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# Abstract book

# Posters

## **CHARACTERISATION OF DIGESTIVE AND ABSORPTIVE FUNCTIONS OF THE ATLANTIC WOLFFISH INTESTINE**

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### **Abstract:**

Atlantic wolffish is a marine cold-water species with a benthic lifestyle that can eat shelled prey, which can be hard to digest. Little is known about their digestive physiology as well as their basic intestinal functions. This study aimed to characterize digestive enzyme activity and ion- and nutrient transport in 3 consecutive intestinal regions. Trypsin-, chymotrypsin- and lipase activity was measured 24 h post feeding. Methionine transport and the electrical parameters TER, TEP and SCC across the intestine was assessed in vitro using Ussing chamber technique. Cellular localization of important proteins for ion- and nutrient transport (NKCC/NCC and NKA) was visualized using immunohistochemistry.

Trypsin, lipase and chymotrypsin was active in all intestinal regions. In the mid/posterior regions the methionine uptake increased ( $p < 0.01$ ). In the same regions, TER decreased ( $p < 0.0001$ ) and TEP was serosa negative which indicates net uptake of anions. NKA and NKCC/NCC was present in the epithelium of all intestinal regions with higher intensity in the anterior region.

Wolffish show digestive enzyme activity along all the intestinal segments studied. A low TER concurrent with high methionine uptake is characteristic for a nutrient transporting epithelium, suggesting that nutrient uptake mainly takes place in the posterior regions. This pattern is opposite to other carnivorous fish who mainly absorb nutrients in the anterior regions. Higher enzymatic activity and nutrient uptake in the posterior regions of the intestine could be an attribute to slow digestion of their shelly diet. The high abundance of NKCC/NCC and NKA in the anterior intestine may indicate predominant ion-coupled fluid uptake in this region, but this needs further investigation.

**ROLE OF MTOR SIGNALLING PATHWAY IN THE CONTROL OF FEED INTAKE BY VALINE IN RAINBOW TROUT.**

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**Abstract:**

In previous studies in rainbow trout, we observed that the branched-chain amino acid (BCAA) valine increased feed intake when centrally administered. Apparently, this effect is caused through the activation of different amino acid sensing systems in brain. These systems are based on: 1) metabolism of BCAA, 2) metabolism of glutamine, 3) taste receptors signalling, 4) GCN2 kinase, and 5) mTOR signalling. However, when the same amino acid is peripherally administered feed intake decreases, with activation of the same sensing systems. Therefore, the aim of this study is to deepen into valine sensing in brain to elucidate how this amino acid is regulating feed intake. We focussed on mTOR signalling, since this protein is reported as an integrator of regulatory signals for feed intake regulation. Thus, we carried out an intracerebroventricular injection, administering saline solution and DMSO alone (control), or containing valine, the mTOR antagonist rapamycin, the mTOR agonist MHY1485, valine + rapamycin, or valine + MHY1485. Feed intake was assessed and then, we repeated the experiment to take samples of brain areas in order to evaluate the mRNA and protein abundance of parameters related to amino acid sensing systems, as well as neuropeptides and transcription factors involved in feed intake control. The results discussed in the context of metabolic regulation of feed intake, also helped to establish the role of mTOR in amino acid sensing.

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ABSTRACT N° ICBF22-397

**TRANSCRIPTOMIC CHANGES ASSOCIATED WITH MATERNAL CARE IN THE BRAIN OF MOUTHBROODING CICHLID *ASTATOTILAPIA BURTONI* REFLECT ADAPTATION TO SELF-INDUCED METABOLIC AND HYPOXIC STRESS**

