

Production of antifreeze glycoproteins in cultured and wild juvenile Atlantic cod (*Gadus morhua* L.) in a common laboratory environment

C.F. Purchase, S.V. Goddard, and J.A. Brown

Abstract: Many fishes accumulate antifreeze proteins or antifreeze glycoproteins (AFGPs) in the blood to increase their chances of survival in cold seawater. Cod (*Gadus morhua* L.) from colder environments have been found to produce more AFGPs than those from warmer areas, but the genetic and environmental contributions to this variation have not been determined. Populations of cultured (from the Grand Banks; Gulf of Maine) and wild (from Fortune Bay; Bonavista Bay) juvenile cod were kept in a common laboratory environment to investigate differences in AFGP production. All the populations were capable of producing AFGPs, and the AFGP levels were similar in cultured and wild cod. The results indicate that high temperatures associated with the production of cultured cod do not negatively affect the ability to produce AFGPs. In addition, young cod from as far south as the Gulf of Maine are capable of producing AFGPs at levels similar to those from the northeast coast of Newfoundland.

Résumé : Plusieurs espèces de poissons accumulent des protéines ou des glycoprotéines (AFGPs) antigel dans leur sang pour augmenter leurs chances de survie dans l'eau de mer froide. Les Morues franches (*Gadus morhua* L.) des milieux plus froids produisent plus d'AFGP que celles qui viennent de régions plus chaudes, mais les contributions génétique et environnementale de cette variation n'ont jamais été déterminées. Nous avons gardé des populations d'élevage (Grands Bancs; golfe du Maine) et sauvages (baie Fortune; baie Bonavista) de morues juvéniles dans des conditions habituelles de laboratoire pour pouvoir étudier les différences dans la production d'AFGP. Toutes les populations étaient en mesure de produire des protéines antigel et les concentrations de protéines étaient semblables chez les quatre populations. Nos résultats indiquent que les températures élevées associées à la production de morues d'élevage n'affectent pas leur capacité de produire des protéines antigel. De plus, les jeunes morues provenant de régions aussi méridionales que le golfe du Maine sont capables de produire des protéines antigel en concentrations équivalant à celles que produisent les morues de la côte nord-ouest de Terre-Neuve.

[Traduit par la Rédaction]

Introduction

Research on the structure of Atlantic cod (*Gadus morhua* L.) populations has intensified following the collapse of northern cod stocks in the early 1990s (Ruzzante et al. 1999). Traditionally, distinct cod populations have been identified by means of morphological characteristics such as vertebral counts (Templeman 1981). These are phenotypic characters that are frequently influenced by the environment and therefore of limited use in identifying genetic differences (Swain and Foote 1999). In the northwest Atlantic, stocks have traditionally been managed as if they are single homogeneous breeding populations occurring over large geographic areas, with only limited localized populations. In recent years, molecular techniques have become a standard means of identifying pop-

ulation structure at the genetic level (Bentzen et al. 1996; Ruzzante et al. 1996). Using these methods, differences in cod populations have been found to occur on finer scales than was previously believed (Ruzzante et al. 1996). However, the degree of sensitivity of detecting differences among stocks often depends on which technique is used (Ruzzante et al. 1999), and a combination of tagging, behavioural, genetic, and phenotypic information would be the optimal way to discriminate between populations (Myers et al. 1997).

Cod and many other cold-ocean fishes produce blood-borne antifreeze proteins or antifreeze glycoproteins (AFGPs) that protect the individual from freezing in water which regularly reaches temperatures below the tissue freezing point (Fletcher et al. 1987; Goddard et al. 1992; Fletcher et al. 1998). These proteins have an affinity for the ice-crystal lattice and are able to bind to embryonic ice crystals and prevent their growth (Davies and Hew 1990). This particular affinity results in the antifreeze proteins depressing the plasma freezing point some 200–300 times more than would be expected on the basis of colligative properties alone, attributes that are of great survival value to fish inhabiting cold ocean environments (DeVries 1983; Davies et al. 1988). A second function, that of protecting cells from depolarization and subsequent damage at low (nonfreezing) temperatures, has also been described for antifreeze proteins (Fletcher et al. 1998).

