep) which were aerated and provided with flowing sea water. The tank room received natural daylight through partially screened south-facing windows and a low level of artificial illumination from laboratory lighting during the working day. The temperature in the indoor holding tanks was controlled within the range 8–10°C, and 10–12 fish were kept in each tank. Very few small cod were available off Lowestoft in the 1969/70 season and the fish used in experiments 15–19 were caught off Conwy, North Wales and brought back to Lowestoft by road. These Irish Sea cod were of similar age and size to those caught off Lowestoft.

Experimental tanks

Cod were kept singly in asbestos-cement tanks (0·6 × 0·6 × 0·5 m deep). The insides of the tanks were treated with "Araldite" epoxy resin to give a hard smooth surface from which it was easy to siphon off faeces and detritus. Each tank had a corrugated transparent PVC cover and a compressed-air line. The water was partially changed twice a week with new water at the same temperature and salinity.

Temperature-controlled rooms

The experimental tanks were kept in temperature-controlled rooms, 10 tanks to a room. Each room was thermostatically controlled to ±0·25°C of a selected temperature within the range 16° to −1·5°C. The temperature of each individual tank was checked daily and the temperature differences between tanks did not exceed 0·4°C. With the exception of the photoperiod experiments 15–19 the controlled temperature rooms received natural daylight through a north-facing window.

Experimental procedure

Cod were taken from the reception or holding tanks and placed singly in the asbestos tanks. In experiments 1–10 inclusive the temperature was reduced slowly to the required level so that the change never exceeded 2°C in 24 h. In the "shock" experiments 11, 12 and 14 the fish were taken from tanks at 4°C and put into tanks at 0°C. In the remaining experiments (13, 15 to 21) the temperature change was spread over several days. In most experiments the cod were held at the experimental temperature for 32–42 days. In others, fish were removed after different intervals to study the timecourse of the blood plasma changes and some fish were held at low temperatures for 80 days to determine the effect of prolonged exposure. Cod do not eat regularly at low temperatures and to make all the results comparable in this respect the fish were not fed during any of the experiments summarized in Table 1.

During the experiments cod sometimes showed a series of five dark vertical bands along each flank which were accompanied by a general paling of the whole fish, but some fish showed a general darkening without bands. These pigment changes occurred in a few fish when they were taken from a tank containing a small shoal and moved into individual tanks. The fish resumed their normal colouration in 12–36 h. We interpreted the pigment change as a fright reaction in response to handling. It has been observed in cod by our colleagues at the Marine Laboratory, Aberdeen, both in the laboratory and at sea (Chapman and Wardle, personal communication).

Length and weight of cod

Length was measured to the nearest cm below and each fish was weighed before and after an experiment on a Torbal balance (model PL/12, capacity 2 kg) to the nearest g. Live cod are difficult to handle and the following method was used for the initial weighing. As soon as the fish was taken out of the water it was loosely wrapped in a wet cloth which covered the head and eyes. When wrapped up even the most lively fish lay still on the balance pan. The fish and wet cloth were weighed in 10–15 s and the fish was immediately returned to its tank. The wet cloth was weighed again, the weight of the fish being given by subtraction. Replicates showed that the "wet cloth" method had a coefficient of variation of 0·12% and that "wet cloth" weight was within 1·0% of the weight of the fish when it was carefully blotted and dried.

Table 2. Summary of statistics to show differences in plasma ΔF and chlorides of fish killed by immersion in a lethal solution of MS 222 Sandoz and fish killed directly by a blow on the head

	Plasma freezing point depression in °C		Plasma chlorides in mequiv/l	
	MS 222 Sandoz	Blow on the head	MS 222 Sandoz	Blow on the head
n	10	10	10	10
$\bar{x}$	−0·658	−0·641	153	152
S.E.	0·006	0·004	0·7	0·9
t	2·545		0·913	
d.f.	18		18	
P	<0·05		–	

Condition factors

These were calculated from the expression $K = 100\ W.L^{-3}$ where W is the weight of the fish in g and L the length in cm.

Killing and blood sampling

When an experiment was ended a fish was quickly netted from its tank and immediately killed by a sharp blow on the head. Cod killed in this way had significantly lower plasma ΔF's but not plasma chlorides, than fish killed by immersion in a lethal solution of MS 222 Sandoz (Table 2). The fish was weighed, its length measured, and prepared for blood sampling. The left operculum was raised and a slit made in the skin immediately anterior to the cleithrum. Skin and musculature were dissected to expose the pericardium, heart and adjacent blood vessels. Blood was drawn from the ventricle with a 5 ml Becton Dickinson vacutainer tube (catalogue no. 3200KA) and a size 22G $1\frac{1}{2}$ inch sterile disposable needle. These tubes contained a trace of sodium heparin as an anticoagulant. Tests with standard NaCl showed that the anticoagulent had no detectable effect on the freezing point. A 3–4 ml blood sample could be obtained from a 40 cm cod. Samples were centrifuged for 25 min at 4·0°C (centrifuge MSE Mistral 6L, head 59548, 2000 rev/min 1730 RCF). The clear supernatant fluid was pipetted into sterile stoppered vials and the fresh material immediately used for freezing point, chloride and, in some cases, glucose determinations.

Water content of muscles

After bleeding the sex and maturity of the fish were determined and the sagittal otoliths removed for age determination. A 20 g block of muscle, free of skin and bone, was removed from the flank below the first dorsal fin. The muscle was weighed, then dried to constant weight at 60°C (Lovegrove, 1962), and the percentage water content calculated from the initial and the final weights.