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**Abstract:**

Maternal care in the cichlid fish *Astatotilapia burtoni* entails protecting eggs and developing fry in a specialized buccal sac. During this mouthbrooding behavior, which can last 2-3 weeks, mothers undergo voluntary fasting accompanied by loss of body mass and major metabolic changes. Following release of fry, females resume normal feeding behavior and quickly recover body mass as they become reproductively active once again. We have sequenced whole brain transcriptomes from females at four time-points throughout their reproductive cycle; 2 days after the start of mouthbrooding, 14 days after the start of mouthbrooding, 2 days after the release of fry, and 14 days after the release of fry. Differential expression analysis and clustering of expression profiles revealed neuropeptides and hormones, including the strong candidate gene *neurotensin*, that suggest molecular mechanisms underlying parental behaviors may be shared across vertebrates. In addition, oxygen transport pathways were found to be dramatically downregulated throughout mouthbrooding while neuroprotective pathways were upregulated, likely to mitigate negative consequences of metabolic depression brought about by fasting. Our results offer new insights into the evolution of parental behavior as well as revealing candidate genes that would be of interest for the study of hypoxic ischemia and eating disorders.

ABSTRACT N° ICBF22-507

**EFFECT OF MACKEREL IN TOMATO SAUCE, MARINATED HERRING AND ANCHOVY TRIMMINGS ON GROWTH, PHYSIOLOGY AND INTESTINAL HEALTH OF RAINBOW TROUT (ONCORHYNCHUS MYKISS)**

Niklas Warwas<sup>1, 2</sup>, Montjouridès Marie<sup>2</sup>, Jonathan Roques<sup>\* 1, 2</sup>, Markus Langeland<sup>3</sup>, Henrik Sundh<sup>1, 2</sup>, Elisabeth Jönsson Bergman<sup>1, 2</sup>, Kristina Sundell<sup>1, 2</sup>

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**Abstract:**

About 70% of fish production undergoes processing before reaching the consumer market, creating significant amounts of side-streams. In addition, about 35% of the output from fisheries and aquaculture becomes food waste. Both food waste and processing side-streams may be of high nutritional value but remain often largely underused. Therefore, our aim is to screen three waste products, from different stages of the refining chain for their nutrient composition and suitability as feed ingredient in diets for rainbow trout, *Oncorhynchus mykiss*.

Three experimental diets were formulated to contain 50% of each test ingredient as the sole source of fishmeal, namely anchovy trimmings (without processing), marinated herring and mackerel in tomato sauce (both refined). The control diet consisted of conventional ingredients including commercial fishmeal. All diets had a protein content of 43-44%. The feeding trial took place in a cold-water, land-based freshwater recirculating aquaculture system over a period of 12 weeks.

Most fish more than doubled their weight at the end of the feeding trial. The highest growth and feeding rates were observed in the treatment group fed with unprocessed anchovy trimmings. Thus, the refining processes of the herring and mackerel may introduce non-palatable and/or anti-nutritional factors leading to reduced growth and impaired barrier function of the proximal intestine. In this study the anchovy trimmings were the most promising side stream for the use as a feed ingredient in its raw form while the mackerel in tomato sauce and marinated herring may require additional treatment before they can be included in diets at high levels.

## **DIVERSE ROLES OF DNA REPAIR GENES AND PROTEINS IN FISH EMBRYO DEVELOPMENT**

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### **Abstract:**

DNA damage caused by exogenous and endogenous factors is a common challenge for developing fish embryos. However, organisms have developed DNA repair pathways that cope with the damage and minimize the persistent mutation risk. The DNA repair pathways consist of proteins that identify and correct damaged DNA. In proliferating cell cultures, such as embryonic stem cells, DNA repair proteins possess several functions. Apart from DNA repair, some of those proteins can be activated in a transcription-dependent but DNA damage-independent manner. A multifunctional role of DNA repair proteins in organogenesis has been claimed in a handful of studies. Many repair proteins expressed in embryos are localized in a particular tissue, play a specific role in organogenesis, and are indispensable for normal embryonic development. Also, few studies showed changes in the DNA repair genes when embryos were exposed to genotoxicants (chemical agents that damage DNA). Thus, it is possible to suggest that there is a link between DNA damage response and phenotype formation in embryos exposed to genotoxicants during early development.

In this study we aim to describe stage-specific changes in DNA repair pathways in fish embryos. Further, the effect of genotoxicants on the repair proteins will be discussed. This study provides an overview on the multifunctional role of DNA repair genes and proteins in fish embryo development.