Received July 20, 2000. Accepted January 12, 2001.

Published on the NRC Research Press Web site on April 2, 2001.

C.F. Purchase,^{1,2} S.V. Goddard, and J.A. Brown. Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF A1C 5S7, Canada.

¹Corresponding author (e-mail: cpurchas@credit.erin.utoronto.ca).

²Present address: Department of Zoology, University of Toronto, 3359 Mississauga Road North, Mississauga, ON L5L 1C6, Canada.

With increasing latitude, winter conditions generally become more severe, therefore in the northern hemisphere one might expect to find that more northerly populations have evolved a higher genetic capacity for antifreeze production (Goddard et al. 1999). Studies have shown that northern populations of certain teleost species do have a greater capacity for producing antifreeze than those living farther south. The winter flounder (*Pleuronectes americanus*) and ocean pout (*Macrozoarces americanus*) are both found in north-west Atlantic waters inhabited by cod. Northern populations of both these species are theoretically genetically capable of producing larger amounts of antifreeze proteins than more southerly populations (Fletcher et al. 1985a, 1985b), since molecular analysis of the antifreeze-gene dosage for both these species indicates amplification of the number of antifreeze-gene copies in northern populations (Davies et al. 1984; Hew et al. 1988).

At the time of writing, analysis of the genetic component of antifreeze production in Atlantic cod cannot be determined, as the required molecular tools have not yet been developed. However, in an experiment where large numbers of juvenile cod from four areas on the coast of Newfoundland were held in a common environment at ambient (subzero) temperatures, Goddard et al. (1999) showed that cod from the most northerly population produced significantly more AFGPs than those from three bays along the northeast coast. This suggests either genetic variability in the capacity for AFGP production or phenotypic variability that is influenced by environmental conditions (temperature) during early development. Levels of antifreeze proteins and AFGPs in different populations of fish reared in a common environment from the egg onwards have not been determined for any species. This would allow the genetic and environmental contributions to antifreeze production to be determined.

Cod aquaculture has received much attention from researchers in recent years. Commercial culture of this species may take two forms. Small cod captured inshore can be kept in sea cages and fed for several months, during which time they increase substantially in body mass and can be sold when the market dictates. Alternatively, juvenile cod can be produced intensively in hatcheries (cultured) from gametes obtained from captive broodstock. Here, larvae and juveniles are reared under elevated temperatures and long photoperiods to allow them to achieve maximum growth rates. Juveniles (>5 g) would then be placed in sea cages to grow to a marketable size.

Winter seawater temperatures in Atlantic Canada regularly fall to near the freezing point of seawater (-1.8°C), and extensive ice cover may also be present (Prinsenberget al. 1997). Without AFGPs, fish tissues generally freeze at -0.5 to 0.8°C if they come into contact with ice (Holmes and Donaldson 1969). It is not known if juvenile cod produced in hatcheries under relatively high water temperatures and long day lengths would be capable of producing AFGPs when placed in sea cages.

Here we performed a common-environment experiment to examine AFGP production in four groups of juvenile Atlantic cod that had reached the laboratory by two different means from four different areas. Two of the populations were reared from eggs stripped and fertilized on a vessel at sea and then brought to the laboratory (cultured cod), while

the other two populations were caught inshore and brought to the laboratory as juveniles (wild cod). These fish had experienced several months' exposure to ambient conditions in coastal Newfoundland waters.

The hypotheses tested were (i) that cultured cod reared under high water temperatures and long day lengths as eggs and larvae are incapable of producing AFGPs as juveniles, and (ii) that under the same environmental conditions, cod from the southern end of their distributional range produce less AFGPs than cod from more northerly areas.

Materials and methods

Collection of fish

Cultured cod

Oocytes and milt were stripped from spawning adults of two geographically distinct groups of cod at sea. Collections were made from the Grand Banks (GB; 46°N , 55°W) and Gulf of Maine (GOM; 42°N , 70°W) in the spring of 1998 (Fig. 1). The GB sample (collected on April 28) consisted of gametes from two females and seven males. Oocytes from the first female were fertilized with milt from four males, and the other three males were used to fertilize oocytes from the second female. Fertilized oocytes were pooled shortly thereafter. The GOM sample (collected on June 28) consisted of gametes from three females and nine males. Gametes from all 12 individuals were mixed together.

