Overwintering of Adult Northern Atlantic Cod (*Gadus morhua*) in Cold Inshore Waters as Evidenced by Plasma Antifreeze Glycoprotein Levels

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Adult Atlantic cod (*Gadus morhua*) are known to produce antifreeze glycoproteins in response to cold temperatures. Our laboratory studies demonstrated that blood plasma levels in adult cod were positively correlated with the number of days they spent in subzero water. Between April 1991 and June 1993, we monitored concentrations of antifreeze glycoproteins in the plasma of late juvenile and adult cod in Trinity Bay, Newfoundland, and used the results to estimate how long cod had been exposed to low water temperatures. A consideration of these data in conjunction with detailed temperature profiles of the area taken over the course of the study allowed us to deduce the distribution of cod in relation to the temperature field. This study provides evidence that (1) blood antifreeze glycoprotein levels can be used to deduce the recent thermal history of cod in the wild and (2) after their inshore summer feeding period, considerable numbers of adult cod overwintered inshore in Trinity Bay in subzero water, producing antifreeze glycoproteins as temperatures fell below 0°C. From May onwards, "cold-adapted" cod moved into warming surface waters, where they became available to an early inshore trap fishery.

Il a été établi que les morues franches (*Gadus morhua*) adultes produisent des glycoprotéines antigels quand elles sont exposées au froid. Nous avons démontré en laboratoire l'existence d'une corrélation positive entre les taux de ces protéines dans le plasma sanguin de morues adultes et le nombre de jours que ces dernières passaient dans de l'eau à une température inférieure à 0°C. D'avril 1991 à juin 1993, nous avons mesuré les concentrations de glycoprotéines antigels dans le plasma de morues pré-adultes et adultes de la baie Trinité, à Terre-Neuve, et estimé à partir des résultats obtenus le temps d'exposition de ces poissons à de basses températures. L'examen des données recueillies et des profils détaillés de température établis dans la région durant la période de l'étude nous a permis de déduire la répartition des morues en rapport avec le champ de température. Les conclusions de l'étude sont les suivantes : 1) les concentrations sanguines de glycoprotéines antigels peuvent être utilisées pour déduire l'exposition thermique récente des morues dans leur milieu naturel et 2) après leur période d'alimentation estivale dans les eaux côtières, un grand nombre de morues adultes avaient hiverné dans les eaux côtières de la baie Trinité, exposées à des températures inférieures à 0°C, ce qui leur faisait produire des glycoprotéines antigels. À partir du mois de mai, les morues « adaptées au froid » ont gagné les eaux de surface, qui commençaient alors à se réchauffer, et pouvaient ainsi faire l'objet d'une pêche côtière printanière à la trappe.

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Atlantic cod (*Gadus morhua*) in the wild generally inhabit waters ranging in temperature from −0.5 to 10°C (Rose and Leggett 1988; Scott and Scott 1988). However, field observations of cod distribution in relation to temperature suggest that their optimal thermal range is between 0 and 5°C (Beverton and Lee 1965; Templeman and May 1965; Lear et al. 1986; Rose and Leggett 1988). Further, Taggart and Frank (1987) gave 2.9°C as the median

temperature for highest cod catches in the Northwest Atlantic and elsewhere.

The adult and juvenile Atlantic cod studied in this work were from the Northern cod stock, defined as those cod populations inhabiting NAFO Divisions 2J, 3K, and 3L off the coast of Newfoundland and Labrador. They will be referred to throughout as Northern cod, or simply cod.

The pattern of migration accepted for the majority of Northern cod allows them to inhabit their optimal thermal range throughout the year (Templeman 1966; Lear and Green 1984; Lear et al. 1986). Adult Northern cod are described leaving inshore summer feeding grounds by late fall – early winter to overwinter offshore in warm (>2°C), deep water below the cold intermediate layer (Petrie et al. 1988). Spawning takes place in spring and is followed by an inshore migration to summer feeding grounds (Templeman 1966). Once inshore, the cod feed primarily on capelin (*Mallotus villosus*), become replenished, and move offshore at the end of the summer to repeat the cycle (Lear and Green 1984; Lear et al. 1986). By overwintering offshore, adult cod avoid exposure to the potentially lethal combination of very low water temperatures (down to −1.8°C) and ice that is a regular winter occurrence in the coastal seas of Newfoundland (Templeman 1966; Symonds 1986; Davies et al. 1988; Petrie et al. 1988; Fissel and Tang 1991).

However, there is evidence to suggest that some adult cod overwinter inshore in Newfoundland bays where water temperatures regularly fall below zero during the winter months (Templeman and Fleming 1965; Wroblewski et al. 1994). Have these fish spent the winter inhabiting cold water, or have they mainly confined themselves to local inshore areas of deep warm water as suggested by Thompson (1943), making only short-duration feeding sorties into the predominantly subzero waters of the bays?

Because continuous monitoring of the overwintering behaviour of significant numbers of free-roaming cod is not practical, the development of alternative methods, such as the use of biochemical or physiological indicators of thermal history, is desirable. We suggest that measuring antifreeze levels in blood plasma is one such appropriate technique.

When exposed to low temperatures, cod produce glycoproteins which are found in the blood and other extracellular fluids. These are termed antifreeze glycoproteins because they increase the freeze resistance of the fish by lowering the plasma freezing point from usual summer values of between −0.6 and −0.8°C (both juveniles and adults) to approximate mean values of −1.5°C in juvenile and −1.2°C in adult cod (Hew et al. 1981; Fletcher et al. 1987; Kao and Fletcher 1988; Goddard et al. 1992). In addition, experiments suggest that antifreeze proteins/glycoproteins protect the integrity of cell membranes at low temperatures (Rubinsky et al. 1990, 1991; Negulescu et al. 1992). This dual function of antifreeze would appear to be of considerable survival value to cod living in very cold, possibly icy waters.