## **EFFECTS OF COPPER EXPOSURE AND HEAT WAVES ON ZEBRAFISH (DANIO RERIO) EMBRYOS**

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### **Abstract:**

Climate change and pollution driven by urbanisation, industrialization and agricultural intensification are two of the most important challenges for wild fish populations. The threat from these stressors is high for developing fish as they are especially vulnerable to sudden environmental changes and unable to escape the harsh conditions.

One of the most detrimental toxins for aquatic organisms are metals, due to their ability to bioaccumulate. Among these, copper has been shown to induce various negative effects, such as gill damage or reduction of reproductive success. Moreover, extreme weather conditions, like heat waves, might exacerbate the detrimental effect of the metals, therefore representing a threat for fish survival, especially when having low dispersal ability, i.e. during their developmental stage.

Although many studies have focused on the effects of temperature on fish development, the thermal capacity of larvae is still largely unstudied. Moreover, most of the studies have focused on either temperature or copper toxicity as single stressors, leaving a lack of knowledge on the combined effect of these two widespread environmental stressors.

In order to uncover the developmental effects of a combination of two stressors, in the current study we exposed zebrafish embryos to different copper concentrations and two different thermal regimes. The exposures simulated a copper exposure in a normal and heat wave scenario. The embryos were checked for morphological features during the development and, upon hatching, their thermal capacity was assessed performing a Critical thermal maximum (CT<sub>max</sub>) challenge.

**EFFECTS OF LOW FISH MEAL DIETS ON GROWTH PERFORMANCE AND INTERMEDIATE METABOLISM OF GILTHEAD SEABREAM (SPARUS AURATA) AND EUROPEAN SEABASS (DICENTRARCHUS LABRAX)**

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**Abstract:**

The growing public interest in environmentally friendly food is leading aquaculture towards a more sustainable approach. Organic aquaculture in terms of sustainability has a pivotal role in global ecology and economy and the feeds containing ingredients with low FIFO (Fish In - Fish Out) ratios are increasingly gaining research interest. Therefore, the aim of this study was to examine the activity of enzymes of intermediate metabolism in the liver of gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*), fed diets with low FIFO mix of ingredients for organic aquaculture. European seabass fed 17% and 20% low FIFO mix (bacterial protein and yeast meal) displayed no statistically significant differences regarding growth rate, compared to the control diet. Similarly, activities of 3-hydroxyacyl CoA dehydrogenase (HOAD) and lactate dehydrogenase (L-LDH) in seabass exhibited no statistically significant differences compared to the control. However, an increase in the enzymatic activity of citrate synthase (CS) in fish fed 20% low FIFO mix, compared to the control diet, was observed. In addition, 25% and 30% low FIFO mix diets statistically improved gilthead seabream growth performance compared to the control group. However, the enzymatic activities of HOAD, L-LDH and CS showed a significant decrease in seabream fed 25% and 30% low FIFO mix, indicating a decreased metabolic capacity, probably due to alternative metabolic resources being activated in this species.

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## **TEMPERATURE PREFERENCE OF JUVENILE ANTARCTIC FISHES**

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### **Abstract:**

Antarctic fish are able to exist at temperatures below the freezing point of seawater by physiologically limiting the formation of ice crystals within cellular tissues using antifreeze glycoproteins. Limited environmental warming may therefore alleviate the energetic costs associated with persisting at sub-zero temperatures, such as ice-suppression and constrained metabolic rates and biochemical processes. Therefore, we hypothesized that Antarctic fish may exhibit a temperature preference towards water temperatures above freezing (0-2°C), rather than those under which they evolved (-2°C). During the 2018 and 2019 field seasons, we examined the temperature preference of two species of benthic rockcods (*Trematomus bernacchii* and *T. pennellii*) from McMurdo Sound. Following collection, fish were placed individually into a 100cm annular temperature preference ring and exposed to a stable thermal gradient ranging from -1.5°C to 6°C. Fish were allowed 2 hours to explore the gradient and their position was monitored via an overhead camera. Following an initial 2-hour experiment, each fish was immediately transferred to an identical arena and the experiment was repeated. Overall, these rockcod species show variability in thermal preference ranging from sub-zero temperatures to 3°C. However, fish tended to choose warmer temperatures in the second temperature preference trial indicating that short exposures to warmer water may increase the temperature preference. These results may indicate that Antarctic fish are prioritizing warmer temperatures, or that after millions of years of stable, sub-freezing temperatures, Antarctic fishes have a reduced capacity to resolve environmental temperatures. These experiments represent the first temperature preference experiments with Antarctic fishes and demonstrate a field-deployable temperature preference chamber for remote field stations.

