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Discover the cold : are Antarctic fish capable of coping with anthropogenic chemicals?

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4. DISCOVER THE COLD: ARE ANTARCTIC FISH CAPABLE OF COPING WITH ANTHROPOGENIC CHEMICALS?

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Objectives

The physiology of Antarctic notothenioid fish departs in many aspects from the physiology of temperate fish species. The adaptation to their extreme environment with stably cold, oxygen-rich waters comprises both gains (e.g., anti-freeze protection) and losses (e.g., inability to mount a heat shock response) of physiological capabilities. A more recent stress factor Antarctic fishes are confronted with is environmental contamination with anthropogenic chemicals. Antarctica receives immissions of persistent halogenated aromatic hydrocarbons (HAHs) by long-range atmospheric transport and global distillation of pollutants in high latitudes. Current trends point to increasing chemical contamination of the Antarctic environment due to the appearance of emerging contaminants, as well as consequences of global warming such as altered atmospheric transport and precipitation and melting of the ice cover. It is known that Antarctic fish accumulate HAHs, however, no knowledge at all is available on the capability of Antarctic fishes to cope with these chemicals, and how vulnerable they are to the toxic activity of these chemicals. The investigation started during the *Polarstern* cruise ANT-XVIII/4 is the first systematic study to relate chemical body burdens to molecular capabilities and possible adverse outcomes in Antarctic fish species. To this end, the project will study

(i) accumulation of dioxin-like, coplanar HAHs in Antarctic fish species in relation to trophic level. We expect that HAH body burdens in Antarctic fishes are more diverse and higher than currently known.

(ii) biotransformation capabilities of Antarctic fish species. We hypothesize that Antarctic fish have limited metabolic capabilities to reduce HAH bioaccumulation.

(iii) expression and functional properties of the arylhydrocarbon (AhR) receptor, as this receptor mediates HAH toxicity and determines HAH sensitivity. We hypothesize that genetic diversity and expression of AhR in Antarctic fish species is comparable to what is known from temperate fish species.

(iv) changes in reproductive parameters of Antarctic fish as possible adverse outcome of the biological action of dioxin-like HAHs. According to the literature from temperate fish species, there exists a robust link between accumulation of dioxin-like HAHs and reproductive disruption. We hypothesize that co-transfer of bioaccumulated HAHs together with body lipids into maturing ovaries and eggs poses a risk to the reproduction of Antarctic fish.

Work at sea

The sampling was conducted in the course of cruise ANT-XXVIII/4, which was a fishery-focused survey using a scientifically sound sampling plan (randomized survey). Fish was sampled by bottom trawl. To minimize the handling stress, only fish netted alive and without macroscopically visible damage was used for our sampling. Fish was anesthetized and dissected immediately to avoid necrotic tissue alterations.

To verify or reject our hypotheses, we applied two sampling designs, design A which examines relationships between HAH accumulation and biological effect indices, and design B which aims to reveal basic molecular and physiological capabilities of the Antarctic fish to cope with dioxin-like HAHs.

Sampling design (A) was applied to find associations between body burdens of compounds with dioxin like- activity, expression of exposure marker CYP1A (early response) and reproductive endpoints (apical response), in relation to physiological and ecological traits (age, sex, lipid contents, position in food web). Two fish species were selected for design A, the mackerel icefish, *Champsocephalus gunnari*, and the Scotia Sea icefish (or blackfin icefish), *Chaenocephalus aceratus*. *C. gunnari* is one of the most important krill feeders of the Antarctic fish community. *C. aceratus* is a rather sedentary form, and a fish feeder when adult. At the time of sampling for our project, it was in final maturation stage, shortly before spawning so that the conditions are ideal to check for gonadal effects as well as for transfer of HAHs into the eggs.

