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Antarctic Notothenioid Fishes Do Not Display Metabolic Cold Adaptation in Hepatic Gluconeogenesis

Leonardo J. Magnoni

ANTARCTIC NOTOTHENIOID FISHES DO NOT DISPLAY METABOLIC COLD ADAPTATION IN HEPATIC GLUCONEOGENESIS

By

Leonardo J. Magnoni

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Advisory Committee:

Bruce D. Sidell, Professor of Marine Sciences, Advisor

Robert Cashon, Assistant Professor of Biochemistry

Ione Hunt von Herbing, Assistant Professor of Marine Sciences

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An Abstract of the Thesis Presented
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Antarctic notothenioid fishes present specializations related to their chronically cold environment, such as high lipid content in tissues (predominantly triacylglycerols, TAG). When TAGs are mobilized, they yield fatty acids and glycerol. Fatty acids are the primary fuel of oxidative muscle tissues. Gluconeogenesis from glycerol has not been studied in Antarctic fishes despite the importance of glycerol as a breakdown product of TAGs.

To assess the possible importance of glycerol as a substrate for gluconeogenesis and to determine whether this pathway and Krebs cycle are metabolically cold adapted, key hepatic enzyme activities were measured in Antarctic notothenioid fishes (*Notothenia coriiceps*, *Gobionotothen gibberifrons* and *Chionodraco rastrospinosus*) and Subantarctic notothenioid fishes (*Dissostichus eleginoides*,

Patagonotothen ramsayi and *Eleginops maclovinus*). Citrate synthase, fructose 1,6-biphosphatase, glycerol kinase, and phosphoenolpyruvate carboxykinase enzyme activities were measured at 1°, 6°, 11°, and 21° C. Levels of specific metabolites in liver (glycerol, glucose and glycogen) and in serum (glycerol and glucose) were measured.

My results indicate that gluconeogenesis and aerobic metabolism are not metabolically cold adapted in livers of Antarctic fishes. Levels of glycerol in plasma and liver were generally similar for all fishes studied, but surprisingly lower than the values reported for other teleost. Maximal activities for all enzymes assayed in livers of notothenioids fishes with Antarctic and Subantarctic distribution were similar when measured at the same temperature (1°C). In addition, energies of activation for all the enzymes, calculated from the slope of Arrhenius plot, were similar between both groups of fishes.

Lack of metabolic cold adaptation in hepatic gluconeogenesis may indicate that this pathway is of low physiological importance in both Antarctic and Subantarctic notothenioids or, more likely, that these two groups are so closely related that insufficient time has elapsed for evolutionary divergence in this trait.

DEDICATION

To my parents, Martha and Néstor.

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INTRODUCTION

Antarctic Isolation and the Origin of the Southern Ocean

The temperature of the Southern Ocean has been declining for the last 55 million years (Clarke and Crame, 1989). However, it was not until the formation of the Drake Passage between the Antarctic Peninsula and South America (about 23 million years ago), that the final barrier preventing the Antarctic Circumpolar Current water from completing its path around Antarctica was removed (Barker and Burrell, 1977). The establishment of this current system permitted the formation of the Antarctic Convergence, a front generated when cold Antarctic surface water flowing northward encounters warmer Subantarctic water flowing southward from the Atlantic, Pacific, and Indian oceans (Laws, 1985). The Antarctic Convergence acts as a barrier to heat flow, thermally isolating the Southern Ocean from other water masses, and restricting migration of ectothermic fauna. The stable cold waters around Antarctica, range from the freezing point of seawater (-1.9°C) to +2°C throughout the year, and provide an excellent setting to study the physiological processes that allow normal biological function at very cold body temperature.

Characteristics of Antarctic Notothenioid Fish

The perciform suborder Notothenioidei dominates the Southern Ocean fish fauna in both numbers of species and biomass. Notothenioids are remarkably diverse in size, body form, habitat, and distribution. Some

notothenioids are found only north of the Antarctic Convergence, e.g. waters surrounding South America, where ocean temperature can be 5-10°C warmer than in the Southern Ocean (Johnston *et al.*, 1998). Eastman (1993) suggests that ancestral notothenioids may have been subjected to vicariant events during the isolation of Antarctica, which resulted in passive allopatric speciation of Antarctic and Non- Antarctic notothenioid species.

Antarctic notothenioids are among the most stenothermal fishes in the world and have a number of biochemical and physiological specializations that are considered to be cold adaptations. These specializations include an increased lipid content deposited in nearly all tissues (Lund and Sidell, 1992), an increased importance of lipids as metabolic fuels (Sidell, 1991; Sidell *et al.*, 1995), and the most well known specialization, the production of antifreeze glycopeptides (AFGPs) compounds (De Vries, 1982).

Lipids are deposited mainly in the form of triacylglycerols (TAGs) (Lund and Sidell, 1992). Fatty acids, one of the products of TAG mobilization, are the preferred fuel for the aerobic metabolism in muscles of Antarctic fishes as is indicated by high activities of enzymes from the β-oxidation pathway (Crockett and Sidell, 1990).

AFGPs are present in all the Antarctic notothenioid fishes that have been studied to date and are composed of amino acids and sugars (De Vries, 1982). They are synthesized in the liver and released to blood and

extracellular fluids (O'Grady *et al.*, 1982). Although AFGPs are entirely excluded from loss in the urine, their conservation is not perfect. Low molecular AFGPs are secreted to the intestinal lumen and lost in part through excretion. Resynthesis of unrecoverable AFGPs may represent a considerable energetic cost (O'Grady *et al.*, 1983).

Protein synthetic rates in liver and white muscle are higher in Antarctic notothenioid fishes than in tropical and temperate zone fishes when compared at the same temperature (Smith and Haschemeyer, 1980). Furthermore, the proportion of the protein synthetic effort devoted to proteins destined to export is greater in Antarctic notothenioid species than that found in temperate fish, and some of these export products could be related to survival under subzero conditions. For instance, AFGP synthesis represents 4% of total hepatic polypeptide synthesis and their half-life is 4 weeks (Haschemeyer and Mathews, 1980). In contrast to protein synthesis, the synthetic pathway for sugar (gluconeogenesis) has not been investigated in Antarctic fishes, even though sugars are involved in AFGPs synthesis.

Gluconeogenesis from Glycerol in Antarctic Fishes: An Underlying Hypothesis

Because liver has a central role in fish intermediary metabolism (Walton and Cowey, 1982), the specializations described earlier presumably may be reflected in the hepatic physiology of Antarctic notothenioid fishes. TAG mobilization produces fatty acids and glycerol.

As mentioned before, fatty acids are the preferred fuel for aerobic metabolism in muscles. However, the physiological fate of glycerol is unknown. I hypothesized that glycerol could be an important precursor for the synthesis of glucose through the gluconeogenic pathway. Gluconeogenesis is a primarily hepatic biosynthetic pathway responsible for the *de novo* synthesis of glucose from precursors such as glycerol, amino acids, lactate and fructose (Moon, 1988). Gluconeogenesis is crucial to glucose homeostasis. Fishes require glucose for the metabolism of certain critical tissues (e.g., nervous system, gills, red blood cells, testes, and renal medulla) and to synthesize certain biological molecules (e.g., mucopolisacharydes, AFGPs), but in general have limited dietary access to carbohydrates (Suarez and Mommsen, 1987). Because synthesis of AFGPs requires amino acids in addition to sugars, it is possible that amino acids will be spared for AFGP synthesis and will be less important than glycerol as substrates for gluconeogenesis.

Metabolic Cold Adaptation: Thermal Effects on Enzymatic Activities

Temperature affects physiological processes by a direct effect on the rates of chemical reactions. Commonly, such effects of temperature on rates of biological activity are quantified by calculating the temperature coefficient or Q_{10} of the process. A decrease in temperature will diminish the catalytic rate of enzymes and may induce biochemical adjustments to compensate for at least part of this reduction in catalytic rate (Dunn, 1987). Therefore, an increase in the catalytic rate of enzymes in limiting

steps of relevant pathways (adaptation) is expected to occur in ectothermic organisms as a compensatory mechanism to overcome the Q_{10} effects of decreasing temperatures. This idea is commonly implied in the concept of metabolic cold adaptation (MCA). However, the concept of MCA as a physiological integrative process is still being tested (Scholander *et al.*, 1953; Wohlschlag, 1960; Holeton, 1974; Clarke, 1991).

Crockett and Sidell (1990) refined the definition of MCA to describe processes that enable an organism living at cold temperatures to have greater active metabolic rate than an organism of similar ecotype from a warmer environment acutely exposed or acclimated to the same low temperature. Data obtained from cardiac, white, and pectoral muscles from Antarctic notothenioid fishes by these authors suggest significant MCA of aerobic energy metabolism that is reliant upon lipids fuels, but lack of such adaptation in pathways of carbohydrate aerobic metabolism. Therefore, it will be valuable to know if other metabolic pathways, such as gluconeogenesis, have accumulated adaptive changes during the evolution of Antarctic fishes.

I hypothesized that the gluconeogenic pathway in Antarctic notothenioid fishes will display MCA, mainly in enzymes involved in glycerol utilization, when compared to Subantarctic notothenioid fishes at the same temperature. To test this hypothesis, I measured maximal enzymatic activities of putatively rate-limiting steps of gluconeogenesis in

livers of each species on the entire range of environmental temperatures for all the species selected.

When evaluating MCA, it is important that comparisons between organisms include not only phylogenically related species, but also ecotypically similar organisms (De Vries and Eastman, 1981). The fishes selected for this study were all sluggish benthic species, with the exception of the Subantarctic *Dissostichus eleginoides*, a mesopelagic species. In spite of this, I decided to include *D. eleginoides* in this comparative study due to inability to sample other Subantarctic notothenioid benthic species, and because some *D. eleginoides* were also capture during bottom trawling.

Objectives of This Study

The aim of this study is to determine whether differences in the gluconeogenic pathway exist between Antarctic notothenioid fishes and notothenioids with Subantarctic distributions. Gluconeogenesis may be a metabolic pathway displaying MCA in Antarctic notothenioid fishes, possibly related to the synthesis of AFGPs. I evaluate whether enzymatic indices of gluconeogenesis from glycerol are higher in livers of Antarctic notothenioid fishes (*G. gibberifrons*, *C. rastrospinosus*, and *N. coriiceps*) than in tissue of Non-Antarctic notothenioid fishes (*D. eleginoides*, *P. ramsayi*, and *E. maclovinus*).

I measured and compared maximal activities of key enzymes in the gluconeogenic pathway (fructose 1,6-biphosphatase, glycerol kinase and

phosphoenolpyruvate carboxykinase) and aerobic metabolism (citrate synthase) at four different temperatures (1°, 6°, 11°, and 21°C). In addition, I determined levels of important metabolites implicated in the gluconeogenic pathway in liver (glucose, glycerol, and glycogen) and plasma (glucose and glycerol).

MATERIALS AND METHODS

Chemicals

Substrates, cofactors and enzymes were purchased from Sigma Chemical Co. (St. Louis, Missouri), ICN Pharmaceuticals Inc. (Costa Mesa, California), and Boehringer- Mannheim (Darmstadt, Germany). All other chemicals were from various commercial sources and were reagent grade.

Experimental Animals

Three Antarctic species and three Subantarctic species were studied; all fish are perciform belonging to the suborder Notothenoidei. The Antarctic species examined were *Gobionotothen gibberifrons*, *Notothenia coriiceps* and *Chionodraco rastrospinosus*. *Gobionotothen gibberifrons*, *N. coriiceps* and *C. rastrospinosus* were captured from depths of 98-215 meters from A*RSV Laurence M. Gould* at sites near Low Island (63° 25' S, 62° 10' W) and Dallman Bay in the vicinity of Astrolabe Needle (64° 10' S, 62° 35' W) off the Antarctic Peninsula during June-July 2001. In addition, some individuals of *N. coriiceps* were captured off the pier at Palmer Station, Antarctica (64° 46' S, 64° 03' W).