Chloride determinations

Chloride concentrations using 0·2 ml of cod plasma were measured with an "Eel"chloride meter (Evans Electroselenium Ltd.) by coulometric titration. Ten replicate titrations on a 200 mequiv/l standard NaCl solution gave a mean of 200·1 ± 0·99 mequiv/l, coefficient of variation 0·5%. As the protein content of the plasma was not determined, the chloride values refer to mequiv/l plasma. Fish blood contains 3–5% by weight of protein (Holmes & Donaldson, 1969) so that the chloride values are probably about 5% lower than those which would have been obtained had they been expressed as mequiv/kg water.

Plasma freezing point depressions

Determinations were made by the Ramsay and Brown (1955) method, using a Beckmann thermometer (H. J. Elliot, E-mil) scaled to 0·01°C. The temperature in the cooling bath was controlled to fine limits and with proper attention to sample size, justified readings to 0·001°C with a magnifying eyepiece: 10 determinations of ΔF for a standard NaCl solution had a standard deviation of ±0·002°C. Plasma samples were always bracketed by standard NaCl samples whose ΔF's were estimated by linear interpolation between the values for 0·2 M and 0·3 M NaCl calculated from the data given by Scatchard & Prentiss (1933).

Plasma glucose

Glucose levels in the plasma were determined by the glucose oxidase method following the procedures given in the Boehringer Mannheim GMBH (1970) work sheet. Eight replicate analyses for one cod gave a mean glucose level of 52 mg/100 ml plasma, standard deviation ± 1 mg.

Results

The first series of experiments (1 to 7) was made to determine the changes in blood plasma ΔF and chloride when cod were acclimated to a range of temperatures from low (−1·5°C) to relatively high (16°C) in normal hypertonic sea water of 32–34‰ S, ΔF −1·72° to −1·85°C (Cox, 1965). In view of Woodhead & Woodhead's (1959) hypothesis that

Table 3. Series 1, summary of the results of experiments 1–7. Cod held for 32–40 days in sea water of 32–34‰S (ΔF −1·74° to −1·85°C), at various temperatures.

Exp. No.	t°C	n	Plasma ΔF in °C		Plasma chloride in mequiv/l		Percentage change in body weight		Percentage water content of the muscles	
			Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
7	−1·5	8	−0·819	±0·007	190	±1·4	−10·7	±0·8	77·9	±0·15
3	0	9	−0·800	±0·007	186	±2.7	− 8·2	±0·5	78·2	±0·30
6	2	10	−0·705	±0·004	170	±1·6	− 9·4	±0·7	79·2	±0·09
2	5	10	−0·669	±0·005	164	±1·5	− 8·0	±0·8	80·0	±0·20
1	8–10	10	−0·641	±0·003	152	±0·9	–	–	–	–
4	13	8	−0·629	±0·003	151	±0·8	−13·7	±1·1	80·1	±0·23
5	16	8	−0·632	±0·005	152	±1·0	−23·1	±1·5	82·1	±0·28

cod remaining in temperatures below 2°C would finally die, particular note was made of the appearance and behaviour of the fish held at the lower temperatures.

Details of the experiments and of the results are summarized in Tables 1 and 3 and shown graphically in Figure 1. Aeration failure led to the death of one fish in exp. 3, and three fish in exp. 5. There was no explanation for the death of a fourth fish in exp. 5 (at 16°C). The loss of 5 fish held at −1·5° C in exp. 7 was caused by a single overnight failure in the cooling system when the temperature fell to −2·0°C and ice formed on the water surface in several tanks. There were 3 other deaths in this experiment, one fish dying from infection, a second with an over-inflated swimbladder, and a third for whose death there was no apparent explanation. Although the cod in exp. 3 and 7 were quiet and inactive, they swam sluggishly about the tanks from time to time, and neither by their colouration nor by their behaviour gave any indication of distress. Mucus secretion apeared to be normal.

The plasma analyses showed an increased ΔF and chloride at the lower temperatures. The cod held at the higher temperatures (13° and 16°C) lost most weight; those held at the lower temperatures (−1·5°, 0° and 2°C) showed a decrease in muscle water content.

The second series of experiments was carried out to observe the behavioural and physiological changes in cod acclimated to different temperatures (0°, 2° and 10°C) in dilute hypotonic sea water of 7–8‰ S, ΔF −0·37° to −0·43°C. The cod were held at 8°–10°C in 32–34‰ S sea water for about 15 days and then