Antifreeze levels in adult cod appear to be regulated entirely by water temperature. When adults are continuously exposed to temperatures of 0°C and below during winter, antifreeze glycoproteins appear in the blood and increase to maximal levels over a period of months, significantly lowering the plasma freezing point. The loss of antifreeze is also temperature dependant. As water temperatures rise above 0°C after the winter, blood antifreeze levels decline until eventually no antifreeze can be detected in the plasma (Fletcher et al. 1987). While antifreeze has, on occasion, been found in the plasma of adult cod acclimated to temperatures between 0 and 2°C, levels have always been low and transitory (Fletcher et al. 1987; S.V. Goddard and G.L. Fletcher, unpublished data). With certain provisos (see Discussion) the relationships between water temperature and the level of plasma antifreeze can be used in the following way to deduce the thermal history of adult cod in the field. First, the presence of significant levels of antifreeze in the blood (sufficient to produce a >0.1°C difference between the freezing and melting point of the plasma) indicates acclimation to 0°C or below. Second, the antifreeze concentration gives an indication of how long the cod have been exposed to cold water. Third, as warm (>0°C) water becomes available to cold-adapted fish (either by migration to other areas or by solar warming in the area occupied), we can estimate the length of time fully cold-adapted cod have been exposed to temperatures above 0°C by the rate of loss of antifreeze from the plasma.

In this study, we have (1) developed a plasma antifreeze/water temperature calibration to estimate the length of time cod have been exposed to low water temperatures, (2) measured the plasma antifreeze levels in cod caught at different times of the year in Trinity Bay, Newfoundland, and the temperature of the water from which they were collected, and (3) used these data, coupled with temperature data obtained from a long-term oceanographic monitoring program in the area, to deduce the thermal history (weeks–months) and distribution, with respect to temperature, of cod in Trinity Bay.

Materials and Methods

General Collection and Analysis of Blood Samples

All blood samples (1–2 mL) were taken from a caudal blood vessel using 3-cc syringes fitted with 21- or 23-gauge needles. Sampling usually took less than 1 min and no anaesthetic was used. Blood samples were placed in Vacutainers containing sodium heparin (Becton Dickinson) and centrifuged for 10 min at 4000*g* to separate cells from plasma. Plasma samples were then stored at −20°C until analysed.

Antifreeze activity was assessed using a nanolitre osmometer (Clifton Technical Physics, Hartford, N.Y.). With this technique, a single ice crystal within the plasma sample is observed under a microscope, and by fine temperature adjustments within the stage holding the sample, the plasma freezing and melting points can be obtained. The difference between the freezing and melting temperature is termed thermal hysteresis and is a direct measure of antifreeze activity (Kao et al. 1986).

Collection of Blood Samples from Cod Held in Laboratory Raceways

From 1984 to 1991, adult cod were collected during the summer (July and August) cod trap fishery in Conception Bay, Newfoundland. From summer until June the following year, these fish were held under ambient light and temperature conditions in a 40 000-L raceway supplied with running seawater pumped directly from a depth of 5–10 m in Logy Bay at the laboratory location (Fletcher et al. 1987). These raceway-held cod were exposed to subzero temperatures from January to April, with plasma antifreeze levels typically

TABLE 1. Sampling dates and days of exposure to subzero temperatures for cod held in the laboratory raceway. Length of exposure to subzero temperatures was obtained by subtracting the Julian day on which the water temperature in the raceway fell below 0°C from the sampling date (Julian days). n = number of fish sampled.

Sampling date	Subzero exposure (d)	n
Jan. 9, 1991	2	10
Jan. 13, 1988	6	11
Jan. 18, 1991	11	9
Jan. 17, 1985	15	8
Jan. 25, 1991	18	9
Jan. 27, 1986	25	6
Jan. 30, 1984	28	5
Feb. 5, 1991	29	10
Jan. 31, 1989	32	7
Feb. 24, 1988	48	18
Feb. 27, 1985	56	10
Feb. 29, 1984	58	5
Mar. 13, 1985	70	5
Mar. 18, 1991	70	10
Mar. 15, 1989	75	10

reaching peak values by mid-March and declining between April and June as water temperatures increased (Fletcher et al. 1987; Goddard et al. 1992). The date on which the water temperature in the raceway first fell below 0°C each winter was recorded (between December 28 and January 7 in all years considered).

During the rising phase of antifreeze production (January–mid-March), blood samples were collected from raceway-held cod. The sampling dates were converted into length of exposure of the animals to subzero temperature. The sampling schedule is presented in Table 1.

Collection of Blood Samples from Cod in the Field

Cod sampled in the field were separated into juvenile (<40 cm) and late juvenile/adult (40+ cm) length categories. The physiology of antifreeze production appears to differ in juvenile and adult cod (Kao and Fletcher 1988; Goddard et al. 1992). Therefore, only data from the latter group were compared with data derived from adult cod held in the laboratory raceway.

Blood samples were collected from cod at a variety of sites (see Fig. 1) in the Random Island area of Trinity Bay on the following dates.

April 8, 1991

Forty seven cod (length 37.5–88.9 cm, 45 fish 40+ cm) were caught in Hatchet Cove, Southwest Arm, with baited longlines (six fish) and gill nets (41 fish). Fish were caught at depths greater than 150 m at water temperatures of −0.6 to −0.7°C.

May 1, 1992

Twenty cod (length 38.7–53.3 cm, 18 fish 40+ cm) were caught in a cod trap in Resolution Cove, Smith Sound. The fish were in the top 20 m of the water at temperatures between 0 and 1°C.