The Subantarctic species examined were *Patagonotothen ramsayi*, *Eleginops maclovinus* and *Dissostichus eleginoides*. *Patagonotothen ramsayi* and *D. eleginoides* were caught from depths between 140 and 176 meters from *R/V Oca Balda* (INIDEP, Argentina) at sites of Atlantic Ocean waters (47° 47' S, 61° 27' W and 48° 48' S, 62° 21' W, respectively)

during February 2001. *Eleginops maclovinus* was caught from Chilean coastal waters (Pacific Ocean) near the localities of La Boca and La Matanza (33° 58' S, 71° 56' W and 34° 20' S, 72° 06' W, respectively) during July 2001.

Except for *E. maclovinus* where gill nets or hook and line were employed and some individuals of *N. coriiceps* where hook and line were employed, all the fish were caught with Otter trawls. Animals were maintained at ambient sea temperatures (1°C for *G. gibberifrons*, *C. rastrospinosus*, and *N. coriiceps*; 6°C for *D. eleginoides* and *P. ramsayi*; and 11°C for *E. maclovinus*) in running seawater tanks on the ships and/or laboratory (Palmer Station, United States, and Las Cruces experimental station, Universidad Católica Pontifica de Chile) for at least 4 hours before sampling. During this period the animals were not fed.

Sample Collection

The fish were removed quickly from the holding tanks with a dip net and stunned by a sharp blow to the head. Prior killing the animal, blood was obtained in ammonium-heparinized syringes from the caudal peduncle or ventricle. Plasma samples were prepared by centrifugation of blood at 10,000 x *g* for 5 min at 4°C. The supernatant was placed in cryogenic tubes and frozen at once in liquid nitrogen.

After sampling the blood, fish were killed by cutting the spinal cord posterior to the cranium. The liver was immediately dissected from the animal and placed in liquid nitrogen. With the exception of some

specimens of *C. rastrospinosus* and *N. coriiceps* (in which livers had different degrees of parasitic infection by nematodes) the total time elapsed from killing the fish to freezing the liver samples was in the range of 1 to 2 minutes for all individuals. In those cases where parasitic infection was found, worms were removed from the liver held on an ice-cold dissection stage prior to freezing. This procedure did not take longer than 5 minutes.

The frozen samples were transported on dry ice to our laboratory at University of Maine and stored at -80°C for subsequent analyses. Numerous papers (Dunn and Johnston, 1986; Moon and Foster, 1989; Foster and Moon, 1991; Ferguson and Storey, 1992; Shikata *et al.*, 1995; Driedzic *et al.*, 1998, Magnoni *et al.*, 2001) report stability of the enzymes under study at ultracold temperatures.

Homogenate Preparation

Frozen liver samples were homogenized at a ratio 9:1 (10% weight/volume) in ice-cold homogenization buffer containing 40 m*M* Hepes (pH= 7.26 at 25°C), 2 m*M* dithiothreitol (omitted for citrate synthase determination), and 1 tablet of protease inhibitor cocktail (Boehringer Mannheim complete™ mini) per 7 ml buffer, using a ground glass homogenizer held on ice. Citrate synthase and phosphoenolpyruvate carboxykinase enzyme activity determinations were performed on crude homogenates after sonication at 35% maximal power in two 15 second burst, with a 15 second cooling interval between them

(Artek-Sonic 300 Dismembrator). For the other enzyme activities and metabolite determinations, the homogenate was centrifuged at 12,400 x *g* for 10 min at 4°C (IEC Micromax), and the supernatants were used. The aqueous phase was drawn from beneath lipid layers when they were present on top of the centrifuged samples. All the metabolite and enzyme activity determinations were performed at least in duplicate.

Glycerol, Glucose, and Glycogen Determinations

Homogenate and plasma supernatants were deproteinized by addition of ice-cooled 6% PCA (perchloric acid) in a proportion 1:3 (supernatant: PCA), kept on ice and mixed repeatedly over a 10 minute period, followed by centrifugation at 10,000 x *g* for 15 min at 4°C. The supernatant was neutralized with ice-cold 5 m*M* K_2CO_3 and centrifuged at 10,000 x *g* for 5 min at 4°C. This remaining supernatant was used for subsequent metabolite determination.

Glycerol Determination. Glycerol concentration was estimated in samples utilizing a kit (UV-method 148270, Boehringer Mannheim). The assay conditions were as follows: 0.32 mg • ml^{-1} NADH, 1 mg • ml^{-1} ATP, 0.5 mg • ml^{-1} phosphoenolpyruvate- CHA, SO_4Mg, 3 U • ml^{-1} pyruvate kinase, 2,75 U • ml^{-1} lactate dehydrogenase and 0.42 U • ml^{-1} glycerol kinase. In this method, the amount of NADH oxidized by a series of coupled reactions is stoichiometric to the amount of glycerol in the sample. NAD^+ formation is determined by the extent of decrease in light absorption at 340 nm.

Glucose Determination. Glucose concentration was determined in samples utilizing a glucose assay kit (GAHK-20, Sigma Chemical Co.) with the following assay conditions: 1.5 m*M* NAD, 1.0 m*M* ATP, 1 U • ml^{-1} hexokinase and 1 U • ml^{-1} glucose 6-phosphate dehydrogenase. The amount of NAD^+ reduced is proportional to the concentration of glucose in the sample. NADH formation is determined by measuring the increase in absorbance at 340 nm (Kunsst *et al.*, 1984).

Glycogen Determination. Liver glycogen levels were assessed using the method of Keppler and Decker (1984), in which the glucose liberated after glycogen breakdown catalyzed by amyloglucosidase was quantified (after subtracting free levels of glucose in liver). The conditions were as follows: 200 m*M* buffer acetate (pH= 4.8 at 20° C) and 9.23 U • ml^{-1} amyloglucosidase (omitted from control) was incubated with 100 μl of supernatant for 120 min at 40° C (total volume 1.1 ml). The reaction was stopped with the addition of 1 ml 3.64% PCA and centrifuged for 5 minutes at 10,000 x *g*. An aliquot of the supernatant was used for glucose quantification as described earlier.

Enzyme Activity Assays

All enzymes were assayed in freshly prepared samples. Activities were measured using a Perkin-Elmer Lambda 40 UV-VIS spectrophotometer. Assay temperature was maintained at 1°, 6°, 11° or 21° C (± 0.1° C) with a Neslab RTE-111 temperature regulated water bath circulating a mixture of ethanol and distilled water (1:1). Reaction rates of enzymes

were determined by increase or decrease in absorbance at 340 nm (412 nm for citrate synthase activity). Cuvettes were preincubated at each specific temperature. A low stream of nitrogen gas in the spectrophotometer chamber prevented water condensation on the cuvettes. Between 5 and 20 μl of supernatants were added to the cuvettes with a pre-established volume (final volume 1 ml) to give a linear rate of change in absorbance over the duration of the assay. Substrate was omitted in controls and background activity was subtracted from that measured in the presence of substrate. Enzymatic analyses were all carried out with substrate and cofactor concentrations yielding maximum reaction velocities, with the reaction mixtures established in preliminary tests to render the highest activity possible. The reactions were started by addition of substrate. Enzymatic activities were measured utilizing imidiazole buffers adjusted to a baseline pH 7.37 at 25°C, and were allowed to follow their intrinsic pH/temperature relationship ($\Delta pK_a/°C$= -0.017), which parallels that of physiological fluids (Somero 1981). The only exception was citrate synthase activity where Tris- HCl buffer was utilized ($\Delta pK_a/°C$= -0.031). All activities are expressed in units, (μmol substrate converted to product • min^{-1}), per gram- wet weight of tissue. The specific conditions for enzyme assays, expressed as final concentrations, were as follows.

Citrate Synthase (CS; EC 4.1.3.7). This assay is adapted from Srere *et al.* (1963) and modified by Hansen and Sidell (1983). The reduction of

DTNB (5,5'-ditnitrobis, 2-nitrobenzoic acid) was followed at 412 nm. The assay medium included 75 m*M* Tris-HCl (pH= 8.1 at 25°C), 0.5 m*M* oxaloacetate, 0.25 m*M* DTNB, and 0.4 m*M* acetyl-CoA. Reactions were allowed to proceed at least for 15 minutes.

Fructose 1,6- Biphosphatase (FBP; EC 3.1.3.11). FBP was assayed in liver according to Mommsen *et al.* (1980), using 50 m*M* imidiazole, 6 m*M* $MgCl_2$, 0.4 m*M* NADP, 2 U • ml^{-1} phosphoglucose isomerase, 2 U • ml^{-1} glucose 6-phosphate dehydrogenase, 0.1 m*M* fructose 1,6-biphosphate, and an AMP trapping system consistent of 0.5 m*M* phosphoenolpyruvate, 0.05 m*M* ATP, 5 U • ml^{-1} myokinase, and 10 U • ml^{-1} pyruvate kinase. The reduction of $NADP^+$ was followed for 40 minutes.

Glycerol Kinase (GK; EC 2.7.1.30). The assay for this enzyme was from Bublitz and Wieland (1962). The reaction mixture included 50 m*M* imidiazole, 1.8 m*M* $MgCl_2$, 4.1 m*M* ATP, 0.49 m*M* NAD, 17 U • ml^{-1} glycerol 3-phosphate dehydrogenase and 6 m*M* glycerol. The reduction of NAD^+ by G 3-PDH was monitored for 30 minutes.

Phosphoenolpyruvate Carboxykinase (PEPCK; EC 3.1.3.11). The assay used for PEPCK is described by Opie and Newsholme (1967) and Petrescu *et al.* (1979). Assay medium was 50 m*M* imidiazole, 1 m*M* $MnCl_2$, 0.15 m*M* NADH, 1.6 m*M* deoxy-guanosine diphosphate, 8 U • ml^{-1} malate dehydrogenase, 5 m*M* phosphoenolpyruvate and 160 m*M* $NaHCO_3$ (saturated with CO_2 and omitted from control). Oxidation of NADH was followed for 15 minutes.

Statistical Analysis

The requirements of normality and homoscedasticity for the analysis of variance (ANOVA) model were examined. Group variance homogeneity was assessed using the modified Levene test, and weighted transformations ($1/\text{standard deviation}^2$) of the data were made where necessary (Neter *et al.*, 1996). Multiple pairwise comparisons of factor level means were performed by the Tukey-Kramer HSD procedure for unequal sample sizes. A level of significance of $P<0.05$ was used in all tests. Statistical procedures were carrying out with the software package SYSTAT 9.0 (SPSS Inc.). All means are reported ± standard errors (± SE).

RESULTS AND DISCUSSION

Metabolite Content in Plasma

The concentration of glucose in blood is dependent upon the relative rates of entry and exit of glucose from this compartment, i.e., the rates of production in some tissues and consumption in others. Blood glucose concentration, therefore, cannot be used by itself as a direct measure of the rate of any process involving carbohydrate metabolism (Suarez and Mommsen, 1987). However, glucose turnover rate is proportional to blood metabolite concentration (Weber *et al.*, 1986), and is assumed that, as observed in higher vertebrates, the maintenance of supracritical blood glucose concentration is an essential physiological characteristic of fishes. Glucose concentrations in plasma of Antarctic and Subantarctic fishes are between the ranges of values reported for other teleost fishes under normal physiological conditions (Moon, 1988; Garin *et al.*, 1987). Although the Subantarctic species *E. maclovinus* has significantly higher values of glucose than the other species examined, a clear distinction between Antarctic and Subantarctic fishes cannot be established regarding plasma glucose concentration (Figure 1). The relatively high values of glucose in plasma observed in *E. maclovinus* compared with the other species in this study could be attributed to handling stress, but because the small sample size (n= 3) it is not possible to resolve this issue.

