

The ICEFISH Cruise Symposium

**Darling Marine Center
Walpole, Maine
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Abstracts

Keynote Address

Aspects of the morphology of phylogenetically basal notothenioid fishes: Where do “Antarctic” features begin to appear?

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The ICEFISH cruise obtained large numbers of phylogenetically basal notothenioids of the families Bovichtidae and Elegendinopidae, a primary goal of the cruise. Since there is uncertainty about the position and relationships of another basal family, the Pseudaphritidae, I will evaluate some of the morphological characters that have been used in analysis and present information on some additional morphological features of basal notothenioids. Histology indicates that Balushkin's (2000) antesupracleithral organ is the thymus, a lymphoid organ present in all gnathostome fishes. It involutes with age in notothenioids and the appearance of its surface epithelium cannot be considered a reliable character because of the variability introduced by regression. Balushkin's (2000) hypoglossal gland, found in *Bovichtus* and *Cottoperca*, is a projection of the mucosa of the oral cavity lateral to the tongue. Histology reveals that it is not a multicellular gland and that its composition does not differ from that of the oral musosa in general – stratified squamous epithelium containing unicellular mucous glands and a few taste buds. It probably develops to fill a gap between the wide tongue and narrow anterior branchial region of bovichtids. Although difficult to visualize, it is a reliable character for bovichtids; non-bovichtids have a gradually tapering tongue and thus lack this structure. Study of ocular morphology reveals that bovichtids, but not other notothenioids, have a persistent embryonic fissure in the choroid and a low falciform process containing a small rete that protrudes through the fissure in the floor of the vitreous chamber. The falciform process is widely distributed in teleosts. The distribution of the latter two characters is consistent with the phylogenetic hypotheses of Lecointre et al. (1997, 2004) and Near et al. (2004) that the Bovichtidae, rather than the Pseudaphritidae (Balushkin, 2000), is the most phylogenetically basal notothenioid family. As skeletal ossification and mineralization are key determinants of buoyancy, and especially neutral buoyancy, relative mass of the skeleton was measured for a sample of notothenioids. A relatively low percentage skeletal mass is evident in *Elegendinops maclovinus* and is a phylogenetically persistent trait in some members of the Antarctic clade. As summarized in a table, several other characters typical of the Antarctic clade of notothenioids also are present in *Elegendinops*, and occasionally in *Pseudaphritis*. These include features of the hemoglobin, vasculature, muscles, brain and sense organs. I will conclude by suggesting some research priorities for the next ICEFISH cruise.

Session 1

Systematics and Life History Strategies of sub-Antarctic Notothenioids and Abyssal Fishes

Observations of demersal fish, benthic composition, and seafloor habitats within the Antarctic Convergence during the ICEFISH 2004 cruise

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A multi-disciplinary research cruise in the Atlantic sector of the Southern Ocean was conducted during the 2004 austral winter as part of the International Collaborative Expedition to collect and study Fish Indigenous to Sub-antarctic Habitats (ICEFISH). Sampling during the ICEFISH cruise included demersal finfish, benthic invertebrates, and information of seafloor composition. Areas sampled within the Antarctic Convergence included Shag Rocks, South Georgia, the South Sandwich Islands, and Bouvetøya Island. A description of demersal finfish and benthic invertebrate composition and distribution from each island group is presented, including information on geological composition of shelf area seabeds. The total number of finfish specimens recorded within the Antarctic convergence during the ICEFISH cruise trawl deployments was 9105 specimens of 29 species. The benthic composition of 31 hauls was analyzed and sorted into 52 taxonomic groupings. Bycatch of these hauls totaled more than 38 metric tons. Sediment samples were taken at 22 locations among the island groups and analyzed for grain size, percent carbonate, and percent organic matter.

A wide contrast in finfish and invertebrate species composition between island groups was observed, with the greatest differences between the South Sandwich Islands and Bouvetøya, where the isolation of the latter island likely has played a role in the differences in species composition. Due to mixed gear selectivity and possible avoidance, it was not feasible to make quantitative estimates of finfish abundance. However, using data from the Blake trawl, estimates of benthic invertebrate densities by station were computed and maps generated that provide information on benthic communities.