May 28, 1992

Twenty-six cod (length 39.8–49.3 cm, 25 fish 40+ cm) were caught in a cod trap in Nut Cove, Smith Sound. Fish were in the top 40 of the water at temperatures between 1.8 and 3.7°C. Subsequent to blood sampling, these fish were transported to the laboratory, tagged, and held in the raceway under seasonally ambient conditions of temperature and photoperiod until July 29, 1992, when blood samples were again taken from the remaining 17 live fish. During this period, raceway temperatures were taken several times a week.

June 18–20, 1992

Thirty-nine cod (length 29.0–67.0 cm, 26 fish 40+ cm) were caught by otter trawling along the bottom of Southwest Arm at depths of 150–180 m and water temperatures between −0.9 and −1.3°C.

June 29–30, 1992

Twenty-six cod (length 26.0–83.0 cm, 12 fish 40+ cm) were caught by jigging in approximately 40 m of water in Newman Sound, Bonavista Bay (see Fig. 1, inset).

September 30 – October 1, 1992

Forty-four cod (length 26.0–49.0 cm, 10 fish 40+ cm) were caught at St. Jones Within, Southwest Arm, either by jigging in 40 m of water (12 fish) or in a capelin trap set in 20 m of water (32 fish). Surface temperatures were approximately 12°C, while temperatures at 40 and 20 m were 2.3 and 8.0°C, respectively. At this time, no cod were caught by otter trawling along the entire length of Southwest Arm. Bottom temperatures along the trawl path ranged from −1.3 to 0°C.

June 2–5, 1993

Sixty-one cod (length 27.0–51.0 cm, 26 fish 40+ cm) were caught by otter trawling in Southwest Arm at depths from 139 to 174 m and temperatures from −0.9 to −1.2°C. This was essentially a repeat of our June 1992 sample.

Temperature Measurements in the Field

Temperature sections were taken in Southwest Arm on January 24, 1991, and out into the deep water of Trinity Bay on January 27–28, 1991. On March 13, 1991, in the Hatchet Cove area of Southwest Arm, temperature/depth profiles were taken both at the edge (to 40 m approximate depth) and half way across (to 160 m approximate depth) the Arm. In all cases, temperatures were recorded with a VEMCO Ltd. Sealog-TD temperature/depth recorder (accuracy = ±0.1°C/±5 m). In addition, between March 1991 and August 1992, water temperatures in the Random Island area of Trinity Bay were monitored in sufficient detail to allow production of monthly temperature sections for Southwest and Northwest Arm (Wroblewski et al. 1993).

Temperatures and depths of capture were recorded on each occasion that cod were collected for blood sampling. Vertical temperature profiles were taken when gill nets, longlines, jiggers, or traps were used. When using the otter trawl, temperature/depth profiles were taken on completion of the tows at either end of the tow path using a wire-mounted Sea-Bird Electronics Inc. Seacat SBE 19-03. The Sea-Bird Seacat equipment was used to take temperature/depth profiles on June 18–20, 1992, June 29–30, 1992, September 30 – October 1, 1992, and June 2–5, 1993, while the VEMCO Sealog equipment was used on April 8, 1991, May 1, 1992, and May 28, 1992.