Fertilized oocytes from GB were kept on board ship for 1 week in a flow-through incubator at $\sim 8^{\circ}\text{C}$ before arriving at Memorial University of Newfoundland's Ocean Sciences Centre. Fertilized oocytes from GOM were brought to shore on the day of collection and kept in a flow-through incubator at 8°C for 1 day before being flown to the Ocean Sciences Centre. Incubation and larval rearing were conducted in 300-L tanks and followed standard laboratory protocols under continuous lighting (Puvanendran and Brown 1999) at a temperature of $\sim 8^{\circ}\text{C}$. Young juveniles were kept in 3000-L tanks at 8 – 12°C under 18 h of light per day and fed ad libitum until they were transferred to the experimental setup at the beginning of December 1998. The fish were fed a soft pellet consisting of a mixture of ground herring (*Clupea harengus*) and Moore Clarke commercial fish pellets. Further details on the collection and early rearing of the fish are given in Purchase (1999) and Purchase and Brown (2000).

Wild cod

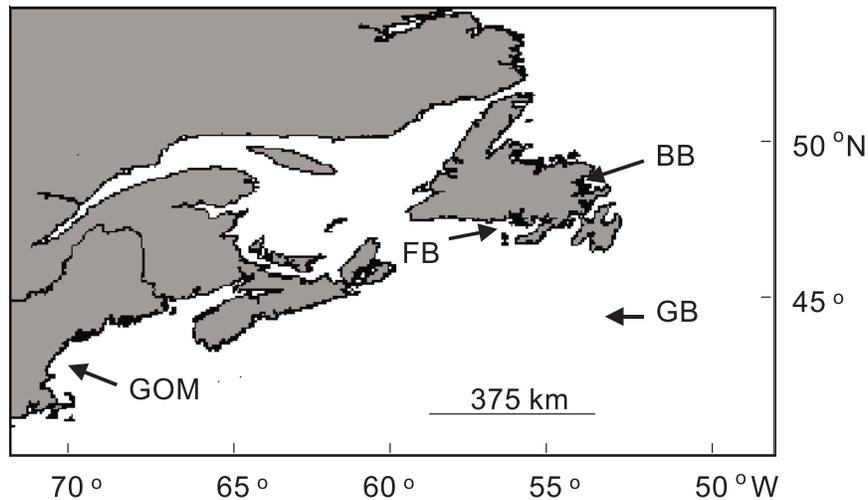
Wild 0+ (young of the year) cod were collected using beach seines from Bonavista Bay (BB; 48°N , 53°W) and Fortune Bay (FB; 47°N , 55°W) on the island of Newfoundland in November 1998 (Fig. 1) and transported to the Ocean Sciences Centre (see Purchase 1999). These fish were weaned onto a pellet food (like the cultured fish) and transferred to the experimental setup at the same time as the cultured juveniles.

Experimental setup

Prior to the start of the experiment (December 9, 1998), all fish were starved for 48 h and measured for total length (cm) and body mass (g). Blood samples were taken from five fish from each population for determining AFGP levels (see below). These fish were not used in the subsequent experiment.

The experimental setup consisted of a 2000-L rectangular tank divided into 10 chambers by mesh screen. These chambers served as experimental "tanks," each of which contained 10 cod from a single population. Of the 10 chambers, 3 contained GB fish, 3 contained GOM fish, 2 contained BB fish, and 2 contained FB fish. The populations were arranged in an alternating manner. To ensure

Fig. 1. The northwest Atlantic Ocean, showing locations of collection sites at the Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB).



that the temperature was the same throughout the tank, ambient seawater entered and exited at two locations and aeration was provided in each chamber.