**Mental barbel variability and structure in the plunderfish *Artedidraco mirus*
(Perciformes: Notothenioidei: Artedidraconidae) from South Georgia,
Antarctica**

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A recent sample (48 specimens from South Georgia and one from Shag Rocks) of the plunderfish *Artedidraco mirus* collected in June 2004 during the ICEFISH Cruise yielded sufficient data to refute two long-held assumptions about this species: 1) it is endemic to South Georgia; 2) its mental barbel is sexually dimorphic (tapered in females and club-shaped with papillae in males). *A. mirus* exhibits three types of mental barbel: A – tapered without a terminal expansion; B – with a narrow terminal expansion composed of simple papillae; C – with a wide terminal expansion composed of convoluted or elaborately branched papillae. There is no correlation between barbel type and age or sex. Barbel histology resembles that of other artedidraconids studied to date (*Dolloidraco longedorsalis*, *Pogonophryne scotti*) in possessing large nerve trunks and blood vessels lateral to a pseudocartilaginous core and dermal papillae with an extensive network of nerves and blood vessels. The high degree of intraspecific variation in artedidraconid barbel structure warrants caution in using this organ as a diagnostic taxonomic character.

New and rare species of snailfishes (Scorpaeniformes: Liparidae) collected during the ICEFISH cruise of 2004

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The ICEFISH cruise of 2004 collected 13 specimens of the fish family Liparidae (Scorpaeniformes) from Burdwood Bank and near South Georgia. These specimens, with the addition of three more from the King Edward Point Laboratory of the British Antarctic Survey, represent nine species. Two of these are new and are described herein: *Careproctus sp. 1* and *C. sp. 2*; the second specimen of *C. minimus* Andriashev and Stein is reported and described, specimens of *C. georgianus* Lönnberg, *C. falklandicus* Lönnberg, *C. ?pallidus* (Vaillant), *Paraliparis copei gibbericeps* Andriashev, *P. gracilis* Norman, and *P. tetrapteryx* Andriashev and Neelov are reported, and live color of *C. georgianus*, *C. falklandicus*, and *P. tetrapteryx* is described and shown.

Session 2

Biochemistry, Molecular and Cell Biology of sub-Antarctic Notothenioids

The adaptive evolution of hemoglobins in the fish family Bovichtidae

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Studies on fish cold adaptation may help to understand whether extreme environments require specific molecular adaptations or select for life styles. Hemoglobins are sensitive to temperature and their properties mirror the thermal conditions encountered by species during their evolutionary histories. This paper will focus on structure, function and molecular phylogeny of the oxygen-transport system of *Cottoperca gobio*, a notothenioid fish of sub-Antarctic latitudes belonging to the basal family Bovichtidae. Unlike most Antarctic notothenioids, it has two major hemoglobins; they share the α -chain, whose amino-acid sequence is similar to those of minor hemoglobins of other notothenioids. Although, in the majority of notothenioids, “embryonic” (minor) α and β globins are expressed in traces in the adult stage, in *C. gobio* a complete “switch” to exclusive expression of the embryonic α -globin gene appears to have occurred in adult fish. The sequences have been used to improve our knowledge on the evolution of notothenioid hemoglobins.

Heterotrimeric G protein subunits of notothenioid fishes: Analysis of the gamma 3 subunit

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Heterotrimeric G-proteins underwent a robust diversification during vertebrate evolution. To date, 21 alpha, 6 beta, and 14 gamma subunits have been cloned in mammals. Why such diversification occurred remains speculative, but it is thought to be related to the diversification of vertebrate information processing systems involving sensory, neuronal, and intercellular communication. In this study, we cloned and sequenced the G protein gamma 3 subunit from representative members of various notothenioid families. Three features were observed. First, the sequences were highly conserved across the notothenioid families. Secondly, the notothenioid sequences had a number of features that distinguished themselves from other fish and vertebrate gamma 3 subunits. Thirdly, an unusual insertion was observed in all notothenioid families except the Elegendinopsidae. Through comparison with known vertebrate G protein subunit, we hope to gain insight on how temperature has impacted G-protein evolution and expand our phylogenetic understanding of the G protein subunit family.