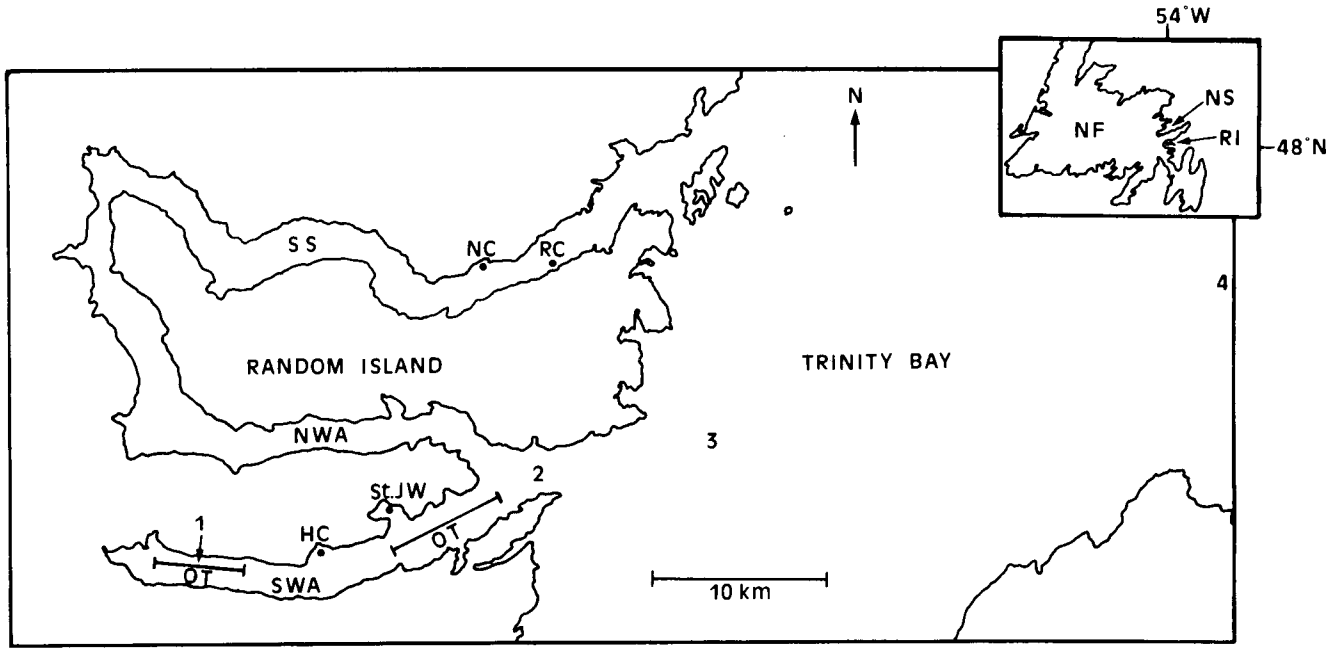

FIG. 1. Chart of the Random Island area of Trinity Bay, Newfoundland, showing transect line (1,2,3,4) along which temperature stations were located. SWA, Southwest Arm; NWA, Northwest Arm; SS, Smith Sound; five fish capture sites, namely HC (Hatchet Cove, April 8, 1991), RC (Resolution Cove, May 1, 1992), NC (Nut Cove, May 28, 1992), OT (Southwest Arm, otter trawl path, June 18–20, 1992, and June 2–5, 1993). St.JW (St. Jones Within, September 30 – October 1, 1992). Inset chart shows location of Random Island (RI) in Trinity Bay, relative to Newman Sound (NS) in Bonavista Bay, where cod were sampled on June 29–30, 1992.

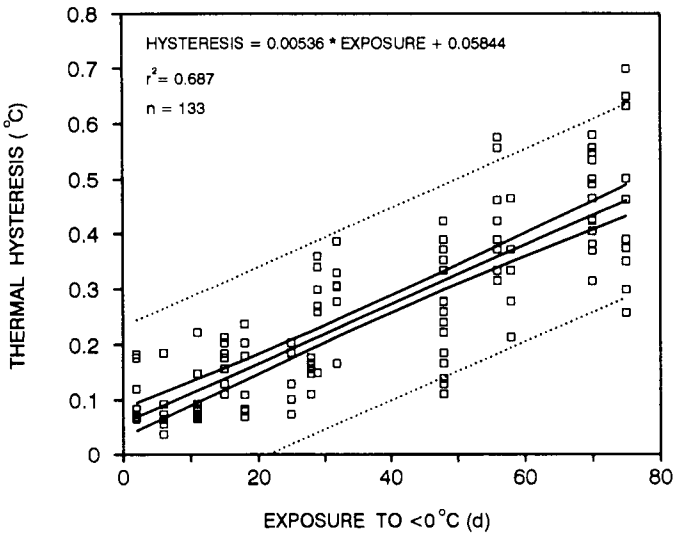

FIG. 2. Relationship between plasma antifreeze levels (thermal hysteresis) and the length of time that adult cod had been living at subzero temperatures. Solid lines are the 95% confidence limits around the regression; dotted lines are the 95% prediction limits for thermal hysteresis in individuals from a sample population of known subzero exposure time.

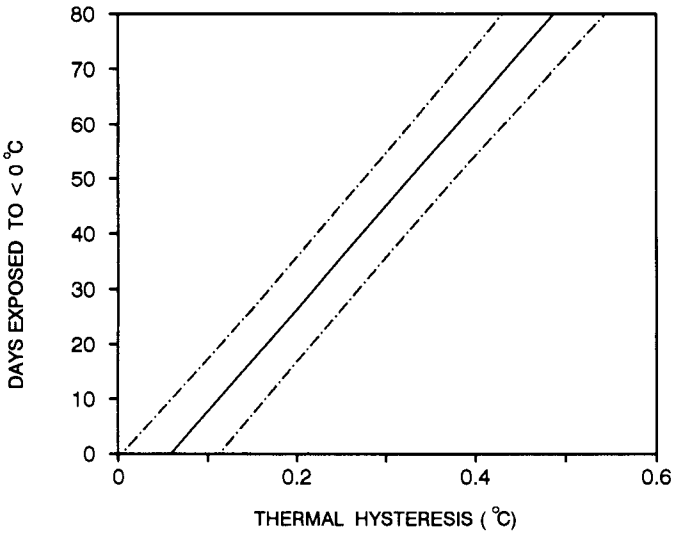

FIG. 3. Expected length of exposure to subzero temperatures for a measured mean value of thermal hysteresis (graph derived from the relationship given in Fig. 2 using inverse prediction statistics). Broken lines are the 95% confidence limits for the expected values for length of exposure of a sample population.

Results

Laboratory Observations

In adult cod held under ambient temperature and light conditions, blood plasma thermal hysteresis (antifreeze) levels were significantly correlated with the duration of exposure to subzero temperature during the rising phase of antifreeze production (Fig. 2). Mean maximal levels of thermal hysteresis (0.46°C) were reached by mid-March, approximately 75 d after first exposure to subzero temperatures. Using the inverse prediction statistics (estimation of X from Y) outlined by Sokal and Rohlf (1981), this relationship can be used as a first approximation in deducing the thermal history of cod in the wild (Fig. 3).

Field Observations

Temperature sections taken at the end of January 1991 showed that while much of the surface water and some of the bottom water in Trinity Bay was below 0°C, a large lens