Sampling design (B) aims to provide a comprehensive assessment of metabolic capabilities and AhR properties of Antarctic fish species, including red- and white-blooded notothenioids. We sampled four species of the Nototheniidae (red-blooded) with different feeding habits - the marbled rockcod *Notothenia rossii*, feeding mainly on krill, *Notothenia coriiceps*, a benthic feeder, the Antarctic toothfish, *Dissostichus mawsoni* which is a fish feeder when adult, and the benthos feeding humped rockcod, *Gobionotothen gibberifrons*. For comparison with species of a different systematic affiliation but also an endemic notothenioid family, we sampled three species of *Channichthyidae* (white-blooded icefish) mentioned above – the mackerel icefish, *C. gunnari*, and the Scotia Sea icefish *C. aceratus*, as well as *Chaenodraco wilsoni*. All these species were object of fisheries surveys since 1975/6 in the Scotia Arc region (CCAMLR). High quality long-term data are available on their biology and ecology, such as geographic distribution, bathymetric range, and life cycle parameters, such as growth, feeding habits, age at maturity, gonadosomatic indices, spawning season, etc. (for review: Kock 2005 a,b).

In addition, we took samples of the following species deviating from the aforementioned species in several aspects of their physiology and ecology. The Antarctic eelpout *Pachycara brachycephalum* is a confamilial species to our native species *Zoarces viviparus* of the North Sea. This Antarctic species is known for cold compensation in enzyme activities and it is suggested that its acclimation pathways are different from those in temperate species and possibly also from the cold-stenothermic notothenioids (Lucassen et al. 2003). Antarctic silverfish *Pleuragramma antarcticum* is a pelagic plankton feeding notothenioid key species in the Antarctic food web. *P. antarcticum* is a member of the Pleurogramminae, the most phylogeneticaly derived subfamily of the notothenioids (Gon & Heemstra 1990). Currently, it was detected that the larvae have insufficient antifreeze capabilities, which are necessary to survive the temperature in their habitat, namely

the lower layer of the platelet ice (Cziko et al. 2006). *Gymnoscopelus nicholsi* belongs to the lanternfishes (Myctophidae). It feeds mainly on euphausiids and is interesting also because it stores lipid extensively subcutaneously and serves as prey for *D. mawsoni* (Gon & Heemstra 1990).

From all species sampled under design B, we collected liver samples for RNA and DNA extraction, for preparation of S9 extracts (to measure biotransformation rates of HAHs) and for biotransformation enzyme analyses.

The selected sampling areas are identical for both sampling designs. They include the Scotia Sea with Elephant Island and the South Shetland Islands as well as areas in Bransfield Strait (CCAMLR Subarea 48.1). Due to the vicinity to South America, this region is supposed to be stronger exposed to anthropogenic chemicals than other areas of Antarctica. For example, atmospheric transport of PCBs from Southern America to the Antarctic Peninsula has been reported (Montone et al. 2003). A further argument supporting the selection of these sampling areas is that they have been studied on xenobiotic contamination in former years (Weber & Goerke 2003, Corsolini et al. 2005).

Preliminary (expected) results

On board of *Polarstern*, we acquired the following data: species, length, weight, sex, weigth of liver, weight of ovary, stage of maturation. For further analyses in the home laboratory, we sampled from fish, according to our sampling designs A and B, respectively: muscle (for chemical analysis), liver (for RT-PCR/cloning/sequencing/heterologous expression, chemical analysis, histology) and gonads (for chemical analysis and histology). For statistical analysis of the associations between HAH accumulation, effect indices, species traits and environmental parameters, multivariate statistics will be employed.