During the experiment, fish were fed pellet food to satiation daily (the diet described above). A simulated natural photoperiod at 44°N (intermediate for all populations) was provided, which included half-hour dawn and dusk periods. The light intensity was 1500 lx during the day and 2 lx during twilight periods. Water temperature (daily) and dissolved oxygen concentration (weekly) were monitored throughout the experiment and did not vary among the chambers. The water temperature was 6°C on December 9, 1998, and showed a declining trend throughout the experiment. From the beginning of February until the end of the experiment, the temperature did not rise above 3.0°C, and when the experiment ended on March 26, 1999, the temperature was 1.5°C (Fig. 2).

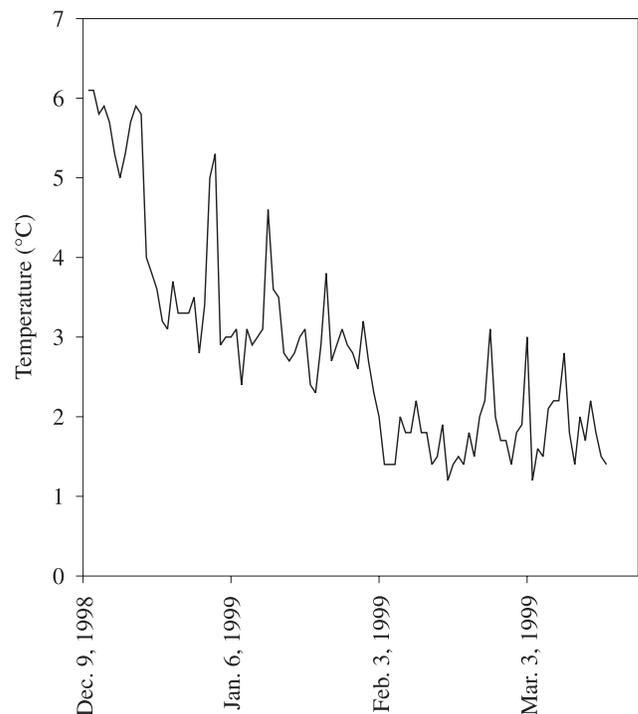
Antifreeze analysis

After 14 weeks under experimental conditions, all fish were starved for 48 h and measured for length and mass. Five cod from each chamber were killed for determination of the hepatosomatic index, percent liver water content, and percent muscle water content as part of another study (Purchase and Brown 2001). Blood samples were taken from 10 cod from each population (chosen randomly among the chambers) for determination of AFGP levels. Samples were taken in the same manner as at the start of the experiment and analyzed in the same way. Blood samples (approximate volume 30–50 µL) were collected from the caudal blood vessel using heparinized microhaematocrit capillary tubes fitted with 26-gauge needles.

Immediately after blood samples were taken, they were spun in a haematocrit centrifuge to separate plasma from cells and refrigerated. Plasma samples were analyzed for antifreeze activity within 12 h of sampling, using a nanolitre osmometer (Clifton Technical Physics, Hartford, N.Y.), by the method described by Kao et al. (1986). This procedure involves microscopic observation of the freezing and melting points of a single ice crystal in the plasma. Antifreeze proteins lower the freezing point of the plasma sample but do not affect the melting point. As a result, there is a difference in the freezing and melting points, termed thermal hysteresis (TH), which is a function of the antifreeze concentration present (Kao et al. 1986; Davies and Hew 1990).

Statistical comparisons of TH levels were made using *t* test. Randomization tests were also performed 10 000 times using bootstrap techniques. Latitudinal comparisons were not made

Fig. 2. Daily water temperatures during the experiment from December 9, 1998, to March 17, 1999.



between wild and cultured populations because of the likelihood of cultured/wild origin being a confounding factor.

Results

Before the start of the experiment the cultured cod had been maintained under a long photoperiod and high temperatures and were not expected to be producing measurable levels of antifreeze at experimental set-up (Fletcher et al. 1987). The presence of antifreeze proteins is indicated by a TH level of $\geq 0.1^\circ\text{C}$ (Goddard et al. 1997). At the start of the experiment, 9 out of 10 cultured fish had a TH level of $\leq 0.1^\circ\text{C}$, while only 1 of these fish had a TH level which indicated that significant levels of AFGPs were being produced.