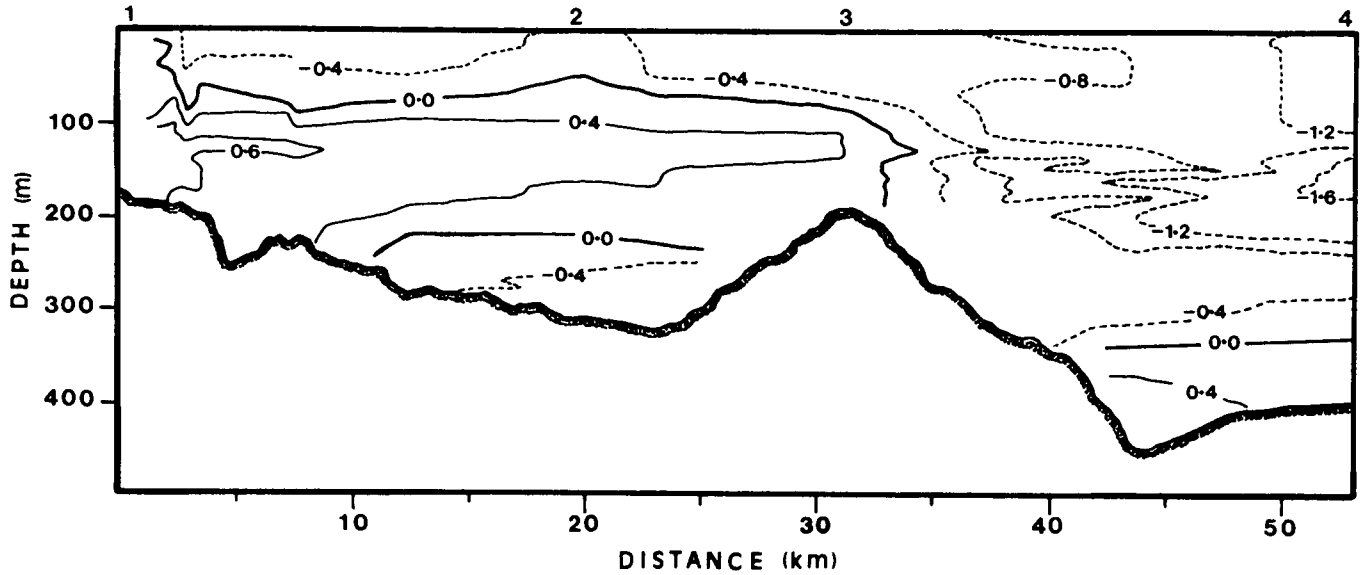

FIG. 4. Temperature section taken at the end of January 1991 along transect line 1,2,3,4, (see Fig. 1) running from the head of Southwest Arm out to the mouth of Trinity Bay, Newfoundland. Solid lines are isotherms >0°C; broken lines are isotherms <0°C.

of warmer water was present in Southwest Arm (Fig. 4). In addition, a pocket of warmer (>0°C) water was recorded deep (>350 m) in Trinity Bay (Fig. 4). By March 1991, however, temperature/depth profiles taken in Hatchet Cove and along a section in Northwest Arm (Wroblewski et al. 1993) revealed uniformly subzero water, with most temperatures at or below −1.0°C. By April 8, 1991, temperature sections showed Southwest and Northwest Arm to be uniformly below 0°C. The cod caught on this date were found in −0.7°C water. These 47 animals had a mean thermal hysteresis level of 0.17 ± 0.01°C (Table 2), suggesting a relatively brief (approximately 19 ± 10 d) exposure to 0°C water (Fig. 3). Fifteen of these fish were identified as belonging to a group of 2690 cod that had been previously caught, tagged, and released in Southwest Arm between January 20 and 27, 1991 as part of the Department of Fisheries and Oceans' (DFO) tagging program. There was no significant difference in antifreeze levels between cod bearing tags (0.19 ± 0.02°C) and the untagged cod (0.15 ± 0.01°C).

Cod caught on May 1, 1992, had high plasma levels of antifreeze with a mean thermal hysteresis value of 0.44°C, indicating exposure to subzero temperatures for approximately 70 ± 10 d (Table 2; Fig. 3). Laboratory experiments have demonstrated that such a high level of antifreeze activity in adult cod is only achieved after long-term exposure to subzero temperatures (Fletcher et al. 1987). The cod caught on May 1 were found at approximately 20 m depth in water that had just warmed to temperatures above 0°C.

Cod caught on May 28 were in the top 40 m in water that had been warming for approximately 4 wk. Their mean thermal hysteresis was 0.25°C (Table 2). Subsequent to sampling, these cod were brought back and held for observation in the laboratory. After 2 mo in seasonally ambient, warm (maximum 9.1, minimum 2.4°C) seawater the remaining cod from this group were sampled again, and all were found to have lost their antifreeze (n = 17; mean thermal hysteresis = 0.063 ± 0.003°C; mean plasma freezing point = −0.69 ± 0.01°C).

On June 18–20, 1992, although surface water temperatures had been above 0°C for almost 50 d, large numbers of cod were caught by otter trawling along the bottom of Southwest Arm in uniformly subzero water. In all cod 40+ cm, thermal hysteresis levels were high (0.43 ± 0.02°C) and plasma freezing points low (−1.34 ± 0.02°C) (Table 2), again indicative of long-term (months) exposure to subzero temperatures (Fig. 3). In cod 30–40 cm in length (n = 13), mean thermal hysteresis was 0.41 ± 0.03°C and mean plasma freezing point was −1.32 ± 0.03°C. There was no significant difference between the <40 and 40+ cm cod in either thermal hysteresis or plasma freezing point ($P > 0.05$ in both cases). None of these values was significantly different from those of the May 1 sample (Table 2) or from maximum values of thermal hysteresis developed in laboratory-held raceway cod continuously exposed to subzero temperatures (Fig. 2).

Between June 29 and 30, 1992, blood samples were taken from 26 cod caught by jigging in Newman Sound, Bonavista Bay. On June 29, 13 cod were caught, seven > 40 cm. While 10 cod had no antifreeze (mean thermal hysteresis = 0.064 ± 0.003°C), three had very high antifreeze levels (length 41, 53, and 83 cm; thermal hysteresis 0.58, 0.28, and 0.63°C, respectively). On June 30, 13 cod were caught (five >40 cm) and none of these 13 fish showed any trace of antifreeze in the plasma (mean thermal hysteresis 0.061 ± 0.003°C). All the cod were caught in approximately 40 m of water at the same site (48.58°N, 53.90°W); however, the temperature/depth profile was considerably different between the two days. On June 29, the bottom temperature was 0.27°C, while the temperature at 30 m was 2.8°C. On June 30, these temperatures were 4.17 and 4.9°C, respectively.