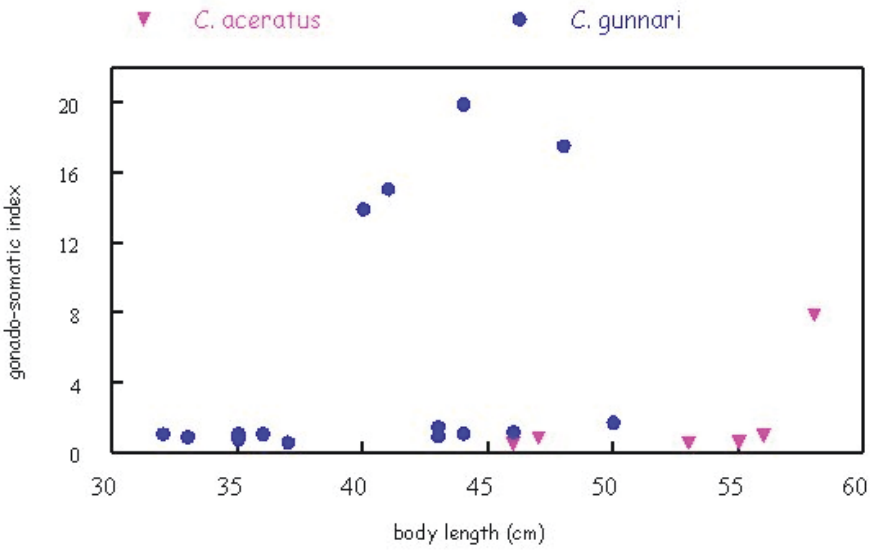

Fig. 4.1: Relationship between gonado-somatic index and body length of C. aceratus *and* C. gunnari.

In total, we sampled 55 *C. gunnari* and 49 *C. aceratus* under sampling design A. Of these, 30 specimen (*C. gunnari*) and 41 specimen (*C. aceratus*), respectively, were sampled off Elephant Island, the other specimen were sampled off South Shetland Islands. For sampling design B, we acquired tissue samples and length-weight data from *Pachycara brachycephalum* (n=3); *Gymnoscopelus nicholsi* (n=10), *Lepidonotothen squamifrons* (n=4), *Chaenodraco wilsoni* (n=12), *Notothenia rossii* (n=15), *Dissostichus mawsoni* (n=7), *Champsocephalus gunnari* (n=15), *Gobionotothen gibberifrons* (n=13), *Notothenia coriiceps* (n=10), *Chaenocephalus aceratus* (n=13), *Pleuragramma antarcticum* (n=45).

Preliminary analysis of the gonado-somatic indices among the fishes of the effect study (sampling design A) revealed that female *C. gunnari* of the same age class are at differing stages of ovarian maturity: while one fraction of the females possessed immature ovaries, the other fraction displayed mature ovaries (Fig. 4.1). Ovarian maturation was paralleled by an increase of liver size, indicative of the role of the liver to provide lipids and lipoprotein for the developing eggs (Fig. 4.2). As ovarian maturation is associated with a major mobilisation and re-distribution of body lipids, and as lipophilic contaminants such as HAHs co-segregate with body lipids, we expect that the two female groups show distinct differences in their levels of HAH levels in the ovaries. This provides an excellent opportunity to study HAH dynamics in relation to fish physiological status, and – as the two female groups belong to the same age class – independent of age-related differences of HAH bioaccumulation.

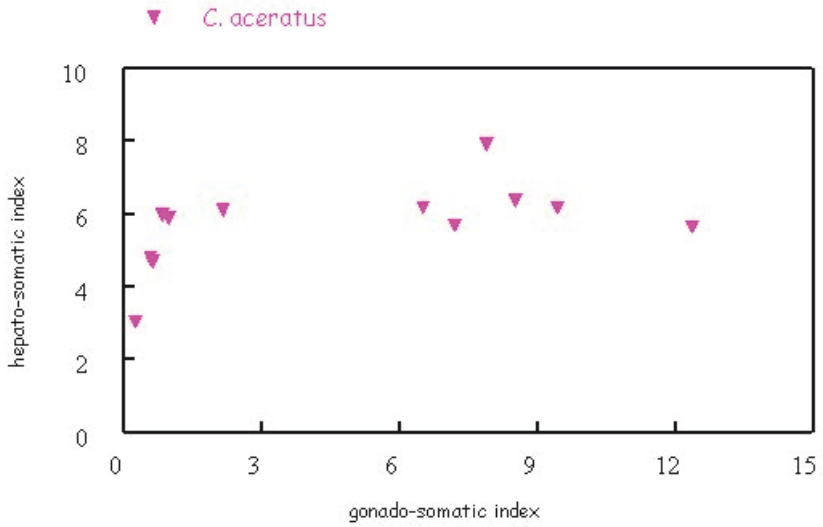

Fig. 4.2: Relationship between hepato-somatic index and gonado-somatic index of C. aceratus.

Data management

All data will be made available by publication in scientific journals. Chemical analyses of tissue samples will be done at EMPA, Dübendorf, molecular and histological examinations will be done at MGU Basel and Centre for Fish and Wildlife Health

Bern. Histological tissue samples will be available upon request from Centre for Fish and Wildlife Health, University Bern, and MGU, University of Basel.

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