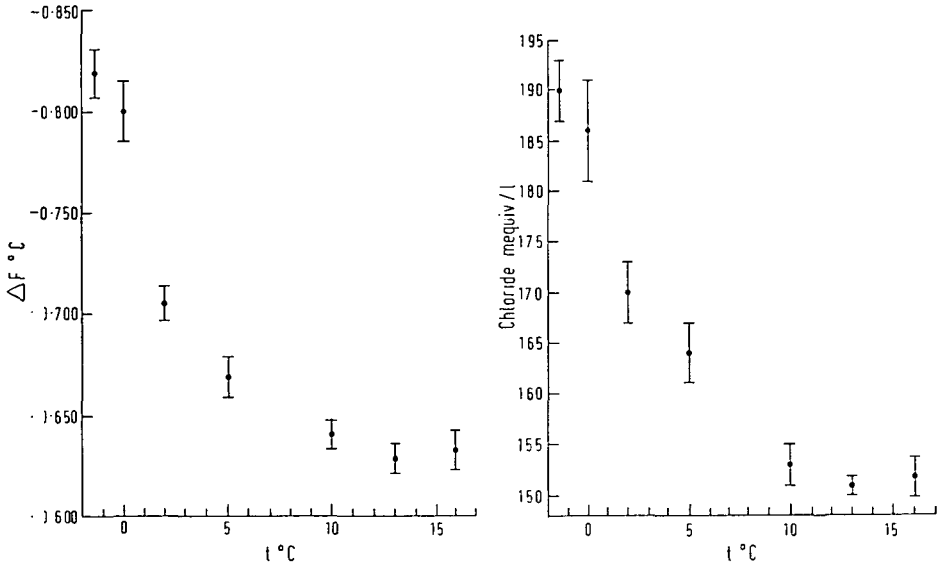

Fig ıre 1. Means and two standard errors for the freezing point depressions (°C) and chlorides (mc quiv/l) of the blood plasma of cod (*Gadus morhua*) kept in normal sea water and held at diff rent temperatures for periods of about 40 days. For further details see Table 3.

Table 4. Plasma ΔF's, plasma chlorides, change in body weight and water content of muscles in cod held for 35 days at temperatures of 10°, 2° and 0°C in low salinity sea water of 7–8‰ S.

Exp. No.	t°C	n	Plasma ΔF in °C		Plasma chloride in mequiv/l plasma		% change in body weight		% of water in muscles	
			Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
8	10	8	−0·598	±0·015	146	±4	−7·54	±1·54	80·30	±0·28
9	2	10	−0·614	±0·015	151	±4	−1.94	±0·68	80·11	±0·34
10	0	10	−0·558	±0·014	128	±4	+1·48	±0·98	80·83	±0·28

transferred to dilute sea water and the temperature was slowly adjusted to the required value. The results for the control (experiment 8) and two experimental groups (experiments 9 and 10) are summarized in Table 4.

There was no difficulty in keeping the fish alive in hypotonic sea water at 0° and 2°C but those held at 10°C were easily excited towards the end of the experiment and great care had to be taken when taking temperatures in the tanks and during routine cleaning. The fish were pale and jumped out of the water if disturbed. As shown in Table 1 there were two deaths. These fish died after convulsions characterized by an arched back, gaping jaws, open opercula, and widely spread pectoral fins.

Although the blood plasma results were more variable than those obtained with fish in normal sea water, the main trend was clear: there was a decrease in plasma ΔF and plasma chloride which was marked in those fish kept at 0°C. The water content of the muscles of the cod kept in hypotonic sea water at 0° and 2°C was significantly greater than that of the cod kept at the same temperatures in normal sea water, suggesting that the low-salinity fish were taking on water. This is substantiated by the percentage changes in body weight for 7 of the 10 fish held at 0°C which gained weight during the experiment although they were not fed. For comparison, cod held at 0°C in normal sea water lost weight (Table 3, experiment 3).

The results from the first two series of experiments may be summarized as follows: cod held at low temperatures for 30–40 days undergo changes in the osmolarity of their blood plasma whose ΔF becomes closer to that of the sea water in which the fish were kept. For the fish held in normal sea water, plasma ΔF and chloride increased, thus confirming the earlier results of Woodhead & Woodhead (1959) and Eliassen et al. (1960). But there were no deaths that could be attributed to osmoregulatory failure. In an experiment to be described later (number 17) 5 cod were held in normal sea water for 80 days at 0°C without any deaths and their blood plasma changes, although similar to those found in experiment 3, were slightly less marked (Table 6).

In experiments 3, 6, 7, 9 and 10 cod were taken from the holding temperature to the experimental temperature slowly, over a period of several days. But at sea, and in particular in the Bear Island area, cod might be subjected to very sudden temperature changes at the fronts between warm (4°C) Atlantic and cold (0°C) polar water (Beverton & Lee, 1965). A third series of experiments was therefore carried out to determine the effect of temperature shock on the plasma changes found in experiment 3. In the "cold shock" experiments 11, 12 and 14, cod were held for 7–24 days in 32–34‰ S water at 4°C and then immediately transferred to sea water of the same salinity but at 0°C. Fish were sampled from 4 h to 50 days after the shock to follow the time course of the plasma changes. The results are shown in Figure 2.

Only one of the 60 cod used in these experiments died and this death (experiment 12, Table 1) was caused by a bacterial infection. For the first few days after the temperature shock most of the fish showed paling and banding and some assumed a mottled appearance. These pigment changes disappeared after 10 days at 0°C. None of the fish, as judged by their locomotory behaviour or orientation, appeared to be stressed by the temperature shock. Mucus secretion appeared to be normal. Figure 2 shows that for the first 5 days following the temperature shock the plasma ΔF and chloride values were within the range previously determined for fish acclimated to 5°C (experiment 2, Table 3). Significant changes were not apparent until 5–15 days after shock and thereafter the values increased towards those previously established for cod held at 0°C for 42 days (experiment 3, Table 3). A temperature shock from 4° to 0°C did not kill the cod, or lead to any sudden plasma changes such as might be expected in the event of an osmoregulatory failure. Figure 2 shows that after 42 days (experiment 12) the ΔF's were markedly less, and the chlorides somewhat lower, than those found in experiment 3. The differences were significant (ΔF's, $t = 6{\cdot}594$, $P < 0{\cdot}001$; chlorides, $t = 2{\cdot}263$, $P < 0{\cdot}05$). The cod used in experiment 3 were killed in December, and those used in experiment 12 were killed in May: there was a seasonal difference between the two experiments. It was possible that the plasma anomalies might be related to the seasonal