By October 1, 1992, no cod were found in the subzero bottom water of Southwest Arm. Cod were caught only in the warm (>2°C) surface waters. None of these fish had antifreeze in their blood plasma (Table 2).

Between June 2 and 5 of the following year (1993), considerable numbers of cod were caught by otter trawling along Southwest Arm in water at temperatures of −0.9 to −1.2°C. Analysis of antifreeze activity in the plasma of 26 cod (>40 cm) gave mean thermal hysteresis and plasma freezing points of 0.42 ± 0.03°C and −1.35 ± 0.03°C, respectively. These values were consistent with those obtained at

TABLE 2. Levels of thermal hysteresis (antifreeze activity) and plasma freezing points in adult and late juvenile cod (40+ cm) found in Trinity Bay, Newfoundland, between April 1991 and June 1993. Values of thermal hysteresis and plasma freezing points are presented as means ± 1 SE. n = number of fish sampled.

Date	Location	Thermal hysteresis (°C)	Plasma freezing point (°C)
April 8, 1991 (n = 45)	Hatchet Cove, Southwest Arm	0.16 ± 0.01	−1.03 ± 0.01
May 1, 1992 (n = 18)	Resolution Cove, Smith Sound	0.44 ± 0.03	−1.33 ± 0.04
May 28, 1992 (n = 25)	Nut Cove, Smith Sound	0.25 ± 0.02	−1.09 ± 0.02
June 18–20, 1992 (n = 26)	Southwest Arm	0.43 ± 0.02	−1.34 ± 0.02
October 1, 1992 (n = 10)	Southwest Arm	0.07 ± 0.01	−0.90 ± 0.01
June 2–5, 1993 (n = 26)	Southwest Arm	0.42 ± 0.03	−1.35 ± 0.03

the same site the previous year (Table 2), again reflecting long-term exposure to subzero temperatures (Fig. 3).

Discussion

Two principal conclusions can be drawn from this work. First, it demonstrates that the laboratory calibration curve relating plasma antifreeze levels to the length of time spent in subzero waters can be used to deduce the distribution of cod in relation to the temperature field. In other words, antifreeze levels can be used as a physiological time tag to estimate length of continuous exposure to subzero water. However, it must be remembered that in order to draw inferences about the behaviour of cod with respect to temperature, it is necessary to have sufficient information on the temperature field. Thus, this technique is particularly valid when used in a situation such as that described in this paper, where groups of cod were monitored within a well-defined area for which long-term temperature data were available. Predictions of residence time in subzero water can be made with the most confidence when mean antifreeze levels are high, indicating long-term acclimation to 0°C and below. Conclusions as to whether cod make short-term incursions from cold to warm or from warm to cold water cannot be drawn using this technique.

Second, the results clearly demonstrate that some cod overwintering in the Newfoundland inshore area spend considerable lengths of time in subzero waters, many opting to stay at these low temperatures for at least 2 mo after surface waters warm to temperatures above 0°C.

There is considerable literature available on preferred temperatures of cod in the wild. While temperature preference apparently changes somewhat with season, geographic location, and fish size, the majority of favoured ranges are reported to fall between −0.5 and 10°C (Martin and Jean 1964; Beverton and Lee 1965; Templeman and May 1965; Woodhead and Woodhead 1965; Minet and Poulard 1973; Rose and Leggett 1988; Scott and Scott 1988). More specifically, cod of the Northern cod stock have been reported to spend most of their time in water at temperatures between 0 and 5°C, making only short-duration forays into either colder or warmer water, for example, in search of prey organisms (Postolakii 1963; Templeman 1965, 1966; Lear et al. 1986). Thus, it might be expected that when faced with a declining temperature regime such as must be experienced in inshore waters as winter approaches, adult and late juvenile cod would attempt to remain in water close to their preferred temperature, either by leaving inshore areas or, as suggested by Thompson (1943), by inhabiting pockets of warmer water inshore. The data presented in this paper, however, suggest the following pattern of behaviour for cod overwintering in the Trinity Bay area of Newfoundland.

During the summer months (June–August), cod inshore feed pelagically on capelin in surface waters (Templeman 1965; Lilly and Fleming 1981; Lilly and Botta 1984). The elements dictating which fish leave and which stay have yet to be identified; however, as winter approaches, some cod migrate offshore, while others do not. Those remaining inshore stay with their acclimation temperature for as long as possible, and, as the general water temperature in the area declines through 0°C, they produce antifreeze glycoproteins in response to the low temperatures they are experiencing, just as cod held in the laboratory raceway do.

Thermal hysteresis levels in cod caught in April 1991 were consistent with comparatively short-term residence in subzero water (from mid-March, approximately). It seems likely that the 15 cod tagged in Southwest Arm in January 1991 had been resident in the area up to the point where they were caught in April 1991. Further, since there was no significant difference in antifreeze levels between the tagged and untagged cod caught in April, it seems reasonable to assume that they had all been there during the winter. Oceanographic data for the area (Fig. 4) show that a lens of warm water was present in Southwest Arm in January, but was gone by March 13 (Wroblewski et al. 1993). Thus, the data from cod caught in April 1991 suggest that they chose to occupy this warmer water while it was available. As the water cooled, the cod acclimated gradually to the increasingly colder temperatures. By the time water temperatures in the area became uniformly subzero (probably some time in March), they were producing antifreeze glycoproteins and could thus tolerate low temperatures (Fletcher et al. 1987).

Of the 2690 cod tagged in Southwest Arm in January 1991, 812 tagged fish (93% of the total recaptured by April 1, 1992) had been caught by August 31, 1991, less than 30 miles from the tagging location, by the inshore fishery.