## **AN EXAMINATION OF THE BASELINE METABOLIC STATUS AMONG FOUR JUVENILE ANTARCTIC FISHES**

Amanda Frazier\*<sup>1</sup>, Andrew Naslund<sup>1</sup>, Milica Mandic<sup>1</sup>, Kenneth Zillig<sup>1</sup>, Anne Todgham<sup>1</sup>

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### **Abstract:**

Polar oceans are experiencing global climate change at some of the fastest rates on Earth and are predicted to undergo significant changes by 2100, including ocean warming, acidification, and sea-ice loss. Unlike more temperate species that may move poleward to find more suitable habitat, polar species do not have migration as an option and must rely on existing physiological plasticity to cope with rapid change. Antarctic species may also be poorly prepared for rapid changes, as they have evolved in extremely stable and isolated environments. Given the critical role that fishes play in linking the upper and lower levels of the food web in Antarctic ecosystems, understanding the vulnerability of fishes is important to predicting larger effects on Antarctic ecosystems. Furthermore, while young developing animals tend to be the most vulnerable to anthropogenic stressors, relatively little is known regarding the ecological roles and baseline physiological differences amongst young Antarctic fish compared to their adult counterparts. In this study we compared the resting metabolic rate, a measure of baseline energy requirements of an organism, of four juvenile Antarctic fish species: *Trematomus bernacchii*, *T. pennellii*, *T. nicolai*, and *Pagothenia borchgrevinki*. We also measured behavioral indices amongst species using the novel object and novel tank tests. By examining baseline metabolic status and behavioral indices across species, whilst taking their ecological context into consideration, we can link important physiological metrics into wider ecological contexts and better predict species performance under future change.

## **BRANCHIAL OSMOREGULATORY RESPONSE OF NOTOTHENIA ROSSII UPON TEMPERATURE AND SALINITY CHANGES**

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### **Abstract:**

Antarctic fish are strictly marine and have evolved in stable thermohaline conditions. Recent climate changes have contributed to rises in water temperature and forecast models indicate the rate of such changes will increase in coastal regions of maritime Antarctica, leading to further ice melting and freshening of shallow waters in enclosed areas.

*Notothenia rossii* were collected in shore waters from King George Island, and acclimated from natural temperatures (0-2°C) to 4-8°C and from 32‰ to 20 and/or 10‰ by addition of freshwater to recirculating tanks, over a period of up to 10 days. Blood samples were used for osmolality and ion-contents, and gills were preserved for determination of NaK-ATPase (NKA) activity, gene expression and for histology and fluorescence immunohistochemistry (FIHC).

Reduced salinity resulted in lower osmolality and decreased NKA activity showing limited hyperosmoregulatory ability. This was accompanied by modifications in the expression of genes coding for ion-transporters (NKA, NKCC, CFTR, NHE), water channels (Aquaporins) and tight junction membrane barrier proteins (Claudins). FIHC shows these fish present a typical distribution of chloride cells in the gill filament, with abundant levels of NKA and NKCC in sea- and brackish water, but do not appear to efficiently upregulate NHE in brackish water. Additionally, high temperature further reduced osmolality, mainly due to reduced sodium and chloride, increasing the osmotic gradient between extracellular fluid and seawater and resulting in up-regulated branchial NKA activity, thus contributing to the energetic demands.