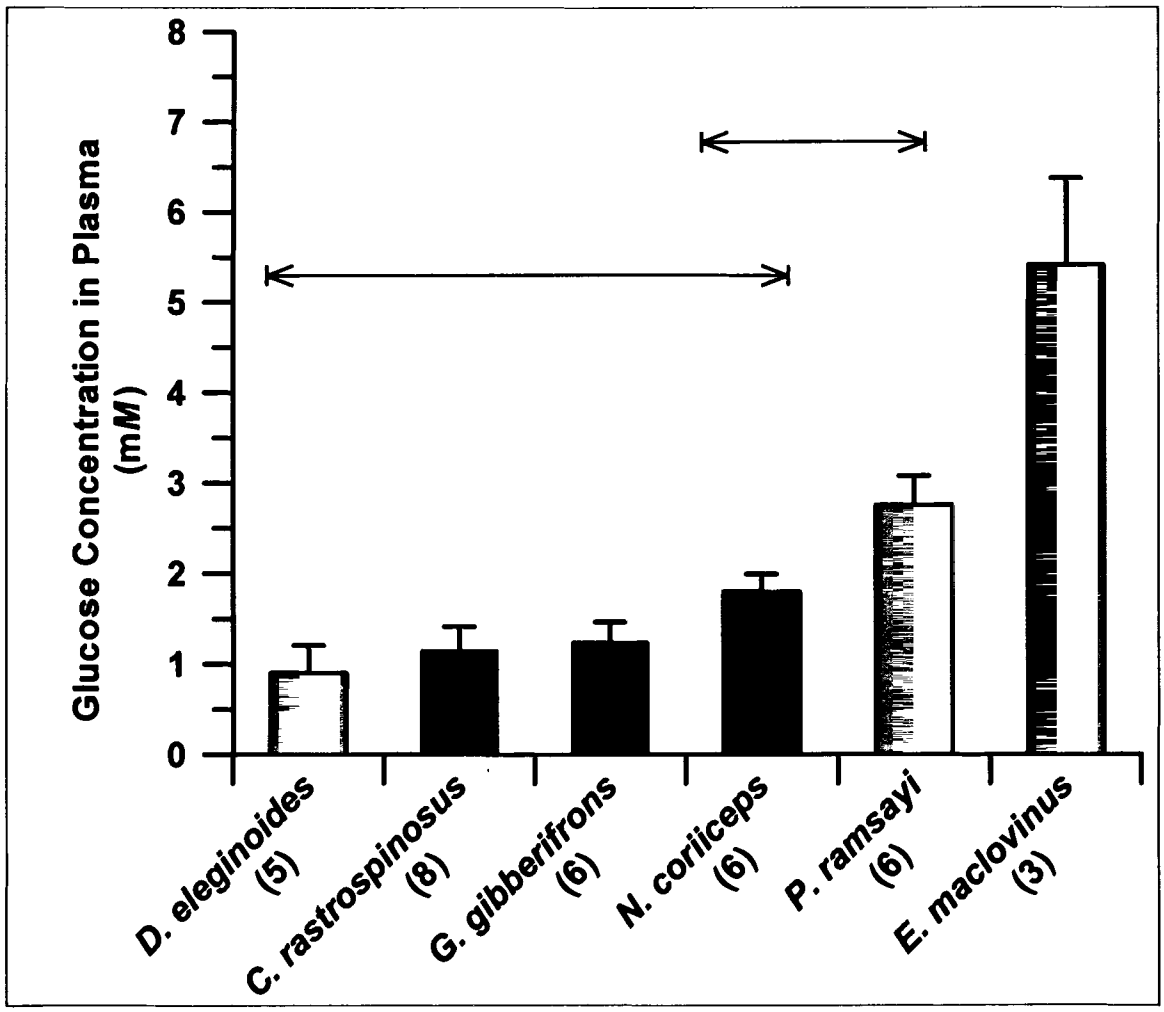

Figure 1. Glucose concentration in plasma of Antarctic (black bars) and Subantarctic (gray bars) fishes. Data are shown as mean ± SE with the sample size between parentheses. The arrow line above the bars denotes groups that are not significantly different ($P<0.05$).

TAG hydrolysis produces glycerol in addition to fatty acids. Considering the importance of fatty acids as metabolic fuels by aerobic muscle of Antarctic notothenioid species, I anticipated finding relatively high levels of glycerol in plasma of these fishes when compared with Subantarctic notothenioid species. However, only the Subantarctic *E. maclovinus* has significantly higher values of glycerol in plasma than the rest of the species (Figure 2). No other significant differences were established between Antarctic and Subantarctic species regarding the concentration of this metabolite in plasma. It is possible that Subantarctic notothenioid fishes also display a predominance in the use of lipids as metabolic fuels that could explain the lack of difference of glycerol concentration in plasma between the groups.

Additionally, glycerol content in plasma for Antarctic species (between 0.05-0.082 m*M*) was surprisingly lower than that reported for other teleosts (*Osmerus mordax*= 109; *Microgadus tomcod*= 0.29, *Liopsetta putmani*= 0.17, Driedzic *et al.*, 1998; *Oncorhyncus mykiss*= 0.38, Bernard *et al.*, 1999; in m*M*). Driedzic *et al.* (1998) attribute antifreeze properties to the high concentrations of glycerol found in plasma of *O. mordax*. However, the other two species in their study containing glycoproteins or macromolecules as antifreeze in blood (*M. tomcod* and *L. putmani*) also have higher levels of glycerol in plasma than Antarctic notothenioid fishes. Bernard *et al.* (1999) conclude that *O. mykiss* do not mobilize TAG reserves during endurance swimming beyond resting levels, and

therefore maintain levels of glycerol and NEFAs in the bloodstream well in excess of oxidative fuel requirements even at rest. However, the pattern of storage of TAGs among tissues is very variable in fishes (Weber and Zwingelstein, 1995), and this factor could affect the concentration of glycerol in plasma. Non-muscular tissues of some fishes (e.g. liver or adipose tissue) may be more important storage locations for TAG than aerobic muscle. In such species, blood concentration of fatty acids and glycerol may exceed those found in species that predominantly store TAGs intramuscularly.

Metabolite Content in Liver

The liver plays a central role in glucose homeostasis, by balancing the uptake, storage and release of this metabolite in the bloodstream (Newsholme and Start, 1973). Livers of *E. maclovinus* and *P. ramsayi* have significantly higher levels of glucose than the other species examined (Figure 3), which is similar to the pattern of glucose levels in plasma from Antarctic and Subantarctic fishes (Figure 1). Assuming a water content of 85%, the concentration of glucose in livers from species other than *E. maclovinus* and *P. ramsayi* was between 2.2 and 5.9 m*M*. These values are, as I expected, very similar to the concentration of glucose detected in plasma of these animals (0.9-5.4 m*M*).

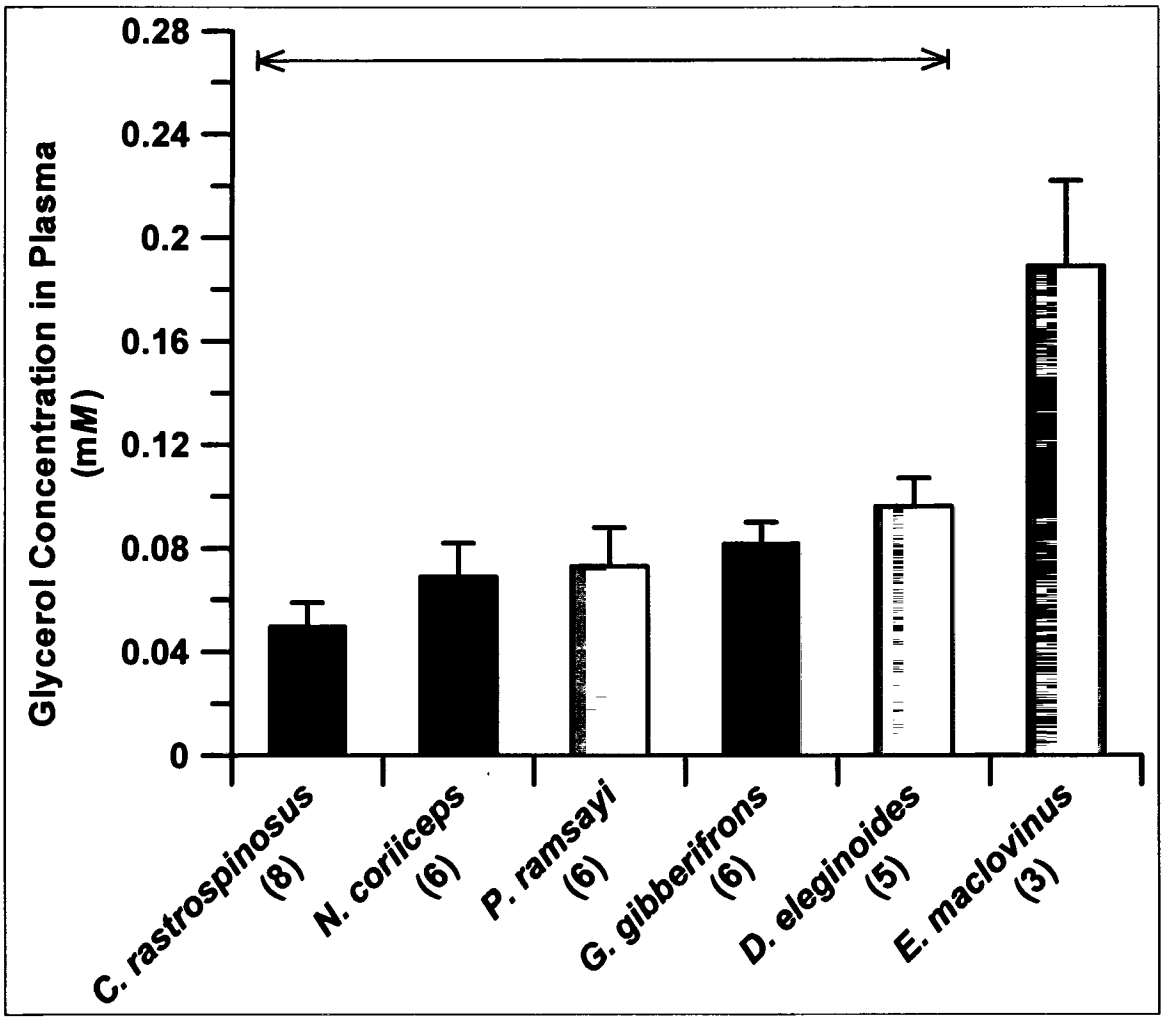

Figure 2. Glycerol concentration in plasma of Antarctic (black bars) and Subantarctic (gray bars) fishes. Data are shown as mean ± SE with the sample size between parentheses. The arrow line above the bars denotes groups that are not significantly different ($P<0.05$).