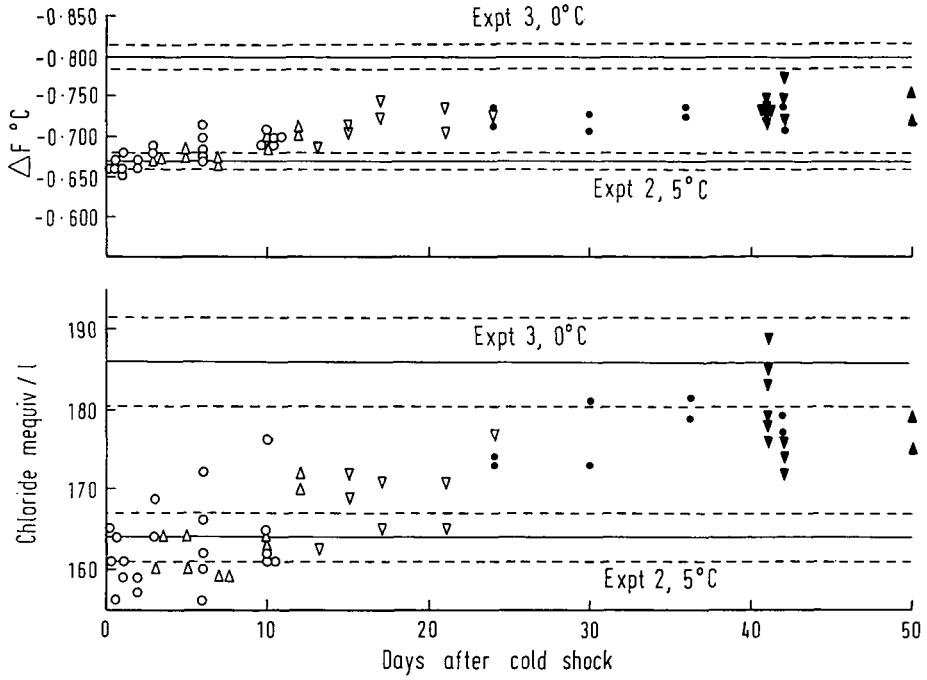

Figure 2. Freezing point depressions (°C) and chlorides (mequiv/l) of the blood plasma of cod (*Gadus morhua*) subjected to cold shock from 4·0°C to 0·0°C in normal sea water. The means and two standard errors for the controls at 5·0°C (exp. 2) and 0·0°C (exp. 3) are also shown. Symbols indicate the month in which the fish were killed:

○ December △ January ▽ February
● March ▲ April ▼ May

factor rather than the initial temperature shock.

The point was taken up in the fourth series of experiments in which "summer" cod held in 32–34‰ S sea water were transferred from 9°–10°C to 0°C slowly over a period of 7 days and kept at the low temperature for 42 days. A comparison between the results of this experiment (number 13), which ended in July, and those of experiments 3 and 12 is made in Table 5. There was no significant difference between the results of experiments 12 and 13 which were therefore combined to form a group of 18 "summer" fish. There were significant differences between the "winter" fish (experiment 3) and the group of 18 "summer" fish. In the "summer" fish the plasma ΔF's and plasma chlorides, and the percentage loss of body weight were all significantly lower, but the percentage water content of the muscles was significantly higher. The results suggest that seasonal differences could have accounted for the differences between the results of experiments 3 and 12.

The differences between "summer" and "winter" fish might be related to photoperiod. In a fifth series of experiments carried out from December to March (winter) and June to July (summer) fish were held in 32–34‰ S sea water and transferred from the holding to the experimental temperature (0°C) slowly over 7 days. The "winter" (experiments 15 and 16) and "summer" (experiments 18 and 19) fish were each divided into two groups held at 0°C for 43 days under light regimes corresponding to short (winter) and long (summer) day-lengths respectively. The controlled temperature rooms were blacked out and light was provided by two 40W tungsten filament lamps to give an intensity of a few m.c. above each tank. In the "winter" group 8 h light was given from 0800 to 1600 h, and in the "summer" group 16 h light from 0800 h to 2400 h. An extra group of "winter" fish (experiment 17) was held at 0°C under a winter light regime for 80 days. Six fish died in this series of experiments: 1 fish in experiment 18 and 5 fish in experiment 19. As shown in Table 1, 5 of the deaths were associated with bacterial infections and in only one case was there no apparent explanation of death. The results for those that survived are summarized in Table 6. They showed that within the "winter" fish there was no difference that could be

Table 5. A comparison between "winter" and "summer" cod held at 0°C in normal sea water (32–34‰S) for 42 days

Experiment no.	Date		Plasma ΔF in °C	Plasma chloride in mequiv/l	% change in body weight	% water content of muscles
12	March to May 1969	n	9	9	–	9
		$\bar{x}$	−0·738	179	–	79·5
		S.E.	±0·005	±1·8	–	±0·2
13	May to July 1969	n	9	9	9	9
		$\bar{x}$	−0·736	176	−5·3	79·8
		S.E.	±0·004	±1·4	±0·4	±0·2
		t	0·299	1·162	–	0·856
		d.f.	16	16	–	16
		P	–	–	–	–
12 + 13	"summer"	n	18	18	9	18
		$\bar{x}$	−0·737	178	−5·3	79·7
		S.E.	±0.003	±1·1	±0·4	±0·1
3	November to December 1967 "winter"	n	9	9	8	9
		$\bar{x}$	−0·800	186	−8·2	78·2
		S.E.	±0·007	±2·7	±0·5	±0·3
		t	7·388***	2·965**	4·513***	4·461***
		d.f.	25	25	15	25
		P	<0·001	<0·01	<0·001	<0·001

attributed to light regime. In the "summer" fish, the group held under the "summer" light regime lost significantly less weight than the group held under the "winter" light regime. But there were no significant differences in plasma ΔF's or plasma chlorides between the two groups of "summer" fish.