Table 1. Thermal hysteresis (TH) levels in juvenile cod from the Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) at the start and end of the experiment.

| Population | Latitude of origin (°N) | Source | TH level (°C) | |
|------------|-------------------------|----------|-------------------------|-------------------------|
| | | | At start of experiment | At end of experiment |
| GB | 46 | Cultured | 0.10 ± 0.02 (0.08–0.18) | 0.31 ± 0.03 (0.11–0.43) |
| GOM | 42 | Cultured | 0.08 ± 0.01 (0.06–0.10) | 0.30 ± 0.01 (0.26–0.35) |
| BB | 48 | Wild | 0.20 ± 0.05 (0.09–0.37) | 0.23 ± 0.02 (0.11–0.32) |
| FB | 47 | Wild | 0.19 ± 0.06 (0.09–0.43) | 0.19 ± 0.03 (0.09–0.39) |

Note: Values are shown as the mean ± standard error, with the range in parentheses; $N = 5$ per population at the start of the experiment and $N = 10$ per population at the end.

However, the majority of the wild-caught fish (which had been kept in the laboratory for several weeks at the same temperature as the cultured cod) had TH levels $>0.1^{\circ}\text{C}$. Total length of GB (13.8 ± 0.38 (mean ± SE)), GOM (8.8 ± 0.04), FB (9.3 ± 0.85), and BB (9.8 ± 0.30) cod was not associated with TH levels within each group. There was no significant difference in TH levels between GB and GOM cod ($t_5 = 1.27$, $p = 0.26$, randomized $p = 0.3341$) or between FB and BB cod ($t_7 = -0.12$, $p = 0.91$, randomized $p = 0.9006$; Table 1).

By the end of the experiment, almost all fish were producing antifreeze proteins and had TH levels of $>0.1^{\circ}\text{C}$. Again, total length of GB (13.6 ± 0.50), GOM (10.5 ± 0.38), FB (9.9 ± 0.19), and BB (11.0 ± 0.46) cod was not associated with TH within each group. Initial and final mean TH levels for FB cod were not significantly different ($p > 0.05$), as was the case for those for BB cod. However, in both groups originating from the wild populations, the standard error among the fish was less at the end of the experiment.

After 14 weeks of common environmental conditions, TH levels did not differ significantly between GB and GOM cod ($t_{11} = 0.24$, $p = 0.81$, randomized $p = 0.8176$) or between FB and BB cod ($t_{15} = -1.16$, $p = 0.26$, randomized $p = 0.2406$; Table 1).

Discussion

The results of this study demonstrate that Atlantic cod from as far south as the Gulf of Maine are able to produce AFGPs when exposed to declining winter water temperatures. We have also shown that cod cultured from the egg in warm conditions are capable of producing antifreeze when exposed to winter temperatures.

AFGP production in cod is related to environmental temperature. Cod do not begin to produce these proteins until the onset of winter (Fletcher et al. 1987). The threshold temperature for AFGP production is higher in juveniles ($2\text{--}3^{\circ}\text{C}$) than in adults ($0\text{--}1^{\circ}\text{C}$) (Goddard et al. 1992), and this is believed to be related to distribution. While adults may overwinter in deeper, warmer water, juveniles generally overwinter in shallower, colder inshore environments. Juvenile are therefore obliged to get ready for winter extremes well in advance of the arrival of such conditions in order to ensure survival (Goddard and Fletcher 1994). Before the start of this experiment, the two populations of cultured cod had been maintained at water temperatures of $\sim 8^{\circ}\text{C}$. They were therefore not expected to show TH levels indicative of AFGP

production. The results support this prediction, as only one cultured cod had a TH level high enough to suggest the presence of AFGPs. In contrast, at the start of the experiment, the majority of the wild fish had plasma TH levels that suggested the presence of low to moderate levels of AFGPs. As these fish had been collected from inshore environments in November, they would likely already have experienced temperatures that had fallen low enough to initiate the production of AFGPs.