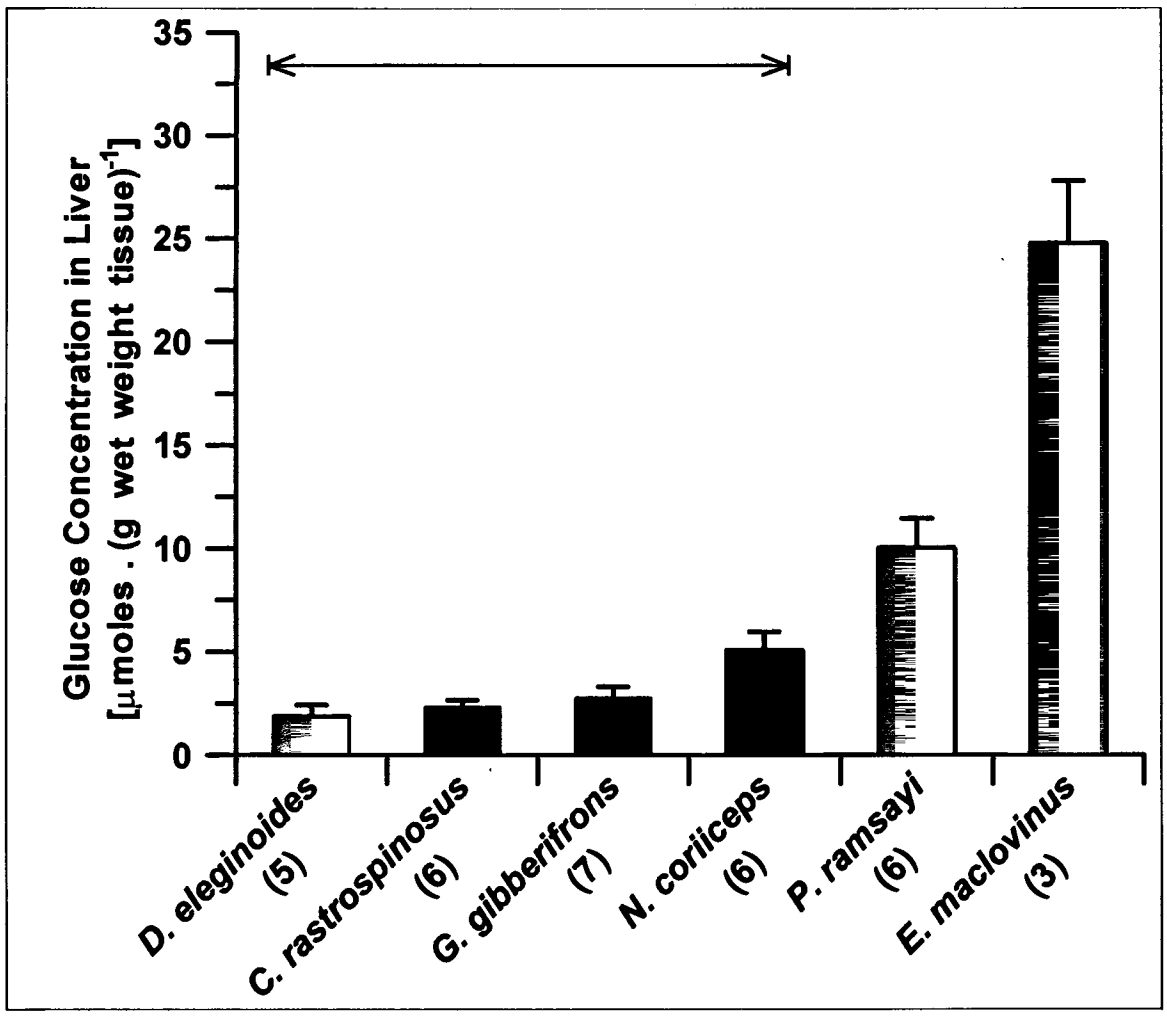

Figure 3. Glucose concentration in livers of Antarctic (black bars) and Subantarctic (gray bars) fishes. Data are shown as mean ± SE with the sample size between parentheses. The arrow line above the bars denotes groups that are not significantly different ($P<0.05$).

Glycogen content in livers of fishes may show great inter- or intra-specific variability (Moon and Foster, 1995; Soengas *et al.*, 1996). However, the content of this metabolite in liver can serve as an indicator of physiological condition of the fish. Glycogen contents in livers of Antarctic and Subantarctic fishes (Figure 4) have generally similar values for all the species [177-275 μmol glycosyl units • (g wet weight tissue)$^{-1}$]. Similar levels of glycogen in livers were reported by Phillips and Hird (1977) for other fishes under normal physiological conditions [*Oncorhyncus mykiss*= 222, and *Anguilla australis occidentalis*= 295, μmol glycosyl units • (g wet weight tissue)$^{-1}$].

I found similarly low levels of glycerol in the livers of both Antarctic and Subantarctic fishes (Figure 5) [0.85-1.35 μmol • (g wet weight tissue)$^{-1}$]. Only glycerol content in liver of the Antarctic fish *C. rastrospinosus* and the Subantarctic fish *P. ramsayi* were significantly different from each other. Assuming a water content of 85 %, the concentration of glycerol in livers of Antarctic and Subantarctic species was between 1 and 1.6 m*M*, at least 12 times the concentration of glycerol detected in plasma of the same fishes.

Driedzic *et al.* (1998) reported high values of glycerol in livers of *O. mordax* and *L. putmani* [96.7 and 21.4 μmol • (g wet weight tissue)$^{-1}$, respectively]. The same authors also detected low values of this metabolite in livers of *M. tomcod* [less than 0.1 μmol • (g wet weight tissue)$^{-1}$]. The alternative, but not mutually exclusive, explanations for

the different glycerol content between liver and plasma can be: loss of this metabolite in the blood through the epithelia (Raymond, 1993), increased recapture of glycerol by the liver from the plasma pool, and/or increased breakdown/ recycling of TAG in the liver (Weber *et al.*, 1999).

Maximal Enzymatic Activities in Livers Measured at 1°C

Unlike glycolysis, which occurs in all tissues, gluconeogenesis in vertebrates is restricted to liver and kidney (Cahill, 1986). PEPCK and FBP, two key enzymatic activities on this pathway, have been reported in a variety of vertebrate and invertebrate species. Both enzymatic activities are considered rate-limiting of the gluconeogenic pathway and are correlated with glucose production (Mommsen, 1986). In addition, the reaction catalyzed by GK is a putative rate-limiting step for the conversion of glycerol to glucose.

Although, CS activity in liver is not directly related to the gluconeogenic pathway, this enzyme is a good indicator of Krebs cycle activity and therefore, of oxidative metabolism (ATP-generation potential). I included determination of hepatic CS activity in this study because the intramitochondrial supply of ATP has been proposed to be limiting in the rate of gluconeogenesis (Savina and Derkachev, 1983).

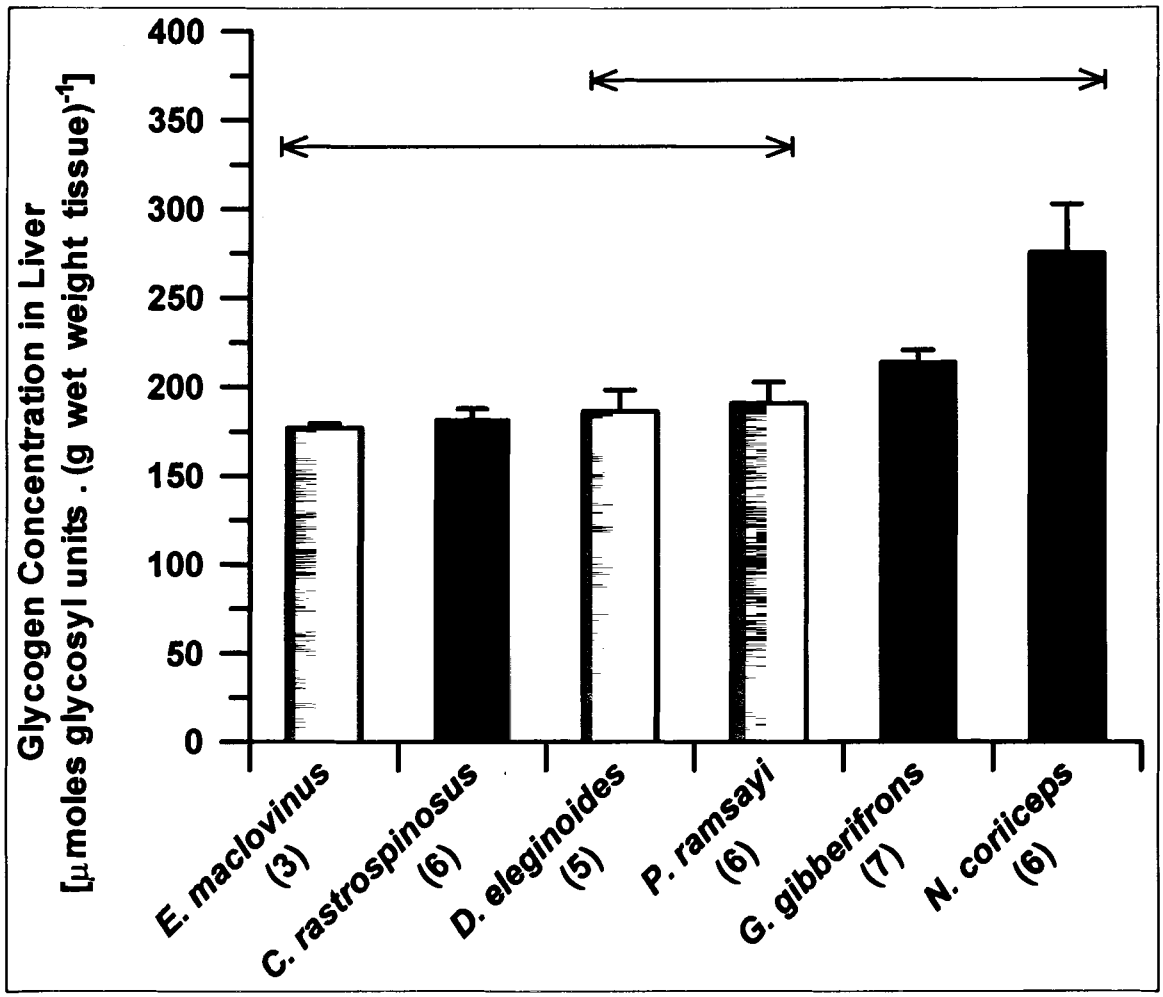

Figure 4. Glycogen concentration in livers of Antarctic (black bars) and Subantarctic (gray bars) fishes. Data are shown as mean ± SE with the sample size between parentheses. The arrow line above the bars denotes groups that are not significantly different ($P<0.05$).