Discussion

Death of cod at low temperatures

In our experiments 146 cod were held in normal sea water (32–34‰ S) for periods from 4 hours to 80 days at temperatures from 2·0° to −1·5°C. As shown in Table 7, 129 of these fish survived; 17 fish died, and in 15 cases death was clearly associated with a known factor and there were only 2 fish (1 in exp. 7 and 1 in exp. 19) for whose death there was no obvious explanation.

In the experiments carried out between October and May there were 10 deaths among the 116 cod held at temperatures of 2°C or below. With the exception of the 1 death in exp. 7, the remaining 9 were related to technical failures, infections or swim-bladder problems, and the results are not consistent with Woodhead & Woodhead's (1959) hypothesis that cod remaining in water below 2°C would die; in exp. 17, 5 cod survived for 80 days at 0°C. Similarly Leivestad (1965) kept cod alive for 60 days at −1·4°C.

At sea cod are caught alive in cold water: below −1·0°C at Bear Island (Beverton and Lee, 1965); at −1·4°C at Newfoundland (Templeman and Fleming, 1965); and off the piers at Lowestoft in 1947 when local sea temperatures ranged from 0·5°C to −0·4°C (Simpson, 1953). There is ample evidence to support Templeman & Fleming's (1965, p. 131) conclusion that cod are resistant to low temperatures and it may be significant that dead cod have not been reported from cold water at Bear Island (Beverton and Lee, 1965, p. 244). But there have been reports of dead cod being caught at sea under circumstances which have been interpreted as showing that they were killed by cold water. Dead cod have been seen floating at the surface (Templeman, 1965), found on beaches (Dannevig, 1930a) and caught in Danish seines (Johansen, 1929) or otter trawls (Lumby and Atkinson, 1929; Simpson, 1953; Woodhead, 1964a). In one of the cases cited by Templeman (1965) the cod may have been supercooled and died after being seeded by ice crystals and Johansen (1929) attributed the death of cod in inshore Scandinavian waters during the severe winter of 1929 to a deficiency of oxygen. During the same winter dead cod were caught in the Southern Bight at Tea Kettle Hole (53° 20′ N 3° 20′ E) where the lowest bottom temperatures were about 2·5°C (Lumby & Atkinson, 1929). In the severe winter of 1947 dead cod were caught in areas of the Southern North Sea where bottom temperatures may not have fallen below 1·0°C and certainly did not fall below 0°C (Simpson, 1953), and in the winter of 1963 dead cod were caught in areas where the bottom temperatures never fell below 2·0°C (Woodhead, 1964b, Figure 2).

These observations would appear to be inconsistent with the results of our laboratory experiments in which cod survived temperatures of −1·5°C for 40 days (Table 1, exp. 7). Dannevig (1930a) reported that cod could survive for at least 28 days at −1·4°C but

Table 6. Series 5, experiments 15–19. Summary of results and statistical comparisons for the photoperiod experiments with cod held at 0°C. For further details see text and Table 1

Experiment no.	Season	Light regime		Duration in days		Plasma ΔF in °C	Plasma chloride in mequiv/C	% change in body weight	% water content of muscles
		Light	Dark						
15	Winter	16 h	8 h	43	n	7	7	7	7
					$\bar{x}$	−0·760	179	−7·8	78·9
					S.E.	±0·005	±1·5	±0·92	±0·2
16	Winter	8 h	16 h	40	n	8	8	8	8
					$\bar{x}$	−0·757	182	−8·5	79·1
					S.E.	±0·008	±1·4	±0·74	±0·1
					t	0·271	1·351	0·627	0·601
					d.f.	13	13	13	13
					P	–	–	–	–
18	Summer	8 h	16 h	40–43	n	9	9	9	–
					$\bar{x}$	−0·737	179	−8·4	–
					S.E.	±0.003	±1·4	±0·68	–
19	Summer	16 h	8 h	40–43	n	5	5	5	–
					$\bar{x}$	−0·738	179	−6·5	–
					S.E.	±0·005	±1·1	±0·38	–
					t	0·156	0·367	2·469*	–
					d.f.	12	12	12	–
					P	–	–	<0·05	–
17	Winter	8 h	16 h	80	n	5	5	5	5
					$\bar{x}$	−0·766	181	−9·8	79·5
					S.E.	±0·006	±1·0	±1·22	±0·1

that 4 fish cooled from 3·5°C to −1·1°C in about an hour died. He concluded that the critical temperature for cod was about −1·0°C and was clearly puzzled (Dannevig, 1930b) by the capture of dead cod in the North Sea during the winter of 1929 in areas where the bottom temperatures were above zero. Dannevig (1930b) considered the possibilities that a sudden change in temperature above the critical level of −1·0°C might have been lethal but noted that this was not supported by his own observations. In our shock experiments cod were not killed by a sudden change from 4·0°C to 0·0°C. Taken as a whole, the facts lead to the conclusion that it is unlikely that the dead cod caught by bottom trawl in the North Sea during the severe winters of 1929, 1947 and 1963 were killed by a direct effect of low temperature.