By the end of the experiment, almost all fish had TH levels that indicated the presence of AFGPs. Juvenile cod from FB had a mean plasma antifreeze level similar to that seen in the fish from BB. Plasma antifreeze levels in the juvenile cod from GOM were not significantly different from those in the fish from GB. Mean TH levels produced by fish in the four study groups were all relatively low (between 0.19 and 0.31°C) compared with the antifreeze-production capacity of juvenile cod in previous studies. Mean TH levels between 0.6 and 0.7°C are commonly observed in juvenile cod collected from Newfoundland coastal waters and exposed to temperatures below -1.0°C over winter (Goddard et al. 1999). We were not surprised to see that the mean TH levels measured in March were low in the four study groups, since the water temperatures experienced by the fish throughout the experiment were comparatively high (by Newfoundland standards). In addition, in the month between capture and experimental set-up, the wild cod were exposed to temperatures higher than they would face in the wild. No studies have been carried out to determine the potential effect of this unexpected elevation of temperature, at a time when a steady decline would normally be experienced, on the pattern of AFGP production in these fish.

In a previous study of antifreeze-production capacity in juvenile cod from Newfoundland waters, Goddard et al. (1999) found that those from the most northerly location were able to produce significantly more AFGPs than the other groups investigated. However, three groups of cod collected from bays along the northeast coast of Newfoundland were not significantly different from each other, and produced similar amounts of AFGPs over the winter cycle. Goddard et al. (1999) concluded that the population living under the most extreme environmental conditions of low temperature and ice cover had experienced significant selection pressure, resulting in an elevation in the antifreeze-production system. The results of our study support the conclusion that this is a genetic difference (as opposed to differences in TH level that might have arisen because of differences in developmental

temperature), as high temperatures during development did not prevent the production of antifreeze proteins.

In the populations from the northeast coast of Newfoundland studied by Goddard et al. (1999), there had been no apparent increase in antifreeze-production capacity, and the populations of cod were very similar in their capacity to produce antifreeze when exposed to very low temperatures over winter. The BB population used in our study is also from the northeast coast of Newfoundland, from a location near one of the populations examined by Goddard et al. (1999), while FB is on the south coast. Growth rates, food-conversion efficiencies, and energy allocation of the BB and FB cod in a common environment are similar (Purchase 1999; Purchase and Brown 2001). In this study, at relatively high temperatures no significant difference in AFGP production could be determined between the BB and FB cod.

The GOM is near the southern end of the distributional range of cod in the northwest Atlantic. Previous studies have shown that cod from the GB and GOM are genetically distinguishable (Taggart et al. 1998) and differ under common environments in larval growth rates, juvenile food-conversion efficiencies, and hepatosomatic indices (Purchase 1999; Purchase and Brown 2000; Purchase and Brown 2001). Winter conditions are much less severe in the GOM than farther north, and these fish may never require AFGPs to protect them from freezing. AFGPs may be energetically costly to produce, and it might be expected that cod from the more southerly, warmer waters of GOM would either be unable to produce AFGPs or would produce them in smaller amounts than their more northerly relatives. However, this study has demonstrated that cod from GOM are able to produce significant quantities of AFGPs at temperatures between 3 and 1°C.

The observation that the cultured cod from the two more southerly groups had developed somewhat higher AFGP levels than the wild groups by the end of the experiment should not be interpreted as any real indicator of AFGP-production capacity. It is possible that cod from the south were experiencing temperatures that would be considered low in their natural environment, while for the Newfoundland fish, the same temperatures would be indicative of a mild winter. In addition, initiation of AFGP production in wild cod, followed by a reversal in temperature cues between November and December, after capture and prior to experimental setup, and then exposure to a gently declining temperature regime would likely have caused perturbations in the AFGP-production system of the wild cod (their initial and final TH levels were very similar).

Although none of the fish in this experiment were subjected to subzero environmental temperatures, which would have fully tested their AFGP-production capacity, they were all held in a common environment. Under these conditions we could see no evidence that would lead us to conclude that cod from the southern end of the distributional range were less able to produce AFGPs than their more northerly conspecifics.

Research in cod aquaculture has increased in recent years. Experimental hatcheries produce eggs, larvae, and young juveniles at elevated temperatures and long day lengths in order to achieve maximal growth rates. These juveniles are then placed in sea cages for on-growing. Depending on the

farm location, once in the ocean environment, these cultured cod may experience water temperatures that are lethal unless they are able to produce AFGPs to protect their cells and tissues from cold and freezing damage. It is not known if ocean conditioning is required during early development to activate the AFGP genes.