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## **WATER BALANCE IN ANTARCTIC FISH: INTESTINAL AND RENAL RESPONSES TO SALINITY OR TEMPERATURE IN NOTOTHENIA ROSSII**

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### **Abstract:**

Antarctic notothenioids are aglomerular, a feature likely inherited from an ancestral over 25Myrs ago. The lack of a renal filtering system is advantageous in marine fish, which need to retain water, and thus water and solutes are excreted via selective, well controlled balance between tubular secretion and absorption. However, in dilute environments this may be detrimental as the obligatory excretion of water will incur in energetic loss. We have addressed the issue looking at drinking, renal activity and urinary production to evaluate the species ability to cope with environmental changes.

Fish were exposed to decreased salinity or increased temperature (HT) for 5 days and blood plasma, urine and tissue samples collected. Osmolality and electrolyte concentration were measured in fluids and Na<sup>+</sup>/K<sup>+</sup>-ATPase(NKA) activity determined in kidney, intestine and urinary bladder. Drinking rates were evaluated using the phenol-red method. Localization of electrolyte transmembrane transporters was performed by immunohistochemistry.

Altered conditions decreased osmolality and increased the urine produced. Urine to plasma ratios and urine composition were greatly altered. Na was the main cation in seawater (SW) and Mg in diluted seawater (DSW). Intestinal and renal NKA activity were increased and strong NKA signal appeared in basolateral side and Mg-transporter in lumen of DSW proximal tubule. HT increased drinking and renal NKA activity. Both conditions increased tight junction proteins and Mg-transporter, and decrease water channels in intestine. On-going analysis are aimed to determine whether the bladder epithelium has a role in urine formation.

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**MOLECULAR AND NEUROAMINERGIC STRESS-RELATED RESPONSES IN THE SUB-ANTARCTIC FISH ELEGINOPS MACLOVINUS EXPOSED TO DIFFERENT THERMAL REGIMES**

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**Abstract:**

*Eleginops maclovinus* is a sub-Antarctic notothenioid that inhabits in several latitudes along both coasts of South America. Contrary to their Antarctic relatives, which evolved in a stenothermal environment, these fish are exposed to a wide range of temperatures. The aim of this study was to evaluate the plasticity of the expression of Heat shock protein and other stress-related genes involved in physiological mechanisms of adaptation and the response of brain neuroamines to acute and gradual changes in temperature.

The specimens were previously acclimatized at 17°C, then 6 experimental groups were used, each of which was challenged with a temperature shock at 10°C or 17°C or 24°C, and sacrificed at 1 or 4 hours, or 8 days after transfer. RNA extraction and cDNA synthesis were performed and gene expression analysed by QPCR. Genes analyzed were hsp60, hsp70, hsp90, Hif1a and cirbp and expression normalized using 18S ribosomal RNA. Monoamines were analyzed by high performance liquid chromatography with electrochemical detection.

HSPs were in general up-regulated by increasing temperatures in liver and headkidney while cirbp appear to be reduced by handling stress and air exposure. There were no evident changes in hif1a expression. Epinephrine and dopamine showed higher values in brains of fish at high temperature, both in those acutely or gradually transferred. Results will be dissected and discussed in the frame of a larger context that includes the plasticity of the thermal response in Antarctic and sub-Antarctic fish.

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## **OSMOTIC RESPONSE OF ANTARCTIC NOTOTHENIROID FISH HARPAGIFER ANTARCTICUS INJECTED WITH CORTISOL AND PROLACTIN**

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### **Abstract:**

Global warming has caused changes in the Antarctic environment, such as an increase in the melting of glaciers which results in a decrease of seawater salinity, or "freshening". The objective of this study was to assess whether endocrine factors may play a role in the response of *Harpagifer antarcticus*, which inhabits very shallow waters in Antarctic Peninsula to adverse conditions of increased temperature and decreased salinity.