If this conclusion is correct, there must be some other explanation to account for the reports of dead cod being caught in numbers up to 3 or 4 per haul in 1947 (Simpson, 1953) and 1 or 2 per haul in 1963 (Woodhead, 1964b). Records held at the Fisheries Laboratory show that some dead fish – including cod – are caught throughout the year. These captures do not appear to be reported by fishermen unless they are actively encouraged to do so.[1]

It seems likely that some dead cod are occasionally caught by trawlers and that this is not unusual. The death of most cod would have been due to natural causes. The chance of catching dead cod will depend, in part, on the rate at which their bodies are eaten by scavengers and decomposed by bacteria, and both these activities would slow down at low temperatures. Other things being equal, more dead cod might be caught in cold winters, not because there are more fish dying, but because each dead fish lasts longer and there are therefore more available to be caught. This hypothesis would go some way to reconcile the results of the laboratory experiments with the observations made at sea. But there could be an increase in natural mortality if some fish died from bacterial or viral infections which they were unable to resist at low temperatures (cf. Boolootian, 1964), or if some fish weakened by parasites were unable to meet any extra metabolic demands which might arise at low temperatures (cf. Kabata, 1970, p. 102). Our records show that among the cod that survived exposure to low temperatures, 8 were infected with *Lernaeocera branchialis* and 1 fish carrying 2 parasites survived

[1] Mr. V. J Bye made enquiries among Grimsby trawler skippers and mates during the winter of 1963. He formed the impression that it was not unusual to catch one or two dead cod in some hauls and that in 1963 the presence was noted and reported because of the widespread enquiries for information on dead fish following the more extensive mortality among soles (By , personal communication). In March 1973 Mr. Scholes sailed on a Lowestoft trawler working in the southern North Sea and reported the capture of 4 dead cod, caught singly, in 63 hauls over a 10 d period. Sea temperatures would have been within the range 5°- 7°C.

Table 7. Survival and death of cod (*Gadus morhua*) held in normal sea water (32–34‰ S) for 4 h to 80 days at temperatures between 2°C and −1·5°C.

Exp. no.	Number of cod		Number of fish that died and related factor				
	Start	Survivors	Cooling failure	Aeration failure	Bacterial infection	Inflated swimbladder	Not known
3	10	9	–	1	–	–	–
6	10	10	–	–	–	–	–
7	16	8	5	–	1	1	1
11	40	40	–	–	–	–	–
12	10	9	–	–	1	–	–
13	10	9	–	–	1	–	–
14	10	10	–	–	–	–	–
15	7	7	–	–	–	–	–
16	8	8	–	–	–	–	–
17	5	5	–	–	–	–	–
18	10	9	–	–	1	–	–
19	10	5	–	–	4	–	1
Totals	146	129	5	1	8	1	2

80 days at 0·0°C (exp. 17). There is no evidence to suggest that parasitism by *Lernaeocera* contributes to the death of cod in cold water.

The survival of cod in cold water might be affected by their condition which can be assessed in terms of the condition factor K (p. 261). The seasonal change in the condition factor of whole (ungutted) cod is not known and the limited data given by Russell (1922) and Lefranc (1970) suggest a range of K from 0·84 to 1·08 for cod in the sea. Table 8 summarizes the data relating to the condition factors of cod kept without food for about 40 days at temperatures of 2°C and below. During this period the mean condition factor fell from 0·995 to 0·906. Thirteen of the cod had initial condition factors which were less than 0·900 and therefore below that of the group mean after 40 days without food. These fish could be fairly described as being in "poor condition" and the results suggest that the survival of cod in cold water may not be affected by condition when $K > 0{\cdot}800$.

Although our results (60 fish) show that cod can survive a sudden temperature change from 4·0°C to 0·0°C, Dannevig (1930a) found that cod (4 fish) died after a rapid change from 3·5°C to −1·1°C. Further experiments are needed to determine the range of sudden temperature changes which cod can survive.

Changes in blood plasma

Our results show that when cod were held in normal sea water at temperatures below 2·0°C their blood plasma had an increased ΔF and an increased salt content. These results agree with those of previous workers on cod (Eliassen et al., 1960; Leivestad, 1965). The plasma changes developed slowly and first became noticable 5–15 days after the fish were transferred to cold water and appeared to level off 35–45 days after the start of the experiment. As shown in Table 9, the plasma ΔF's and chlorides in cod held at 0·0°C for 80 days were not significantly different from those found in cod held at the same temperature for 40 days.

All teleosts examined show changes in the salt content of their serum or plasma when held at low temperatures (Umminger, 1969a): in marine species the concentration of salt increases but the reverse usually occurs in freshwater species. Furthermore, marine species which live in cold or polar waters typically have blood with a high salt content. Although the increased salt concentrations are accompanied by an increase in osmolarity, the chlorides contribute a lower proportion of the osmotically active substances that are present. If the increased

Table 8. Numbers of cod with condition factors grouped at intervals of 0.050 before and after being held in normal sea water (32–34‰ S) at 2°C or less for 36–43 days without food.