The intron structure of *ldh-a* genes from notothenioid fish

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The *ldh-A* genes from notothenioid fish have been widely studied as part of attempts to understand cold-adaptation in enzymes. A large number of the coding regions have been characterized and show very little variation in either nucleotide or amino acid sequence implying considerable selective pressure on these loci. We describe here the nucleotide sequences of six introns from within the coding regions of *ldh-a* from a temperate notothenioid fish, *Notothenia angustata* and the polar notothenioid, the Antarctic toothfish *Dissostichus mawsoni*. The six introns are from about 100 to more than 1000 bp in length and show different patterns of variation. Three introns show only a small proportion of nucleotides and are conserved, whereas the remaining three are much more variable in sequence and contain a significant number of indels. The high level of sequence conservation in three introns suggests some selective constraints whereas the other three introns may be useful in assessing variation within this group of fish.

Characterization of leukocytes from some Antarctic and sub-Antarctic teleost species

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Cell preparations derived from head kidney, spleen, and intestine of the notothenioid species *Dissostichus eleginoides*, *Notothenia coriiceps*, and *Trematomus hansonii* have been investigated. Cells were fixed in paraformaldehyde and analyzed by flow cytometry and optical microscopy. The three species displayed typical leukocyte populations composed of lymphocytes, granulocytes, monocytes/macrophages and thrombocytes with a peculiar organ distribution and unusual flow cytometric patterns.

Unfixed cell preparations deriving from head kidney (HK), spleen, peripheral blood leukocytes (PBL), gills, and intestine of *Trematomus bernacchii* (*Trber*) were analyzed by flow cytometry and indirect immunofluorescence with an anti-sea bass immunoglobulin (Ig) that cross-reacts with all teleost species investigated. Results showed that the percentages of Ig-bearing cells in organs of *Trber* were 20.2 in HK, 11.3 in spleen, 24.8 in PBL, 7.9 in the intestine, and 18.5 in the gills. Flow cytometric patterns of cells obtained from these organs revealed a different tissue distribution of cell populations.

PBL from the erythrocyte-free icefish species *Chionodraco hamatus* (*Cham*) were cultured for 24 hours at 0-2 °C with bacterial lipopolysaccharide and lectins in the presence and absence of calf serum, and then the cells were counted and analyzed by flow cytometry. Results showed that *Cham* leukocytes spontaneously proliferated in the absence of stimulants, and added stimulants differentially affected proliferation. Flow cytometric analysis of proliferating cells revealed peculiar PBL populations affected by culture conditions.

This work represents a first characterization of cells involved in immune reactions in the species investigated.

Session 3

Invertebrate Biology

Comparison of seastar (Asteroidea) fauna across island groups of the Scotia Arc

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The islands sampled during ICEFISH 2005 span the Antarctic Convergence, an ostensible oceanographic barrier to exchange of genetic material for shelf species. We focused on seastar fauna from otter and beam trawl bycatch because seastars are ecologically important in Antarctic waters, where they are often the most abundant group of organisms, and because their taxonomy is relatively well defined. We expected that the seastar fauna from islands on the high latitude side of the convergence would show more similarity to each other than to islands on the low latitude side. An alternative model is isolation-by-distance, from which we would predict that the island furthest from others would have the most unique fauna. For shelf-depth Asteroidea of the Scotia Arc region, neither model was supported, instead, the patterns of distribution appear to be driven by a few ubiquitous species.

Infauna of isolated islands: A study on macrofauna from the ICEFISH 2004 Cruise

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Two of the resounding themes in Antarctic research have been the role of the polar front in structuring communities and understanding the circumpolar distribution of the fauna. The sub-Antarctic islands yield insight to these two topics yet little quantitative work has been done on many of the remote island ecosystems. Macro-infauna provide a community that can be sampled rapidly and accurately. Here we describe the infaunal community off of Bouvetøya Island, the most remote sub-Antarctic South Atlantic island. The fauna is similar to that of other sub-Antarctic islands, being a combination of cosmopolitan and Antarctic species. The shallow station, at 40m water depth, was characterized by motile amphipods and ephemeral polychaete species similar to those found in the heavily ice disturbed continental shallows. The rest of the stations, 200-500m, were dominated by a more stable, diverse community dominated by polychaetes. These results suggest connectivity with other islands as well as the main Antarctic continent even though it is spatially distinct. These data constitute the first semi-quantitative macrofaunal results from Bouvetøya Island.