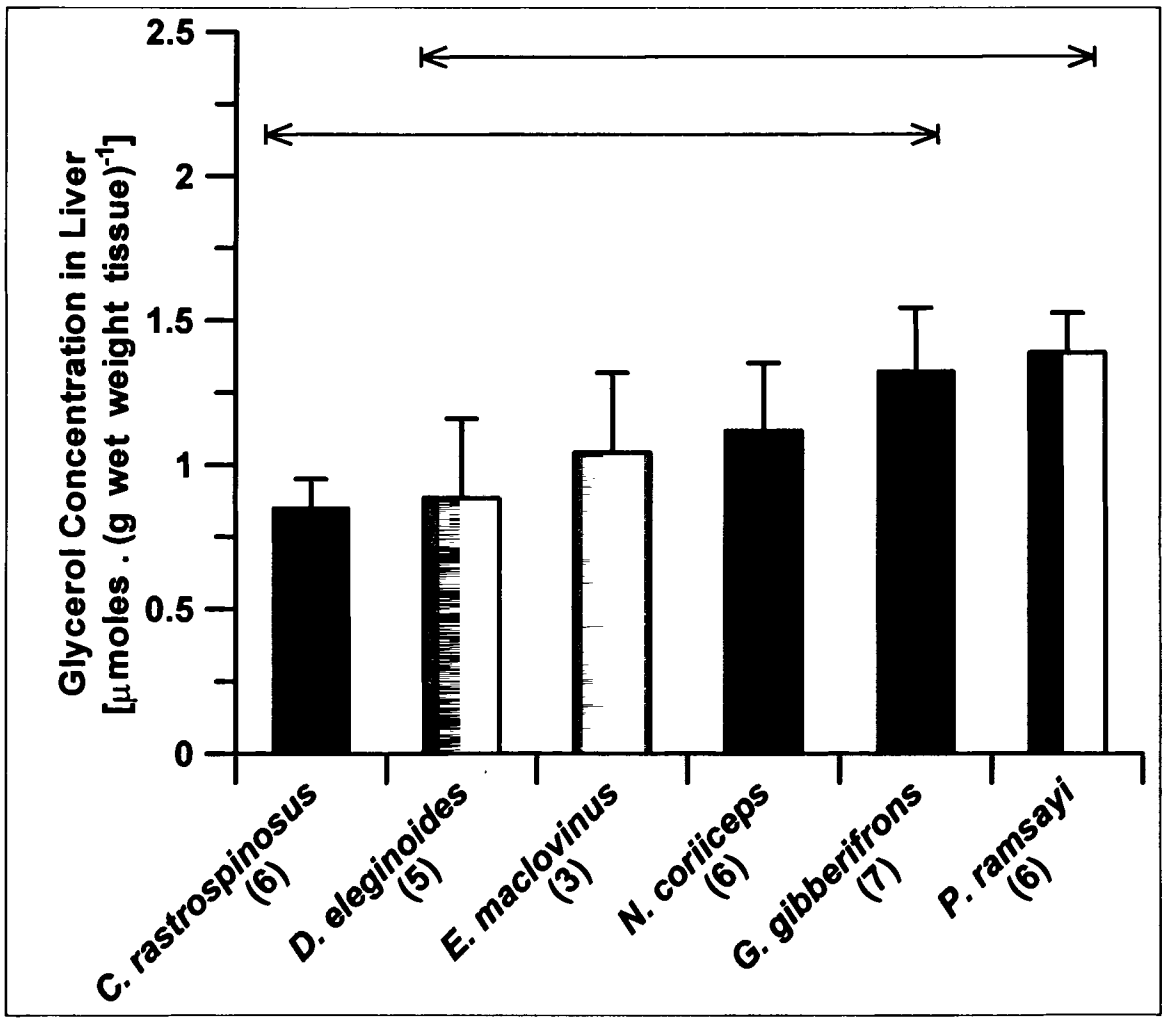

Figure 5. Glycerol concentration in livers of Antarctic (black bars) and Subantarctic (gray bars) fishes. Data are shown as mean ± SE with the sample size between parentheses. The arrow line above the bars denotes groups that are not significantly different ($P<0.05$).

Two types of adaptations for high rates of enzymatic catalysis at low temperature are possible (Somero, 1991). First, higher intracellular concentrations of enzymes can guarantee an increased number of catalytic sites, compensating for the reduced rate of activity per site due to Q_{10} effects. Second, fewer copies of the enzyme may be produced that have higher rates of catalysis per active site. Both compensation mechanisms will be reflected as an increase in the maximal activities of the enzymes per gram wet weight of tissue.

If the hepatic enzymes mentioned previously are metabolically cold adapted, a higher maximal enzymatic activity per gram of tissue is expected to occur in Antarctic fishes with respect to Subantarctic fishes when measured at 1°C. To my surprise, livers from Antarctic notothenioids fishes show lower or similar levels of maximal activity than Subantarctic notothenioids fishes for all the enzymes assayed in this study at that temperature.

FBP is normally localized in the cytosol and is under strict regulatory control by metabolites (Villanueva and Marcus, 1974). With the exception of *E. maclovinus,* liver of *C. rastrospinosus* have a lower maximal activity of FBP than the rest of the species. Nevertheless, maximal activities of FBP in livers of Antarctic and Subantarctic fishes were, in general, similar for all the species (Figure 6).

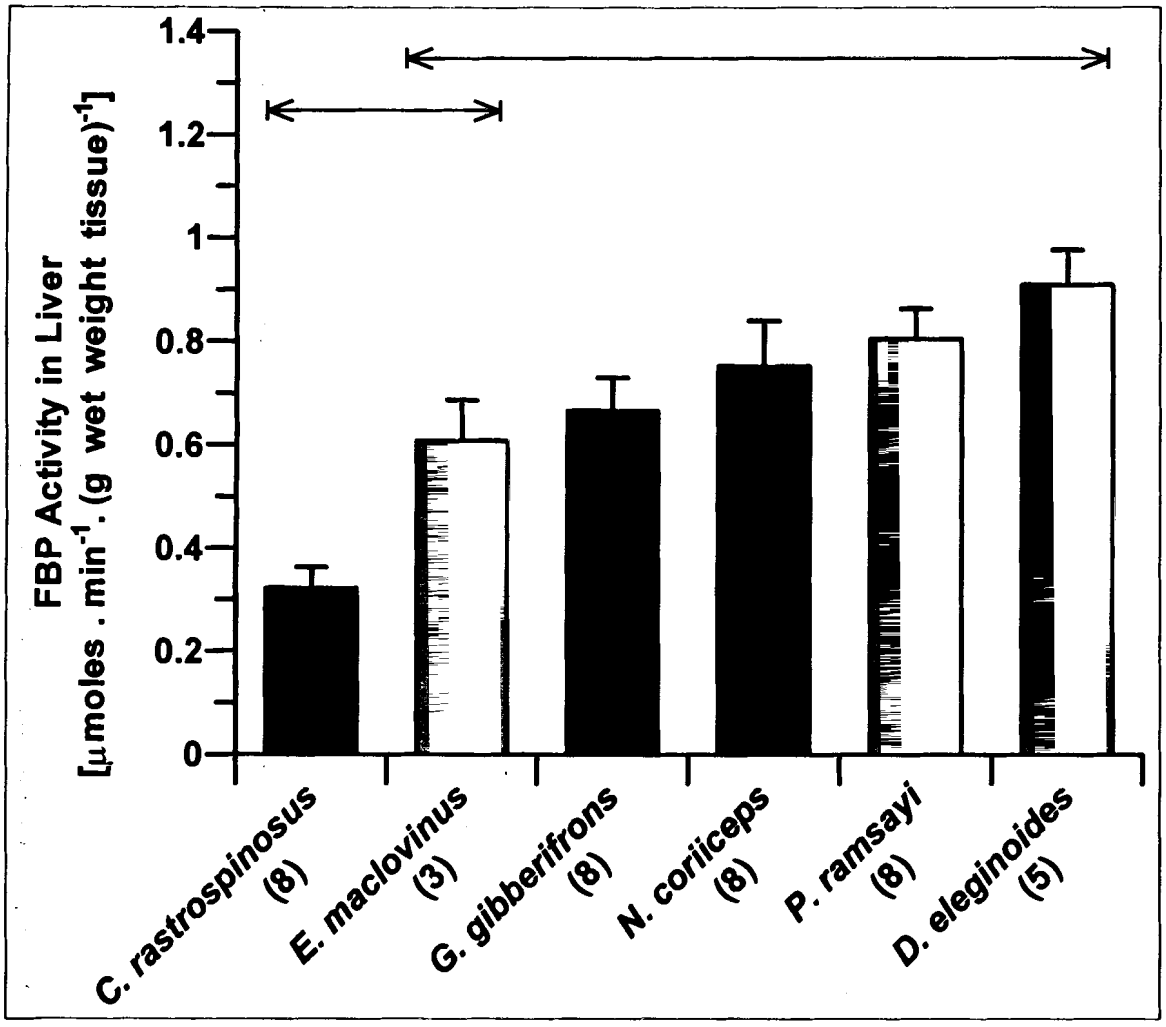

Figure 6. FBP maximal activity in livers of Antarctic (black bars) and Subantarctic (gray bars) fishes measured at 1°C. Data are shown as mean ± SE with the sample size between parentheses. The arrow line above the bars denotes groups that are not significantly different ($P<0.05$).

PEPCK has sites of intracellular localization that vary according to organ, species, and physiological conditions (Suarez and Mommsen, 1987). However, in this study PEPCK activity was analyzed in crude homogenates after sonication, which allows me to quantify both cytosolic and mitochondrial forms. PEPCK maximal enzymatic activity in livers of the Subantarctic fishes *D. eleginoides* and *P. ramsayi* are significantly higher than the rest of the species when measured at 1°C (Figure 7). PEPCK maximal enzymatic activity in livers of *E. maclovinus*, *C. rastrospinosus*, *G. gibberifrons* and *N. coriiceps* do not significantly differ from each other.

No significant differences were found among maximal GK activities in livers of Antarctic and Subantarctic fishes. Activities were similarly low for all the species [0.008-0.016 at 1°C; 0.032-0.048 at 11°C, μmol • min^{-1} • (g wet weight tissue)$^{-1}$], near the limit of detection of the method. However surprising, the result is not rare. Foster and Moon (1986) did not detect GK activity in liver of *Myxine glutinosa* when the assay was performed at 10°C. The same authors (1990) found very low levels of GK activity in liver of *Perca flavescens* [0.030 μmol • min^{-1} • (g wet weight tissue)$^{-1}$] when measured at 15°C.

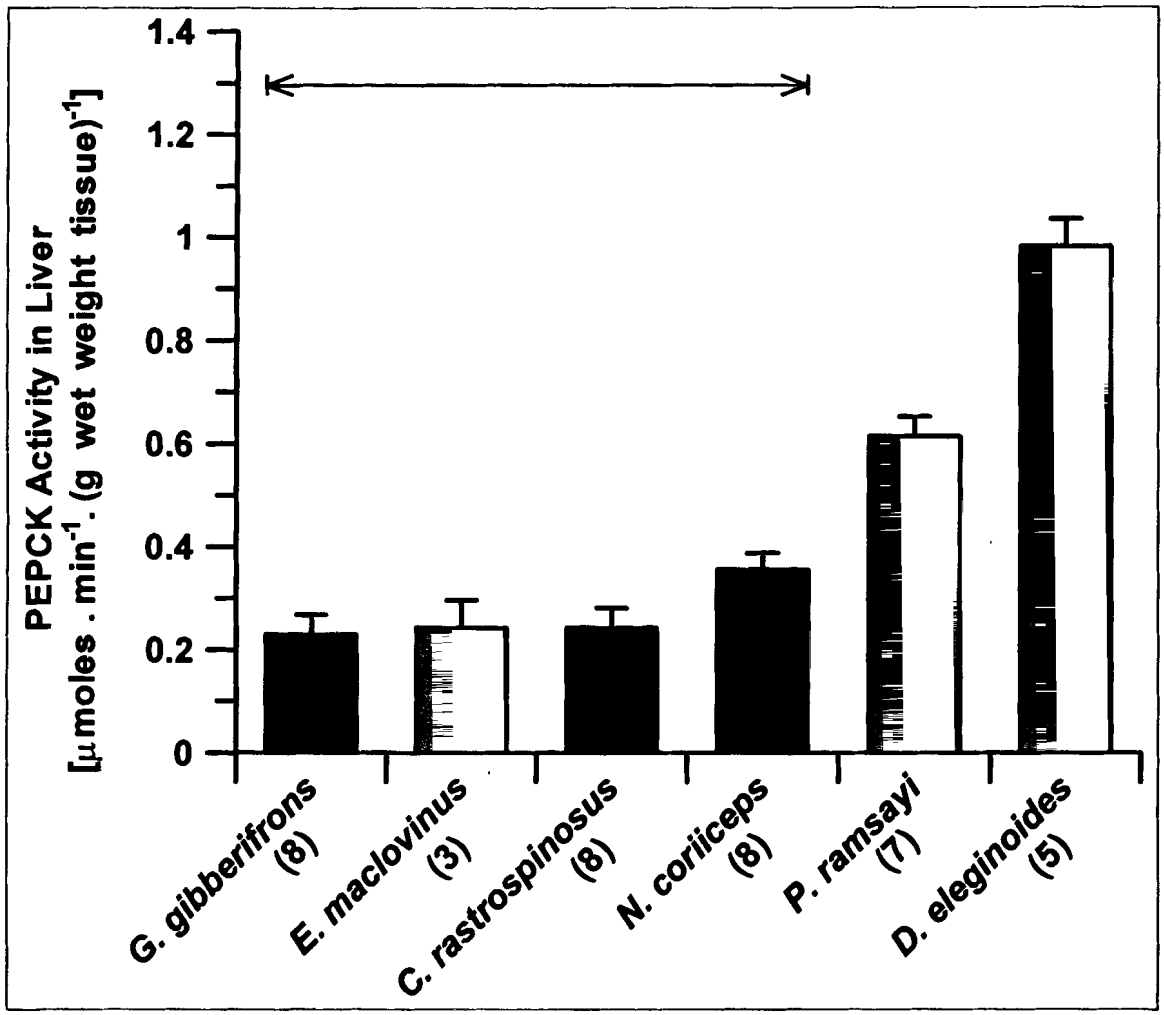

Figure 7. PEPCK maximal activity in livers of Antarctic (black bars) and Subantarctic (gray bars) fishes measured at 1°C. Data are shown as mean ± SE with the sample size between parentheses. The arrow line above the bars denotes groups that are not significantly different ($P<0.05$).