	Condition factor $K = 10\ WL^{-3}$												
	0·700 to 0·749	0·750 to 0·799	0·800 to 0·849	0·850 to 0·899	0·900 to 0·949	0·950 to 0·999	1·000 to 1·049	1·050 to 1·099	1·100 to 1·149	Over 1·150	n	Mean K	S.E.
Initial n	–	–	3	10	15	12	21	14	6	4	85	0·995	±0·01
Final n	2	3	15	19	20	13	11	1	–	1	85	0·906	±0·009

Table 9. A comparison between the plasma ΔF's and chlorides found in cod held at 0·0°C in normal sea water (32–34‰ S) for 40 and 80 days within the period December to March 1970.

Exp.	Days held	Number of cod	Plasma ΔF in °C		Plasma chlorides in mequiv/l	
			$\bar{x}$	S.E.	$\bar{x}$	S.E.
15	40	7	−0·760	±0·005	179	±1·5
17	80	5	−0·766	±0·006	181	±1·0
	t		0·825		1·155	
	d.f.		10		10	
	P		NS		NS	

salt content arose from an osmoregulatory failure, the proportion of the total osmotic activity contributed by chloride would be expected to increase. Following this line of argument, Leivestad (1965), Umminger (1969a) and DeVries (1971) have concluded that the increased salt content found in marine teleosts should not be regarded as indicating osmoregulatory failure.

Our results give no critical data on this point because the protein content of the plasma was not determined. If there was no significant change in the protein content of the plasma with temperature – as Umminger (1969b) found for *Fundulus heteroclitus* – the plasma chlorides accounted for a slightly lower proportion of the observed freezing point depression of the plasma in the cod held at low temperature. The stability of the new chloride level (Table 9) and the survival of cod at low temperature supports the view that there is no osmoregulatory failure.

The chloride changes found in cod held at low temperatures in normal sea water could have been brought about by loss of water from the blood which might be accompanied by partial dehydration of the whole fish, and the decrease in muscle water content at low temperatures is consistent with this suggestion. Our colleagues at the Ministry of Agriculture, Fisheries and Food's Radiobiological Laboratory at Lowestoft are studying water flux in cod and its relation to temperature. Some data on the percentage water content of whole cod have been made available to us and the results (Table 10) show that fish held for 21 days at 0° and 4°C have a lower water content than those held at 10°C. Our own experiments with cod held in hypotonic sea water also show overall changes in water content. The cod held at 0·0°C showed a marked reduction in plasma chloride and the data summarized in Table 4 suggest that this may have been brought about by dilution of the blood. The fish were certainly taking up water because they gained weight, although they were not fed.

If the increased chloride concentrations found in cod held in normal sea water at low temperatures are not indicative of an osmoregulatory failure, the question arises as to their significance. Leivestad (1965) suggested that the increase would reduce the metabolic cost of osmoregulation and DeVries (1971) has reviewed the role of inorganic ions – and other solutes – in relation to freezing resistance. But the true significance of the changes may lie elsewhere. Behrisch (1972) has drawn attention to the increased activity of certain enzyme systems in relation to the concentration of univalent cations and suggested that this may be of importance in thermal acclimation in poikilotherms. Similarly the increased levels of plasma glucose found in some fish at low temperatures may, as suggested by Umminger (1969b), be associated with some metabolic advantage rather than being directly related to improved resistance to freezing. We made a limited number of glucose determinations on cod held in normal sea water at −1·0°C for 36–40 d which gave plasma glucose levels of 96 mg/100 ml (n = 4, S.E. = ±9) as compared with levels of 22·5 mg/100 ml (n = 10, S.E. ± 1·9) for fish held at 10°C for 40 d. The significance of this increase is not altogether clear, but it is of interest to note that at West Greenland cod may winter in some fjords – such as Ikertok – below the sea ice (Hansen, 1949) and here an increase in plasma glucose might have a selective advantage in offering a greater resistance to freezing. Thus the increased glucose levels found in cod – which seldom encounter situations where it would be used as an anti-freeze – could be regarded as an example of pre-adaptation. It would be of interest to study the blood plasma–temperature relations in the warmer water gadoids – such as the hakes – to compare the temperate, boreal, and polar species of the group as a whole from this point of view.

Table 10. The percentage water content of whole cod kept for 21 days in normal sea water (32–34‰ S) at 0°, 4° and 10°C (data from Pentreath & Hewitt, personal communication).

Experiment	1	2	3
Temperature	0°	4°	10°
Number of fish	6	9	5
% Water content			
$\bar{x}$	78·00	78·68	80·38
S.E.	±0·212	±0·413	±0·397
t	1·48	2·95**	
d.f.	13	12	
P	NS	<0·01	
Exp. 1 & 2 pooled			
n		15	5
$\bar{x}$		78·41	80·38
S.E.		±0·270	±0·397
t		4·087***	
d.f.		18	
P		<0·001	

Table 11. Plasma ΔF's and plasma chlorides (mequiv/l) for cod held in normal sea water (32–34‰ S) for 40–43 days at 0·0°C and killed in different months

Exp.	Days held at 0·0°C	Month in which fish were killed	n	Plasma ΔF °C	Plasma chloride in mequiv/l
3	42	Dec	10	−0·800	186
16	40	Jan	8	−0·756	182
15	43	Mar	7	−0·760	179
12	42	May	9	−0·738	179
13	42	July	9	−0·736	176
18+19	40–43	July	14	−0·737	179

Table 11 summarizes the available data relating plasma ΔF and plasma chloride in cod held at 0·0°C during different months. There appears to be a seasonal difference in that the winter fish have a greater ΔF and a higher salt content than summer fish. At 0·0°C, the only temperature for which comparable data are available, the differences are small and were not influenced by day length under the conditions of the experiments. However, the timing of the light regimes used (long day, light from 0800 h to 2400 h, short day, light from 0800 h to 1600 h) may not have been appropriate to simulate the effects of natural illumination. Further investigations are required to determine the cause and significance of these seasonal differences.