Relationships between liparids and *Paralomis* crabs off South Georgia

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The lithodid crab *Paralomis formosa* occurs in the South Atlantic and the waters around South Georgia and is frequently caught by long-line fishing. We report the discovery of an egg mass of an unidentified species of liparid fish in the branchial chamber of the crab. Non-destructive *in situ* imaging demonstrates significantly reduced gill volume due to displacement of gills by the eggs. Partial necrosis of the most heavily affected gills also indicates at least locally insufficient flow of haemolymph through the gills. In addition to the local impact of the eggs, the increased hydrodynamic flow-through resistance due to the presence of the parasitic egg mass further reduces the availability of oxygenated seawater even to the unaffected gills. Preliminary molecular data indicate that *Paralomis formosa* is parasitized by a single species of Liparidae with close ties to the genus *Careproctus*.

Inferring the population history of Antarctic serolid isopods using molecular markers

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Until now, little about the colonization history, migration and speciation of benthic invertebrates in the Southern Ocean is known. Resolution and explanatory power of the molecular data routinely available is too weak to make inferences about these processes. Using a geographically widespread species from the *Ceratoserolis trilobitoides* species complex, we compared several methods to isolate simple sequence repeats (SSR, microsatellites) for subsequent studies requiring resolution at the population level. Radioactive screening methods of non-enriched libraries yielded several microsatellites but proved labour-intensive and the handling of radioactivity requires equipment not normally present in a standard molecular lab. Two non-radioactive methods were also used. While with the PIMA protocol using six different (di- and trinucleotide) repeat probes not a single microsatellite could be isolated, 50-60% of the colonies screened with an innovative cross-hybridization technique contained microsatellites.

Session 4

Cytogenetics, Phylogeny, and Population Biology of sub-Antarctic Notothenioids

Karyotypes of basal lineages in notothenioid fishes: the genus *Bovichtus*

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A comparative cytogenetic study was performed in the family Bovichtidae, the most basal lineage of the Antarctic fish suborder Notothenioidei. Three species of the genus *Bovichtus* with very different distributions in the Southern Ocean were studied, namely *B. diacanthus* (from Tristan da Cunha Island), *B. variegatus* (from Otago Peninsula, New Zealand) and *B. angustifrons* (from Tasmania). Chromosomes were analyzed using both conventional karyotyping and cytogenetic mapping of ribosomal genes through fluorescence *in situ* hybridization. The three species showed a very conservative karyotype composed of all telocentric chromosomes (diploid number = 48; Fundamental Number = 48). The chromosomal distribution of ribosomal genes differed from those of all other notothenioid species studied to date; rather than present on a single chromosome, the 28S and 5S rDNAs were separated on two different chromosome pairs. Separation of two classes of ribosomal genes seems to be the most widespread condition in teleosts, including the bovichtids, whereas the remaining notothenioids show a derived separation.

Molecular evolution in nototheniid fishes

Phylogeny

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Cytogenetics

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Most of the interrelationships among notothenioid genera have been solved, except within the family Nototheniidae, where unresolved nodes persist despite a number of recent molecular and morphological phylogenetic studies. By using several nuclear and mitochondrial markers, two “hot” problems have been investigated: (1) interrelationships among five nototheniid clades (group *Gobionotothen*, group *Notothenia/Paranotothenia*, group *Trematomus/Pagothenia*, group *Lepidonotothen/Patagonotothen*, “pelagic” group (*Dissostichus*, *Pleuragramma*, etc.); (2) species phylogeny within the genus *Trematomus*. Results from these molecular phylogenies, including for the first time a resolved tree for *Trematomus*, are compared with chromosomal patterns of those animals. The relative positions in chromosomes of 5S rRNA genes, 28S rRNA genes and immunoglobulin genes obtained through Fluorescent *In Situ* Hybridization technique support several interesting clades, among which is a sub-clade of *Trematomus* species.