The data presented support lack of MCA in the gluconeogenic pathway in livers of Antarctic fishes when compared with Subantarctic notothenioid fishes. Surprisingly, Antarctic notothenioid fishes do not display any hallmarks of an increased use glycerol as a gluconeogenic precursor. It is possible that the decreased importance of carbohydrate metabolism in this group of fishes, as demonstrated for glycolysis by Crockett and Sidell (1990), could be related to the lack of difference in gluconeogenesis between Antarctic and Subantarctic notothenioid fishes. Decreased utilization of carbohydrates for aerobic metabolism by tissues will result in a relative increase in availability of glucose and glycogen for anabolic processes such as synthesis AFGPs, and therefore lower requirements of precursors such as glycerol or amino acids for synthesis of glucose through the gluconeogenic pathway. In addition, skeletal muscle of vertebrates may present very low activities of gluconeogenic enzymes, but there is no evidence that this process has importance in maintaining the blood pool of glucose (Connet, 1979). Therefore, aerobic muscle probably does not have a relevant role in the synthesis of glucose from precursors in Antarctic fishes.

Regarding the pathway of production of energy from aerobic metabolism, CS maximal activity was demonstrated to be metabolically cold adapted in muscles and brain from a number of Antarctic notothenioids fishes when compared to temperate fishes (Crockett and Sidell, 1990; Kawall *et al.*, 2002). However, in this study, CS maximal

enzymatic activities in livers of Antarctic notothenioid fishes do not display MCA when compared with related species with Subantarctic distribution. In general, Antarctic and Subantarctic notothenioid species have similar levels of CS activity in liver when assayed at 1°C (Figure 8). *D. eleginoides* has high values of CS activity in liver. A similar pattern is observed on the hepatic FBP and PEPCK enzymatic activities (Figure 6 and 7). This is not surprising, since as mentioned before, *D. eleginoides* is a more active mesopelagic fish than the other species examined (Eastman, 1993).

Decreased CS activity in livers of Antarctic fishes, when compared to Subantarctic fishes, may correlate with lower capacity of Krebs cycle and reduce ability to supply ATP to hepatocytes. Low rates of ATP production may limit gluconeogenesis, an anabolic pathway that requires energy for the synthesis of glucose from different precursors.

Arrhenius Plot and Activation Energy of Hepatic Enzymes

The first step in order to analyze adaptation in structure and reactivity of enzymes to temperature is obtaining the thermodynamic parameters from the reaction (Russell, 2000). Comparison of these thermodynamic parameters between enzymes from Antarctic species and orthologs from temperate zone fishes may reveal patterns of evolutionary adaptation to cold temperature.

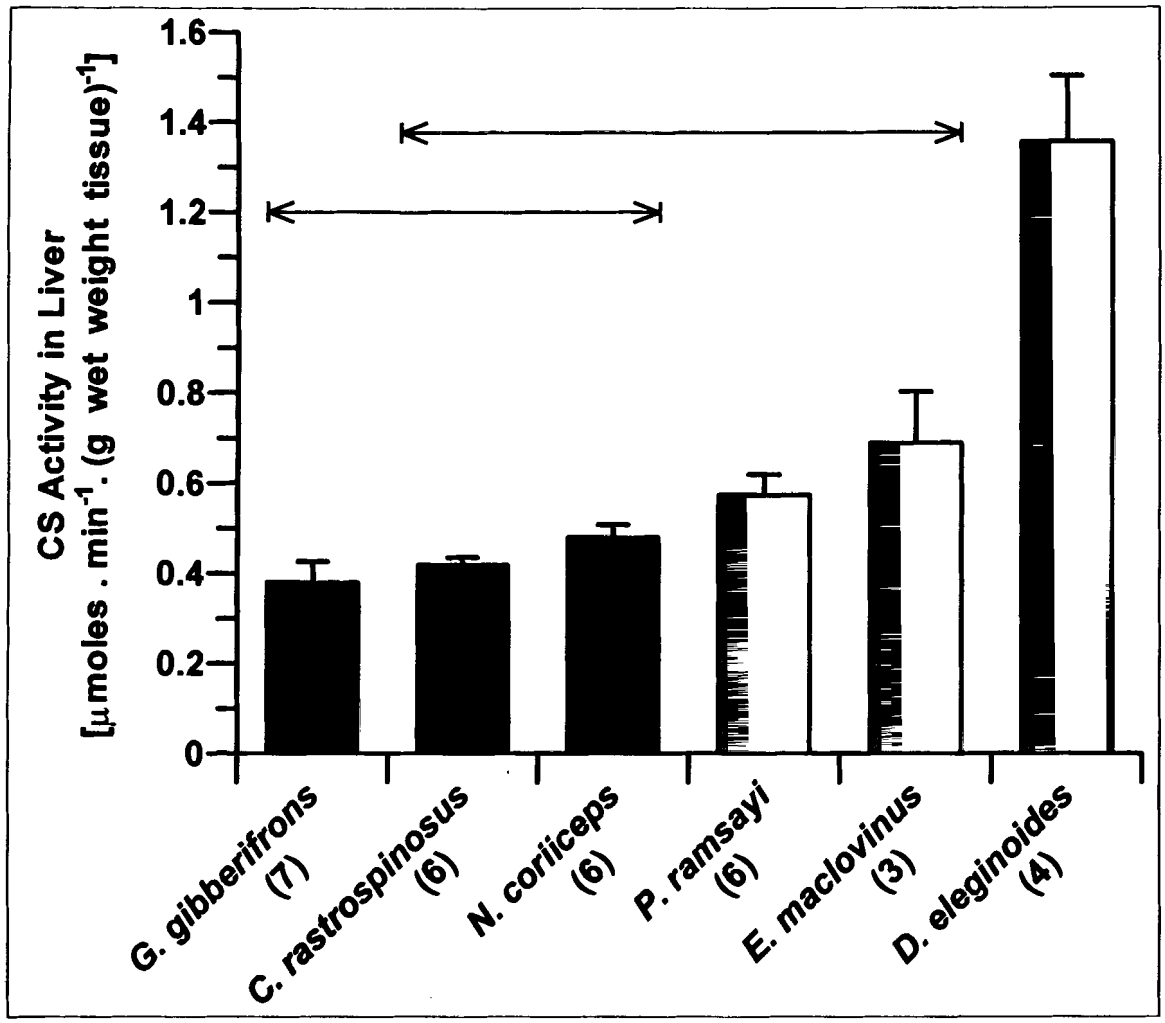

Figure 8. CS maximal activity in livers of Antarctic (black bars) and Subantarctic (gray bars) fishes measured at 1°C. Data are shown as mean ± SE with the sample size between parentheses. The arrow line above the bars denotes groups that are not significantly different ($P<0.05$).

Measurements of maximal activities at different temperatures are used to calculate the energy of activation (E_a) of the enzymatic reaction from the slope ($-E_a/R$) of the Arrhenius Plot ($\log_{10}$ maximal enzymatic activity as a function of $1/T$, where R represents the universal constant for gases and T the absolute temperature). These calculations are important in order to estimate the enthalpy of activation ($\Delta H^{\ddagger}$), that together with the entropy of activation ($\Delta S^{\ddagger}$), will determinate the free energy of activation of the process ($\Delta G^{\ddagger}$) (Lehrer and Barker, 1970).

$$\Delta H^{\ddagger} = E_a - R \cdot T \qquad\qquad \Delta G^{\ddagger} = \Delta H^{\ddagger} - \Delta S^{\ddagger} \cdot T$$

I did not purify the enzymes of this study and therefore could not estimate $\Delta G^{\ddagger}$ and $\Delta S^{\ddagger}$ of the processes. However, the E_a or $\Delta H^{\ddagger}$ can be used to compare enzymes from Antarctic and Subantarctic organisms. A possible strategy for cold adapted enzymes is to decrease the $\Delta H^{\ddagger}$ value in order to reduce the temperature dependence of the maximal activity (Lonhienne *et al.*, 2000). In fact, a decrease of $\Delta H^{\ddagger}$ or an increase of $\Delta S^{\ddagger}$ has the common effect to increase the maximal enzymatic activity, and both strategies may occur during the adaptation of enzymes to low temperatures.

No differences were observed between Antarctic and Subantarctic species in the slope value and E_a calculated from Arrhenius plots, when maximal activities of each enzyme are compared at different temperature.

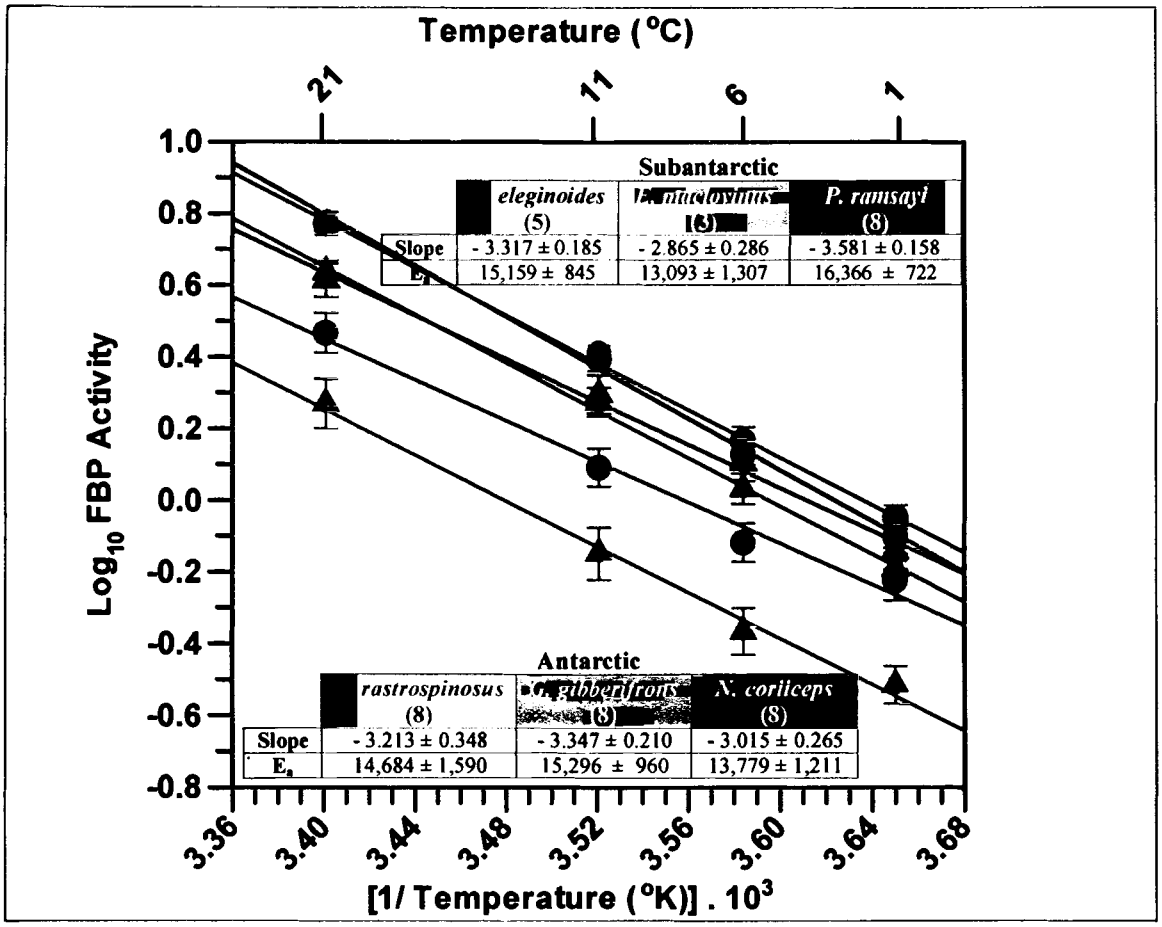

Figure 9. Arrhenius plot for maximal FBP activity in livers of Antarctic and Subantarctic fishes. The insert tables show the slope calculated from Arrhenius Plot (Slope) using least-squares regression, and mean activation energy (E_a) for FBP. Data are shown as mean ± SE, sample size between parentheses. E_a= -2,300 · R · (Slope), where R= 1.987 cal · mol^{-1} · $^oK^{-1}$.