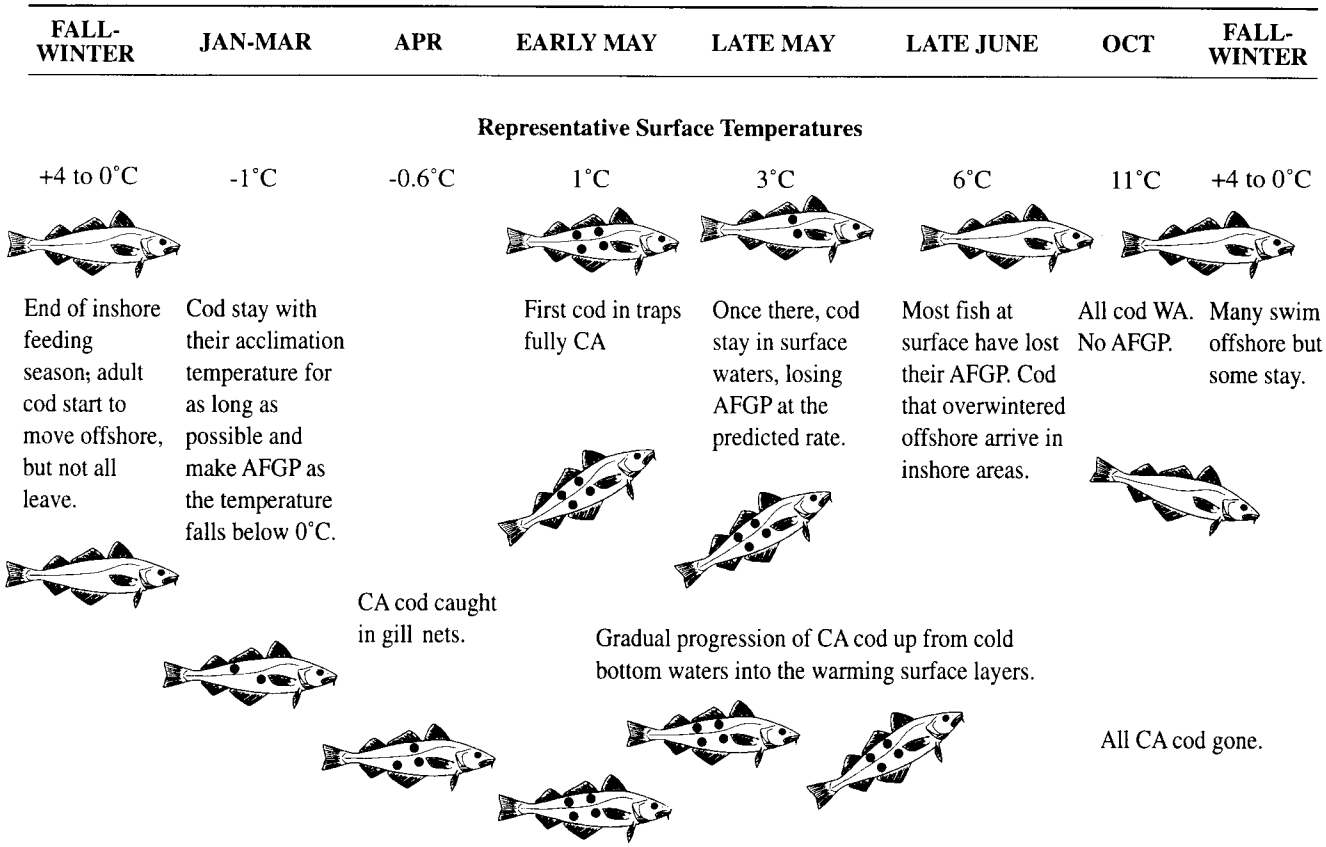

FIG. 5. Data-supported conceptualization of cod distribution in relation to temperature and depth, year-round in Trinity Bay, Newfoundland, as inferred from antifreeze levels and information on temperature and depth of capture obtained between April 1991 and June 1993. AFGP, antifreeze glycoproteins; WA, warm adapted; CA, cold adapted. Degree of cold adaptation (antifreeze level) is represented by solid circles, such that fish with no circles are warm adapted, with no antifreeze; those with two or three circles are either gaining or losing antifreeze; those with four circles are fully adapted to low temperatures, with mean antifreeze levels at maximum.

This suggests either that considerable numbers of cod had remained inshore since January or that the tagged cod had homed back to the tagging area following dispersal. Between January and March 1992, nine of the group tagged inshore in January 1991 were caught in the offshore winter fishery, suggesting that a fish overwintering inshore one year may move offshore to overwinter another year.

On May 1, 1992, cod were caught in surface waters that had just warmed to temperatures above 0°C. Individual thermal hysteresis values in these fish were uniformly high, and the mean level was consistent with mean maximal levels produced by cod held at temperatures below 0°C in the laboratory for approximately 75 d or more (Fig. 2; Table 2). In other words, the wild fish had been in subzero water since at least February 15. During the winter of 1992, the last pocket of water warmer than 0°C in the Random Island region of Trinity Bay was recorded on February 13 (Wroblewski et al. 1993). Thus, oceanographic data corroborate the physiological data and together they strongly suggest that all the adult cod caught in a cod trap on May 1 had been resident in cold inshore water throughout the winter.

In contrast with the cod caught during this study in Trinity Bay in May, a group of 20 adult (>45 cm) cod caught on May 16, 1991, on the northern Grand Banks in water >300 m deep and at temperatures between 1 and 3°C had no trace of antifreeze in the plasma (Howse 1993). Mean thermal hysteresis in these fish was 0.06 ± 0.002°C. The complete absence of plasma antifreeze in all these fish was not unexpected, as they were found in comparatively warm water; however, it further supports the contention that the cod sampled in Trinity Bay on May 1, 1992, had been overwintering at subzero temperatures.