Our results suggest that low temperatures during early development are not required for AFGP production later in life, as the cultured fish (two populations) were kept under long day lengths and at temperatures $\geq 8^\circ\text{C}$ from the early egg stage. Thus, AFGP production in juvenile cod is not negatively affected by the elevated temperatures and long photoperiods used in hatcheries. It would be beneficial to examine if cultured cod kept in hatcheries under high temperatures through their first winter are capable of producing AFGPs the following winter at sea. However, our results suggest that conditioning at sea is not required for the production of AFGPs in cod, therefore we might expect that they would be able to produce these proteins regardless of the age at which they were put in sea-cages.

In conclusion, this study has demonstrated that cod populations of southerly origin have the ability to produce AFGPs under appropriate conditions, and that early exposure to low environmental temperatures is not necessary to trigger the ability to produce AFGPs at low temperatures. This result is of considerable significance to aquaculture, since it indicates that juvenile cod reared under intensive hatchery conditions are capable of producing AFGPs when placed in sea cages for on-growing.

Acknowledgments

We thank those who helped in the collection of the fish, the staff of the Ocean Sciences Centre for their help in conducting this experiment, and C. March and Dr. M. Kao for taking the blood samples and determining TH levels. Funding for this study was provided by a Natural Resource Development Scholarship from the Government of Newfoundland and Labrador and a Graduate Fellowship from Memorial University of Newfoundland to C.F.P. and a grant from the Natural Sciences and Engineering Research Council of Canada to J.A.B.

References

- Bentzen, P., Taggart, C.T., Ruzzante, D.E., and Cook, D. 1996. Microsatellite polymorphism and the population structure of cod (*Gadus morhua*) in the north west Atlantic. *Can. J. Fish. Aquat. Sci.* **53**: 2706–2721.
- Davies, P.L., and Hew, C.L. 1990. Biochemistry of fish antifreeze proteins. *FASEB J.* **4**: 2460–2468.
- Davies, P.L., Hough, C., Scott, G.K., Ng, N., White, B.N., and Hew, C.L. 1984. Antifreeze protein genes of the winter flounder. *J. Biol. Chem.* **259**: 9241–9247.
- Davies, P.L., Hew, C.L., and Fletcher, G.L. 1988. Fish antifreeze proteins: physiology and evolutionary biology. *Can. J. Zool.* **66**: 2611–2617.
- DeVries, A.L. 1983. Antifreeze peptides and glycopeptides in cold-water fishes. *Annu. Rev. Physiol.* **45**: 245–260.
- Fletcher, G.L., Haya, K., King, M.J., and Reisman, H.M. 1985a. Annual antifreeze cycles in Newfoundland, New Brunswick, and