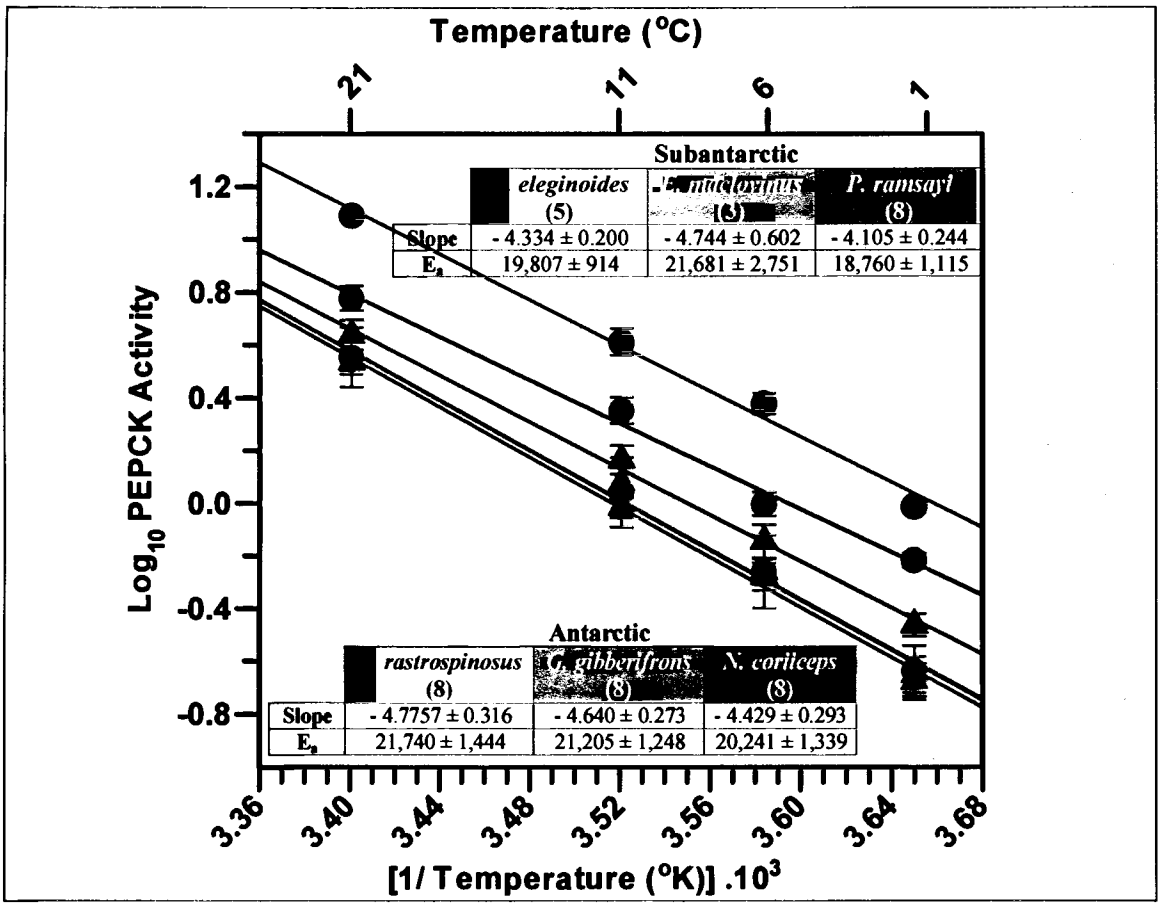

Figure 10. Arrhenius plot for maximal PEPCK activity in livers of Antarctic and Subantarctic fishes. Further details as in legend to Figure 9.

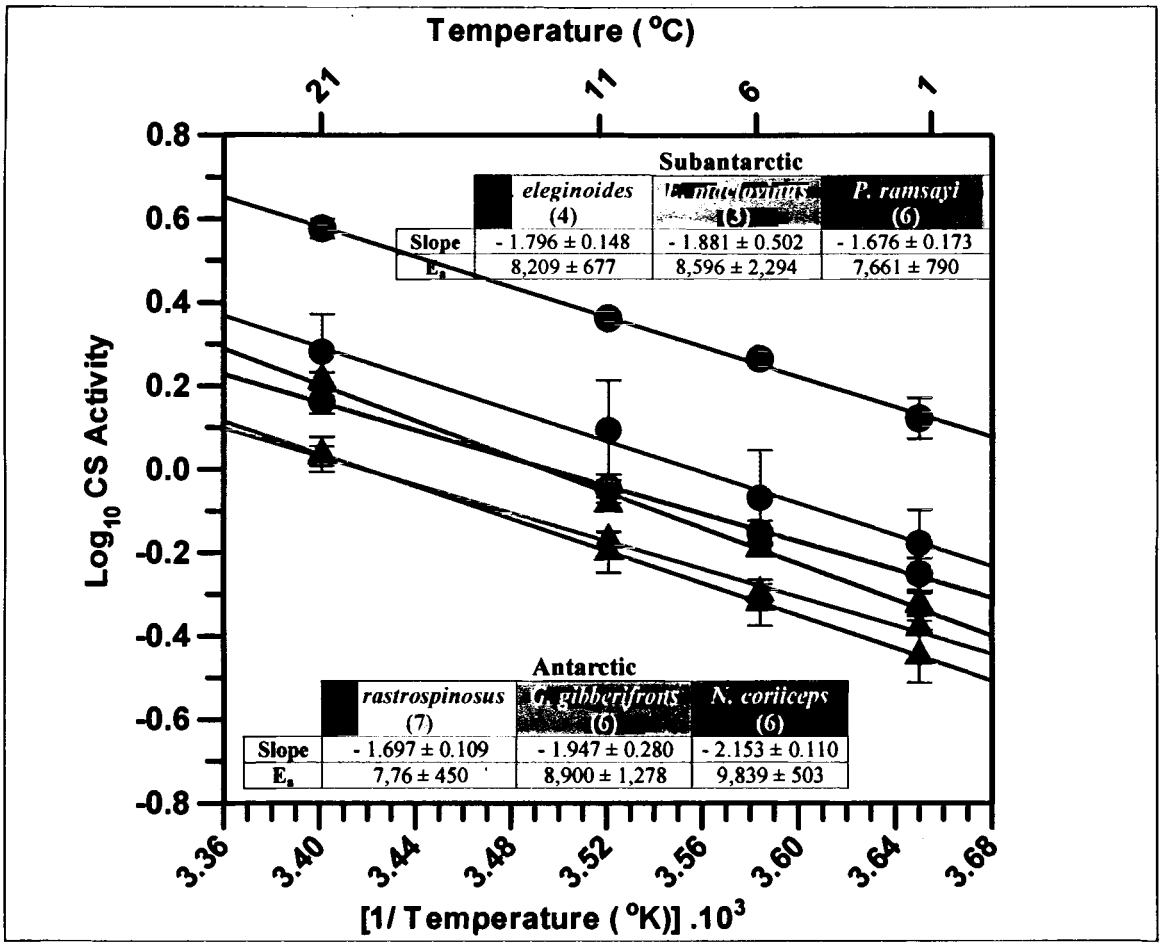

Figure 11. Arrhenius plot for maximal CS activity in livers of Antarctic and Subantarctic fishes. Further details as in legend to Figure 9.

The slopes from Arrhenius plots and the E_as for the hepatic enzymes FBP (Figure 9), PEPCK (Figure 10), and CS (Figure 11) were not significantly different among the notothenioid species examined (Appendix A). In addition, when non-transformed data for enzyme activities were analyzed by non-linear regression, no significant differences were found among estimated values for E_as of hepatic enzymes from any of the species (Appendix B). It is possible that the enzymes under study will have similar values for $\Delta G^{\ddagger}$ in livers of Antarctic notothenioid and Subantarctic notothenioid fishes, supporting the conclusion of lack of cold adaptation of enzymatic activities measured at 1°C presented in the previous section.

CONCLUDING REMARKS AND FUTURE WORK

The data presented in this comparative study on glycerol content, enzymatic activities and thermal sensitivities of FBP, PEPCK, GK, and CS do not support the concept of MCA on the gluconeogenic pathway and aerobic metabolism in livers of Antarctic notothenioid species. Lack of MCA in hepatic gluconeogenesis may indicate that this pathway is of low physiological importance in both Antarctic and Subantarctic notothenioids. Other possible explanation is that insufficient time has passed for evolutionary divergence in gluconeogenesis between these two closely related groups of notothenioids. In support of this idea, comparative study on mitochondrial rDNA sequences suggests recent divergence between Antarctic and Subantarctic notothenioids fishes (1.7-7 million years ago), later than the formation of the Antarctic Convergence (Stankovic *et al.*, 2002).

D. eleginoides does not contain AFGPs in its fluids. However, no data are available about presence of AFGPs in *P. ramsayi* and *E. maclovinus*. Clarke and Johnston (1996) suggested that the absence of AFGPs in Subantarctic species correspond to secondary losses. We cannot discard a hypothetical production of AFGPs in those *P. ramsayi* and *E. maclovinus*, but this is unlikely to occur, because other South American and New Zealand notothenioid species found north of the Antarctic Convergence (similar water temperature) do not contain AFGPs (Eastman, 1993).

Fields and Somero (1998) detected significantly higher maximal activities of lactate dehydrogenase from muscles of Antarctic fishes (*N. coriiceps*, *C. rastrospinosus*, and *G. gibberifrons*) than those of Subantarctic species (*E. maclovinus*, *P. magellanica*, and *P. tesellata*). Their work and this study constitute the only known studies comparing maximal enzymatic activities in the intermediary metabolism of Antarctic and Subantarctic notothenioid fishes. Additional comparative studies of hepatic enzymes between notothenioids with different distributions, including these involved in amino acid and lipid metabolism, will be essential to evaluate possible adaptive changes on other metabolic pathways than gluconeogenesis.

The importance of glycerol remains unclear in Antarctic notothenioid fishes. Additional *in vivo* and *in vitro* experiments utilizing radiolabeled compounds may help resolve this issue.