Ecological aspects

Cod held in hypotonic sea water (7–8‰ S) at 10°C appeared to be very excitable and as shown in Table 3, two fish died. One of the survivors showed a marked reduction in plasma chloride and plasma ΔF. We concluded that a combination of low salinity and relatively warm water was unsuitable for cod and could be fatal. At temperatures of 5·0°–6·0°C cod become agitated at salinities less than 4·6‰ and die at salinities less than 2·7‰ (Odense, Bordeleau & Guilbault, 1966). These results are of interest in connection with the cod in the Baltic Sea. We have examined the series of monthly charts of temperature (Lenz, 1971) and salinity (Bock, 1971) for the Baltic. If cod were to be excluded from water of less than 7‰ S and warmer than 10°C, the seasonal warming of the surface water could have a marked effect on their distribution in the Gotland Sea and nearby areas. During the summer months the cod would be forced to leave the shallow water, and in particular coastal areas such as the Gulf of Riga. In August a substantial volume of water from the surface down to 20 m could be unsuitable for cod which would be forced into deeper water towards the oxygen deficient zone below the permanent halocline at a depth of about 60 m (Fonselius, 1969). Oxygen levels below the halocline in the Gotland Sea are usually less than 1·0 ml/l, as compared with a critical level of about 2·7 ml/l for cod (Sundnes, 1957). The cod could be thought of as being progressively squeezed between a thickening layer of low-salinity warm water and a core of more-saline, cool, but oxygen-deficient water. The extent to which seasonal changes in the distribution of Baltic cod can be interpreted in relation to environmental factors is not known and the present results reinforce Otterlind's (1966) plea for more work in this area.

The survival of cod in cold water in the laboratory is of interest in connection with their distribution in the Barents Sea. At Bear Island paying catches of cod taken by otter trawl are often associated with bottom temperatures greater than 2·0°C (Graham et al., 1954). Woodhead and Woodhead (1959) suggested that the relation between the distribution of cod and the 2·0°C isotherm reflected the limit to some physiological process. It now seems more likely that the significant factor is the steepness of the gradient across the front between the warm Atlantic and cold Arctic water masses rather than the position of a particular isotherm. The bottom temperature charts (for example, Lee, 1952, Figs. 10, 12, 18, 21 and 22; Cushing, 1959, Fig. 35; and Beverton and Lee, 1965, Figs. 5, 8, 9 and 10) show that the 2·0°C isotherm often appears to mark the steepest part of the gradient and it is here that cod, if present in the area, are concentrated and catches are greatest. The suggestion that the distribution of cod was related to temperature gradients was repeatedly made in the contemporary unpublished cruise reports written by the Naturalists-in-Charge when the RV "Ernest Holt" was working in the Bear Island area. For example, Graham, summarizing an Arctic Team discussion held at the Lowestoft Laboratory noted that "gradients of temperature seemed possibly more important for good catches than actual temperature" (Report for "Ernest Holt" cruise 9/1949). Beverton, returning from a cruise to the Bear Island area in July and August wrote that "the position of the 2·0°C isotherm coincided closely with the limits of their (cod) distribution except in the SE Gully where the temperature gradient was least steep" (Report for "Ernest Holt" cruise 5/1956). There are many other similar unpublished observations.

We suggest that a bottom temperature of 2·0°C only appears to be limiting and that this arises because the isotherm often marks the steepest, and thus the most readily detectable, part of the gradient. At Bear Island, Graham et al. (1954) reported a bottom

temperature change of 6·25°C in 3 cables, equivalent to a minimum gradient of 0·01°C/m. It seems possible that cod can detect temperature gradients of this order (Harden Jones, 1968, p. 192). The behavioural response could be an ortho- or klinokinesis, or a klinotaxis, and the fish could pile up on the warm side of the front. The corollary of this hypothesis would be that in the absence of a steep gradient cod, if present, would not be limited in their distribution by temperature and those fish that wander into water colder than 2·0°C would not die. It has already been noted (p. 265) that there are no reports of dead cod having been caught in cold water at Bear Island.

Summary

1. Cod held in normal sea water for 40 d at temperatures below 2°C show an increased plasma ΔF and chloride level. Cod do not die when held at 0°C for up to 80 d and there is no evidence to suggest that the plasma changes follow from an osmoregulatory failure.

2. Cod held in dilute hypotonic sea water show a decreased plasma ΔF and chloride level. Cod survived at low temperatures but warm (10°C) low salinity sea water may be lethal.

3. The blood plasma changes observed in cod held in hyper- and hypotonic sea water may be brought about, in part at least, by the loss and uptake of water respectively.

4. Cod survived cold shock from 4°C to 0°C in normal sea water and these results are not consistent with reports of cod being killed by low temperatures at sea.

5. The distribution of cod in relation to temperature observed in northern waters is probably determined by the response of the fish to the gradients across the fronts between warm and cold water rather than a direct lethal effect of low temperature. But in the Baltic Sea cod trapped in pockets of warm low-salinity wa er may die.

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