Population genetics of the mackerel icefish (*Champscephalus gunnari*)

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The mackerel icefish (*Champscephalus gunnari* Lönnberg 1905) is widely distributed south of the Antarctic Convergence and over shelf areas surrounding sub-Antarctic islands. We examined DNA sequence variation in three mitochondrial genes and four nuclear genes in four populations from the Atlantic Ocean sector and one population from the Indian Ocean in order to evaluate population structure. The Heard Island population is genetically distinct but most closely related to South Atlantic populations. Significant heterogeneity among South Atlantic Ocean populations is observed for both mtDNA and nuclear genes, with the South Shetland Islands population distinct from Shag Rocks and South Georgia Island. We were not able to detect genetic differentiation between Shag Rocks and South Georgia Island (in contrast to previous studies), perhaps due to small sample size. The mitochondrial and nuclear markers developed here will enable a more powerful analysis of population structure.

Session 5

Physiology and Ecology of sub-Antarctic Notothenioids

Evolution of a stress response: Lessons from a cold-adapted fish

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In response to acute rises in temperature, several species of notothenioid fishes of McMurdo Sound, adapted to constantly near-freezing waters, do not up-regulate the expression of a family of stress proteins called heat shock proteins (Hsps). The mRNA from *hsp70* can be detected in the tissues of three species of field-caught Antarctic fish, suggesting that these genes, normally inducible in most taxa, are expressed constitutively in some Antarctic species. These same genes are inducible in closely related, temperate species from New Zealand. To determine whether Hsp genes are constitutively expressed in fishes from other regions of the Southern Ocean, northern blots were conducted on control and heat shocked tissue from two species collected near the Antarctic Peninsula, *Bovichtus dicanthus* and *Notothenia coriiceps*. Furthermore, a cDNA microarray developed from clones from a eurythermal gobyfish was used to probe gene expression patterns in these fish.

**Age and growth in a sub-Antarctic fish *Patagonotothen ramsayi* (Regan, 1913)
(Nototheniidae)**

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The Falklands' rockcod *Patagonotothen ramsayi* were aged successfully using whole and sectioned otoliths. Marginal increment analysis illustrated that one opaque and one translucent zone was laid down each year. Counting daily rings in juvenile fish and back calculating to their assumed hatch dates validated the first annulus. Readings taken from scales and otoliths showed good agreement with no significant difference between them ($P>0.05$). Inter- and intra-reader comparisons also showed good agreement. The maximum observed age was found to be 14 years and the calculated von Bertalanffy growth model $L_T = 33.77(1 - e^{-0.25\text{year}(t+1.07)})$ showed that *P. ramsayi* is a relatively slow growing fish that attains 5~6 cm L_T in its first year and after which grows approximately 3 cm per year until 4 years. Males seemed to have a slightly lower growth rate but attained a greater maximum size.

Do neuromuscular currents of sub-Antarctic fish show temperature adaptation?

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Miniature end plate currents (MEPCs) are brief sub-threshold electrical events recorded at the neuromuscular junction, triggered by spontaneous release of quanta of neuromuscular transmitter (acetylcholine in fish and other vertebrates). The time course of MEPCs is prolonged at low temperatures, but several species of high-Antarctic nototheniid fishes show nearly complete temperature compensation.

The work reported here was done to determine the level of temperature compensation in MEPCs of sub-Antarctic nototheniid fishes. MEPCs were recorded over a temperature range of 0° to 12°C from the inferior oblique extraocular muscle dissected from 7 species collected during the 2004 cruise of the *Nathaniel B. Palmer*. The best recordings were from 4 specimens of *Notothenia rossii*; results from 1 specimen are reported here.