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APPENDICES

APPENDIX A

Activation Energy of Hepatic Enzymes Calculated from Arrhenius Plot

		E_a	95% confidence interval
Subantarctic fishes	*D. eleginoides* (5)	15,159 ± 845	13,383 - 16,935
	E. maclovinus (3)	13,093 ± 1,307	10,181 - 16,005
	P. ramsayi (8)	16,366 ± 722	14,889 - 17,842
Antarctic fishes	*C. rastrospinosus* (8)	14,684 ± 1,590	11,436 - 17,931
	G. gibberifrons (8)	15,296 ± 960	13,336 - 17,256
	N. coriiceps (8)	13,779 ± 1,211	11,302 - 16,255

Table A1. Activation energy (Ea) for FBP in livers of Antarctic and Subantarctic fishes calculated from the Arrhenius plot. Data are shown as means ± SE, sample size shown in parentheses. E_a is calculated as describe in Figure 9.

		E_a	95% confidence interval
Subantarctic fishes	*D. eleginoides* (5)	19,807 ± 914	17,878 - 21,735
	E. maclovinus (3)	21,681 ± 2,751	15,551 - 27,810
	P. ramsayi (8)	18,760 ± 1,115	16,480 – 21,041
Antarctic fishes	*C. rastrospinosus* (8)	21,740 ± 1,444	18,791 - 24,689
	G. gibberifrons (8)	21,205 ± 1,248	18,657 - 23,753
	N. coriiceps (8)	20,241 ± 1,339	17,507 - 22,975

Table A2. Activation energy (Ea) for PEPCK in livers of Antarctic and Subantarctic fishes calculated from the Arrhenius plot. Data are shown as means ± SE, sample size shown in parentheses. E_a is calculated as describe in Figure 9.

		E_a	95% confidence interval
Subantarctic fishes	*D. eleginoides* (4)	8,209 ± 677	6,758 - 9,660
	E. maclovinus (3)	8,596 ± 2,294	3,485 - 13,683
	P. ramsayi (6)	7,661 ± 790	6,021 - 9,301
Antarctic fishes	*C. rastrospinosus* (7)	7,756 ± 450	6,719 - 8,792
	G. gibberifrons (6)	8,900 ± 1,278	6,272 - 11,528
	N. coriiceps (6)	9,839 ± 503	8,790 - 10,883

Table A3. Activation energy (Ea) for CS in livers of Antarctic and Subantarctic fishes calculated from the Arrhenius plot. Data are shown as means ± SE, sample size shown in parentheses. E_a is calculated as describe in Figure 9.

APPENDIX B

Activation Energy of Hepatic Enzymes Calculated from Non-linear Regression Model

		E_a	95% confidence interval
Subantarctic fishes	*D. eleginoides* (5)	14,842 ± 1,186	12,350 - 17,334
	E. maclovinus (3)	13,988 ± 1,789	10,002 - 17,972
	P. ramsayi (8)	15,330 ± 914	13,461 - 17,200
Antarctic fishes	*C. rastrospinosus* (8)	15,610 ± 2,795	9,902 - 21,319
	G. gibberifrons (8)	14,581 ± 1,208	12,114 - 17,048
	N. coriiceps (8)	12,852 ± 1,613	9,553 - 16,151

Table B1. Activation energy (Ea) for FBP in livers of Antarctic and Subantarctic fishes calculated from non-linear regression model. Data are shown as means ± SE, sample size shown in parentheses. Parameters A and E estimated by least-square regression from the non-linear model $Y = A \cdot EXP(-E/T)$, where the depended variable (Y) is enzymatic activity in μmol • (g wet weight tissue)$^{-1}$ and the independent variable (T) is temperature in °K. E_a is calculated as E • 1.987 cal • mol^{-1} • °K^{-1}.

		E_a	95% confidence interval
Subantarctic fishes	*D. eleginoides* (5)	18,348 ± 1,126	15,971 - 20,724
	E. maclovinus (3)	19,373 ± 8,059	1,417 - 37,329
	P. ramsayi (8)	16,654 ± 1,590	13,397 - 19,911
Antarctic fishes	*C. rastrospinosus* (8)	18,799 ± 2,455	13,784 - 23,813
	G. gibberifrons (8)	19,609 ± 1,591	16,360 - 22,859
	N. coriiceps (8)	18,977 ± 2,283	14,314 - 23,640

Table B2. Activation energy (Ea) for PEPCK in livers of Antarctic and Subantarctic fishes calculated from non-linear regression model. Data are shown as means ± SE, sample size shown in parentheses. Further details as in legend to Table B1.

		E_a	95% confidence interval
Subantarctic fishes	*D. eleginoides* (4)	8,079 ± 574	6,849 - 9,310
	E. maclovinus (3)	8,167 ± 2,083	3,525 - 12,809
	P. ramsayi (6)	7,742 ± 751	6,185 - 9,299
Antarctic fishes	*C. rastrospinosus* (7)	7,889 ± 580	6,689 - 9,189
	G. gibberifrons (6)	8,725 ± 1,162	6,336 - 11,114
	N. coriiceps (6)	10,293 ± 532	9,189 - 11,398

Table B3. Activation energy (Ea) for CS in livers of Antarctic and Subantarctic fishes calculated from non-linear regression model. Data are shown as means ± SE, sample size shown in parentheses. Further details as in legend to Table B1.

APPENDIX C

Metabolite Concentrations in Plasma and Liver of Antarctic and Subantarctic Fishes

		Plasma		Liver		
Metabolite		**Glucose [a]**	**Glycerol [a]**	**Glucose [b]**	**Glycerol [b]**	**Glycogen [c]**
Subantarctic Fishes	***D. eleginoides***	0.901 ± 0.307 (5)	0.096 ± 0.011 (5)	1.864 ± 0.558 (5)	0.886 ± 0.275 (5)	186.161 ± 12.056 (5)
	E. maclovinus	5.409 ± 0.963 (3)	0.189 ± 0.033 (3)	24.757 ± 3.026 (3)	1.044 ± 0.276 (3)	176.892 ± 2.494 (3)
	P. ramsayi	2.758 ± 0.320 (6)	0.073 ± 0.015 (6)	10.035 ± 1.418 (6)	1.387 ± 0.138 (6)	190.440 ± 11.996 (6)
Antarctic Fishes	***C. rastrospinosus***	1.155 ± 0.263 (8)	0.050 ± 0.009 (8)	2.323 ± 0.350 (7)	0.850 ± 0.101 (6)	181.740 ± 5.775 (6)
	G. gibberifrons	1.245 ± 0.222 (6)	0.082 ± 0.008 (6)	2.792 ± 0.533 (7)	1.324 ± 0.220 (7)	214.167 ± 6.445 (7)
	N. coriiceps	1.798 ± 0.192 (6)	0.069 ± 0.013 (6)	5.088 ± 0.899 (6)	1.118 ± 0.234 (6)	267.963 ± 6.445 (6)

Table C1. Metabolite concentrations in plasma and liver of Antarctic and Subantarctic fishes. Values are means ± SE, sample size between parentheses. Concentrations are expressed in: a, mM; b, μmoles · g^{-1} wet weight tissue; and c, μmoles glycosyl units · g^{-1} wet weight tissue.

APPENDIX D

Enzymatic Activities in Liver of Antarctic and Subantarctic Fishes

Temperature (°C)		1°	6°	11°	21°
Subantarctic Fishes	*D. eleginoides* (5)	0.909 ± 0.067	1.490 ± 0.138	2.527 ± 0.214	6.004 ± 0.464
	E. maclovinus (3)	0.608 ± 0.079	0.774 ± 0.101	1.254 ± 0.159	2.988± 0.392
	P. ramsayi (8)	0.805 ± 0.058	1.364± 0.086	2.598± 0.144	6.050 ± 0.375
Antarctic Fishes	*C. rastrospinosus* (8)	0.322 ± 0.041	0.468 ± 0.077	0.785 ± 0.140	2.040 ± 0.357
	G. gibberifrons (8)	0.666 ± 0.063	1.112 ± 0.109	1.933 ± 0.173	4.426 ± 0.336
	N. coriiceps (8)	0.752 ± 0.088	1.337 ± 0.166	2.082± 0.257	4.296 ± 0.476

Table D1. Fructose 1,6-biphosphatase (FBP) activity in liver of Antarctic and Subantarctic fishes performed at four different temperatures. Values are means ± SE, sample size between parentheses. Enzymatic activity is expressed in μmoles product produced · min^{-1} · g^{-1} wet weight tissue.

Temperature (°C)		1°	6°	11°	21°
Subantarctic Fishes	*D. eleginoides* (5)	0.981 ± 0.054	2.441 ± 0.213	4.186 ± 0.493	12.429 ± 0.593
	E. maclovinus (3)	0.243 ± 0.054	0.608 ± 0.184	1.203 ± 0.323	3.831 ± 0.973
	P. ramsayi (8)	0.614 ± 0.038	1.032 ± 0.106	2.361 ± 0.280	5.711 ± 0.513
Antarctic Fishes	*C. rastrospinosus* (8)	0.243 ±0.038	0.554 ± 0.070	1.285 ± 0.209	3.570 ± 0.419
	G. gibberifrons (8)	0.230 ± 0.038	0.574 ± 0.067	0.992 ± 0.091	3.246 ± 0.235
	N. coriiceps (8)	0.356 ± 0.032	0.764 ± 0.091	1.543 ± 0.169	4.591 ± 0.529

Table D2. Phosphoenolpyruvate carboxykinase (PEPCK) activity in liver of Antarctic and Subantarctic fishes performed at four different temperatures. Values are means ± SE, sample size between parentheses. Enzymatic activity is expressed in μmoles product produced · min^{-1} · g^{-1} wet weight tissue.

Temperature (°C)		1°	6°	11°	21°
Subantarctic Fishes	*D. eleginoides* (5)	1.355 ± 0.147	1.845 ± 0.049	2.310 ± 0.074	3.791 ± 0.205
	E. maclovinus (3)	0.688 ± 0.114	0.911 ± 0.203	1.329 ± 0.305	1.993 ± 0.364
	P. ramsayi (6)	0.573 ± 0.046	0.714 ± 0.045	0.915 ± 0.069	1.475 ± 0.097
Antarctic Fishes	*C. rastrospinosus* (6)	0.418 ± 0.016	0.509 ± 0.027	0.681 ± 0.030	1.088 ± 0.062
	G. gibberifrons (7)	0.379 ± 0.047	0.501 ± 0.056	0.658 ± 0.069	1.119 ± 0.109
	N. coriiceps (3)	0.480 ± 0.028	0.647 ± 0.023	0.831 ± 0.029	1.644 ± 0.072

Table D3. Citrate synthase (CS) activity in liver of Antarctic and Subantarctic fishes performed at four different temperatures. Values are means ± SE, sample size between parentheses. Enzymatic activity is expressed in μmoles product produced · min^{-1} · g^{-1} wet weight tissue.

BIOGRAPHY OF THE AUTHOR

Leonardo Julian Magnoni was born in Rosario, Argentina on May 27th, 1967. He and his family moved to Mar del Plata in 1976, running away from mosquitoes. He attended High School, graduating as a Technician in Chemistry in 1985. After spending some years working in different jobs, he decided to be a "Licenciado" in Biology, attending Universidad Nacional de Mar del Plata. It was there, he found his first love: animal physiology. The fruit of this love was his first paper on physiology of crabs. Followed by several years of work and some study, he graduated in 1997 with honors. After his crab romance, he discovered the fascinating underwater world. For a short time, he became an amateur sailor. He joined the Institute for Fisheries Research and Development, working as a scientist aboard vessels in South Atlantic waters. In 1998, he obtained a scholarship to attend postgraduate courses on the Marine Biology and Aquaculture program at Universidade de Vigo in Spain. He published his second paper working on metabolism of rainbow trout. In 1999, he obtained a Fulbright scholarship to pursue a Masters at University of Maine, and attempted to learn some English at University of South Carolina. In 2001, he spent winter season at Palmer Station (Antarctica). Now, Leonardo is moving further north. He will pursue a Ph.D. in Biology at the University of Ottawa working in comparative animal physiology. Leonardo is a candidate for the Master of Science degree in Marine Biology from The University of Maine in August, 2002.