- Long Island winter flounder *Pseudopleuronectes americanus*. Mar. Ecol. Prog. Ser. **21**: 205–212.
- Fletcher, G.L., Hew, C.L., Li, X., Haya, K., and Kao, M.H. 1985b. Year-round presence of high levels of plasma antifreeze peptides in a temperate fish, ocean pout (*Macrozoarces americanus*). Can. J. Zool. **63**: 488–493.
- Fletcher, G.L., King, M.J., and Kao, M.H. 1987. Low temperature regulation of antifreeze glycoprotein levels in Atlantic cod (*Gadus morhua*). Can. J. Zool. **65**: 227–233.
- Fletcher, G.L., Goddard, S.V., Davies, P.L., Gong, Z., Ewart, K.V., and Hew, C.L. 1998. New insights into fish antifreeze proteins: physiological significance and molecular regulation. Soc. Exp. Biol. Semin. Ser. No. 65. pp. 239–265.
- Goddard, S.V., and Fletcher, G.L. 1994. Antifreeze proteins: their role in cod survival and distribution from egg to adult. Int. Counc. Explor. Sea Mar. Sci. Symp. No. 198. pp. 676–683.
- Goddard, S.V., Kao, M.H., and Fletcher, G.L. 1992. Antifreeze production, freeze resistance, and overwintering of juvenile Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **49**: 516–522.
- Goddard, S.V., Morgan, M.J., and Fletcher, G.L. 1997. Influence of plasma antifreeze glycoproteins on temperature selection by Atlantic cod (*Gadus morhua*) in a thermal gradient. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 88–93.
- Goddard, S.V., Kao, M.H., and Fletcher, G.L. 1999. Population differences in antifreeze production cycles of juvenile Atlantic cod (*Gadus morhua*) reflect adaptations to overwintering environment. Can. J. Fish. Aquat. Sci. **56**: 1991–1999.
- Hew, C.L., Wang, N.-C., Joshi, S., Fletcher, G.L., Scott, G.K., Hayes, P.H., Buettner, B., and Davies, P.L. 1988. Multiple genes provide the basis for antifreeze protein diversity and dosage in the ocean pout, *Macrozoarces americanus*. J. Biol. Chem. **263**: 12049–12055.
- Holmes, W.N., and Donaldson, E.M. 1969. The body compartments and distribution of electrolytes. In Fish physiology. Vol. 1. Edited by W.S. Hoar and D.J. Randall. Academic Press, New York. pp. 1–98.
- Kao, M.H., Fletcher, G.L., Wang, N.C., and Hew, C.L. 1986. The relationship between molecular weight and antifreeze polypeptide activity in marine fish. Can. J. Zool. **64**: 578–582.
- Myers, R.A., Barrowman, N.J., and Hutchings, J.A. 1997. Inshore exploitation of Newfoundland Atlantic cod (*Gadus morhua*) since 1948 as estimated from mark-recapture data. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 224–235.
- Prinsenbergh, S.J., Peterson, I.K., Narayanan, S., and Umoh, J.U. 1997. Interaction between atmosphere, ice cover, and ocean off Labrador and Newfoundland from 1962 to 1992. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 30–39.
- Purchase, C.F. 1999. Inter-population differences in growth and energy allocation of northwest Atlantic cod (*Gadus morhua* L.) revealed by common environment experiments. M.Sc. thesis, Department of Biology, Memorial University of Newfoundland, St. John's, Nfld.
- Purchase, C.F., and Brown, J.A. 2000. Inter-population differences in growth rates and food conversion efficiencies of young Grand Banks and Gulf of Maine Atlantic cod (*Gadus morhua* L.). Can. J. Fish. Aquat. Sci. **57**: 2223–2229.
- Purchase, C.F., and Brown, J.A. 2001. Stock-specific changes in growth rates, food conversion efficiencies, and energy allocation in response to temperature change in juvenile Atlantic cod. J. Fish Biol. **58**: 36–52.
- Puvanendran, V., and Brown, J.A. 1999. Foraging, growth and survival of Atlantic cod larvae reared in different prey concentrations. Aquaculture, **175**: 77–92.
- Ruzzante, D.E., Taggart, C.T., Cook, D., and Goddard, S. 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. Can. J. Fish. Aquat. Sci. **53**: 634–645.
- Ruzzante, D.E., Taggart, C.T., and Cook, D. 1999. A review of the evidence for genetic structure of cod (*Gadus morhua*) populations in the NW Atlantic and population affinities of larval cod off Newfoundland and the Gulf of St. Lawrence. Fish. Res. **43**: 79–97.
- Swain, D.P., and Foote, C.J. 1999. Stocks and chameleons: the use of phenotypic variation in stock identification. Fish. Res. (Amst.), **43**: 113–128.
- Taggart, C.T., Ruzzante, D.E., and Cook, D. 1998. Localized stocks of cod (*Gadus morhua* L.) in the northwest Atlantic: the genetic evidence and otherwise. In The implications of localized fishery stocks. Edited by I. Hunt von Herbing, I. Kornfield, M. Tupper, and J. Wilson. Northeast Regional Agricultural Engineering Service, New York. pp. 65–90.
- Templeman, W. 1981. Vertebral numbers in Atlantic cod, *Gadus morhua*, off the Newfoundland shelf and adjacent areas, 1947–71, and their use in delineating cod stocks. J. Northwest Atl. Fish. Sci. **2**: 21–45.