A total of 462 MEPCs were extracted from the original data files for this one specimen and analyzed to determine the exponential decay rate (and time constant, τ) of each MEPC. An Arrhenius plot and regression were used to describe the effect of changing temperature on the decay phase of all the MEPCs: $-\ln\tau = 27.783 - 6063/K$, yielding an Arrhenius temperature coefficient (μ or apparent E_a) of $-50.4 (\pm 3.15 (2 \text{ S.E.})) \text{ kJ mol}^{-1} \text{ deg}^{-1}$.

At temperatures near 0 °C, the *N. rossii* MEPCS had τ ranging from about 2000 to 4000 μsec , similar to values extrapolated for temperate teleosts, and longer than MEPCs from high Antarctic nototheniids. Based on these results, it seems that *N. rossii* is not compensated for low temperature to the same degree as the high Antarctic species.

Session 6

Other Topics

Aspects of gonadal morphology in the South Georgian plunderfish *Artedidraco mirus* (Perciformes: Artedidraconidae)

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Research on the reproduction of Antarctic fishes has been mainly focused on macroscopic evaluation of the maturity, fecundity, egg size and first maturity length while microscopic studies have been limited to only a few species. In particular no data are available on plunderfishes of the family Artedidraconidae. In order to present a preliminary description of gonads in the South Georgian plunderfish *Artedidraco mirus*, morphological observations on ovary and testis were carried out, using both macroscopic and histological approaches. All fishes were collected during the ICEFISH cruise off the island of South Georgia in June, 2004. The ovaries were typically hollow in structure; from the ovarian wall, connective tissue projected into the ovarian cavity, forming ovigerous folds in which oocytes were located. The testes appeared of the lobular-type; the lobules were limited by connective tissue. The occurrence of specimens showing post-spawning features suggests that the spawning process was concluding when the fishes were caught.

Swimming kinematics of notothenioid fishes of the sub-Antarctic region

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Notothenioid fish species are derived from a common benthic ancestor and have undergone an extensive adaptive radiation including re-invasion of pelagic habitats. Interestingly, despite the extent of this adaptive radiation, notothenioid fishes largely swim with their pectoral fins in a mode called labriform swimming. This study documents the swimming behavior of a range of notothenioid species. The spontaneous swimming activity of 48 individual fish representing 14 species of notothenioids was observed during the 2004 ICEFISH expedition aboard the *Nathaniel B. Palmer* using a custom-built swimming observation tank. Some cases were recorded using two synchronized cameras to capture detailed 3D movement and the general kinematics of all species was captured using an overhead video camera. Analysis of this data is currently in progress to extract the relevant kinematic parameters. The analysis for six individual *Notothenia rossii* has been completed. Upon completion of the full analysis of all recorded species it will be possible to look for phylogenetic and ecological correlations and to compare these data to what is known of temperate labriform swimmers in similar ecological niches. It will also be possible to make comparisons with models of labriform swimming kinematics that make predictions about kinematics adapted for high maneuverability or sustained swimming and energy conservation.

Wintertime occurrence of picophytoplankton in the Southern Ocean

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Data on the distribution of picophytoplankton from the Southern Ocean are still relatively scant and primarily collected during the austral spring and summer. During the ICEFISH expedition conducted during austral winter, 2004, we examined the abundance of picophytoplankton in surface waters along a 366 km E/W transect at ~55°S latitude between the South Sandwich Islands and Bouvetøya Island and along a 2780 km N/S transect from Bouvetøya Island to Tristan da Cunha Island. In waters less than 1° C, we repeatedly observed two distinct populations of picoeucaryotes that were readily resolved by flow cytometry but no Phycoerythrin (PE)-containing cells or *Prochlorococcus*. PE-containing cells were, however, observed along our N/S transect once water temperatures exceeded 1.3° C, placing their southernmost limit of distribution close to the Antarctic Polar Front. *Prochlorococcus*, which occurred in waters >10° C and which were relatively common in surface waters around Tristan da Cunha, were not observed south of the Sub-Antarctic front. Based on this data set, it appears that both PE-containing picophytoplankton and picoeucaryotes are present in sub-Antarctic waters year-round.

Concluding Forum

The International Polar Year: ICEFISH 2007 and Beyond

Cinzia Verde, Guido di Prisco, and H. William Detrich