The observations in Trinity Bay during the month of May also provide evidence that inshore overwintering cod become available to the inshore fishery before the arrival of cod from offshore, traditionally estimated to occur after the beginning of June. Solar warming of surface waters to temperatures above 0°C (as happened at the end of April in 1992) may be a signal for cold-adapted fish to start moving into shallow waters where they become available to the trap fishery.

Once antifreeze production ceases, its rate of removal from the plasma is temperature dependent, the biological half-life being approximately 15 d at 5°C, 37 d at 1°C, and almost 100 d at 0°C (Fletcher et al. 1987). Although the May 1 fish were caught at the surface at temperatures >0°C, they had maximal antifreeze levels. This is not surprising because the surface waters only reached temperatures above 0°C at the end of April (Wroblewski et al. 1993). However, by May 28, the mean antifreeze level found in cod caught at the surface had fallen to approximately 57% of that present in the cod sampled on May 1 (an approximate half-life of

34 d). Individual values of thermal hysteresis were consistent with the hypothesis that fish in this sample had been maximally cold adapted but were now losing their antifreeze protection. Using the results for rate of loss of antifreeze against temperature presented by Fletcher et al. (1987), it can be calculated that these fish must have spent the whole 28 d from May 1 to 28 in water temperatures between 1 and 5°C. In other words, rate of loss of antifreeze suggests that once in the warmer surface waters, these cold-adapted cod were staying there rather than returning to the subzero bottom waters in the Random Island area. By the end of summer, previously cold-adapted cod would have lost all their plasma antifreeze while inhabiting the warmer waters near the surface. This contention is supported by the fact that continued exposure to summer temperature conditions (in the laboratory) resulted in a complete loss of antifreeze from the plasma of the cod brought back from Nut Cove. Thus, they exhibited the response typical of all cod held under such conditions in the laboratory during past years (Fletcher et al. 1987).

However, in both 1992 and 1993, cod trawled from the bottom of Southwest Arm in June were found to be maximally cold adapted, suggesting that they had been resident in subzero water for at least 75 d, and presumably for the entire winter (i.e., from some time between January and February). These cod probably represented the remainder of the deepwater group from which the cold-adapted cod caught in April and May originated. It seems likely that from the end of April onward there is a gradual movement of cold-adapted inshore overwintering cod out of the subzero bottom water and into the warming surface layers, possibly in search of food. This movement cannot be motivated simply by the presence of warm water, as some fully cold-adapted cod were still on the bottom in late June, at least 50 d after the surface water warmed above 0°C. The fish caught on June 29–30, 1992, in Newman Sound suggest a possible mechanism for the movement of cold-adapted cod from cold bottom water to warm surface water. Bay-scale oceanographic events bringing cool bottom water and associated cod up into the shallows may play a part in this progression.

By the end of September, trawling along the bottom of Southwest Arm resulted in failure to catch any cod. Cod were only caught at depths <50 m, in temperatures considerably warmer than 0°C, and none of these animals showed any evidence of plasma antifreeze. Either all cold-adapted cod had moved out of Southwest Arm or they had all moved nearer the surface to feed in warmer water and, in the process, had lost all their antifreeze. Of these two alternatives, the latter seems the most likely, particularly because the cod caught in April 1991 were still in the process of becoming cold adapted.

The results of our work have been summarized in a diagram showing cod distribution in the Random Island area of Trinity Bay with respect to temperature, depth, and time using the field data collected between 1991 and 1993 (Fig. 5).

Rose and Leggett (1988) suggested that cod 40+ cm may not be capable of surviving prolonged exposure to temperatures outside the preferred range of −0.5 to 8.5°C, although they found juvenile (15–20 cm) cod living at temperatures near −1°C. During the winter of 1991–92, water temperatures in Northwest and Southwest Arm, Trinity Bay, were consistently low, with the majority of the water column being below −0.6°C (Wroblewski et al. 1993). Indeed, cod were caught in April 1991 at water temperatures between −0.6 and −0.7°C, while in June 1992, cod were trawled from water at temperatures below −1.0°C. The ability of adult cod to survive extreme low temperatures for extended periods in the ocean can, therefore, no longer be in question. However, it is known that when held under the same environmental conditions, antifreeze levels in adults are not high as those found in juvenile cod (Goddard et al. 1992). Thus, at times of extreme low temperature coupled with the presence of ice in the water column, adult cod may exhibit patterns of behaviour that minimize the danger of ice crystal damage, such as occupying deep water and reducing their swimming activity to a minimum.

A further point to note is that mean maximum thermal hysteresis developed by the inshore overwintering cod sampled in the field matched, almost exactly, that developed in winter by cod that had been obtained from the summer (July–August) trap fishery in Conception Bay over a period of 8 yr and overwintered in the laboratory (Table 2; Fig. 2). The July–August trap fishery has always been considered to depend largely on the landward migration of cod that have overwintered offshore on the slopes of the Banks (Templeman 1966; Lear et al. 1986). Thus, it seems likely that many of the cod used to obtain the calibration curve data were part of this landward migration. Therefore, the data suggest that there is little difference in the capacity of offshore and inshore overwintering adult cod to tolerate subzero winter water temperatures, and thus, in terms of low temperature tolerance alone, all adult cod from the Newfoundland/Labrador region appear to have the capacity to overwinter inshore successfully.

An understanding of the physiology of antifreeze production in cod of the "Northern" stocks coupled with extensive oceanographic monitoring of the relatively small study area has allowed us to reach some conclusions on the distribution of cod within the temperature field in Trinity Bay, Newfoundland. With the proviso that calibration curves are obtained for each stock considered, this method may be applicable to investigations of the distribution of other northern populations of cod in relation to low temperature.

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