For this, fish were acclimated in tanks at 2°C and 8°C and injected intraperitoneally with 150 µL saline (control), cortisol (15ug/g) or prolactin (15ug/g), and transferred to 33PSU (SW) and 10PSU (LSW), a challenge extended to 48hrs post-injection. Plasma levels of cortisol and osmolality were determined and the Na<sup>+</sup>/K<sup>+</sup>-ATPase H<sup>+</sup>-ATPase activities evaluated in gills and posterior kidney. Expression of membrane transporters was evaluated in osmoregulatory tissues by QPCR.

Results showed osmolality was elevated at both groups at 8°C, SW or LSW. Fish subjected to changes from 2°C SW show increased cortisol with those at 8°C LSW showing highest levels and this group also showed the highest mortality. Gill NKA activity did not show significant differences at 8°C or LSW when compared to respective control groups but differences were observed in renal NKA activity due to temperature or salinity. Further analysis is ongoing to evaluate the role of endocrine regulation.

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**RESULTS OF LONG-TERM STUDY OF THE BURBOT (*LOTA LOTA L.*, 1758)  
REPRODUCTION: THE SPAWNERS MIGRATION, THE LARVAE DOWNSTREAM  
MIGRATION AND THEIR SURVIVAL DURING THE TRANSITION TO  
EXOGENOUS FEEDING IN THE FLOODPLAIN SYSTEM**

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**Abstract:**

The data of long-term (1996-2021) study of the burbot reproduction in spawning tributaries of the lower reaches of the Ob River (Western Siberia) are summarized.

The extension of spawners feeding and spawning migrations in the Ob River basin is up to 1000 or more kilometers. If conditions for summer feeding were favorable (high level of the flooding, low water temperature), the hepatosomatic index (HSI) of spawners has become high, the spawning migration has started later and burbot has used lower spawning grounds. If feeding conditions were unfavorable (low HSI), spawners have started the upward spawning migration earlier and have used the upper spawning grounds. Due the long migration and low HSI, spawners (mostly females) might miss the spawning season.

The larvae migration from the places of the eggs incubation could take several days determining the behavior of larvae in the stream. At the age of up to 3 days, when the swim bladder has not yet functioning, the presence of rheoreaction and negative geotaxis have contributed to the maintenance of buoyancy. To entering the turbulence zone and to start the downstream migration the larvae had to use the muscular efforts for moving against the vector of gravity. The average number of downstream larvae was about 1.5 and 3 billion, respectively, in the Voikar River (a tributary of the lower reaches of the Ob River) and in the lower reaches of the Ob in total.

The number of feeding larvae about 11 days old in the floodplain system of the Ob River during studied years has varied from 0.13 to 1.32% of the downstream larvae number. The average number of the feeding larvae in the Voikar River has been 12.4 millions. The survival of larvae has determined by the availability of starter food - phytoplankton, which concentration has depended from the combination of water temperature and illumination at the feeding grounds.

**VARIATION IN REPRODUCTIVE TIMING BETWEEN LACUSTRINE AND RIVERINE ENVIRONMENTS IN THE GREAT LAKES BURBOT**

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**Abstract:**

Burbot (*Lota lota*) are a cryophilic freshwater fish native to many cold-water systems in the Northern Hemisphere. Burbot are endangered or extirpated in some regions of their native range due to habitat changes, climate change or over exploitation. A purported increasing recreational fishery matched with lack of management is leading to interest in Lake Superior burbot. Currently little is known about Lake Superior burbot, particularly basic life history such as migration, reproductive timing, and spawning site selection. In this study we investigated the reproductive timing and spawning area selection in Lake Superior. Riverine samples were obtained using hoop nets placed in tributaries of Lake Superior in mid-winter. Lacustrine samples were collected using set lines as well as rod and reel on several Lake Superior embayments during the ice-cover season. Captured burbot were weighed, measured and screened for oocyte development. Preliminary results suggest that final maturation occurred at different times between riverine and lacustrine environments, with riverine spawning in January and lake spawning later in spring. Additional information documents adult spawning sizes, duration of the reproductive window, and habitat selection during the reproductive period as well as rate of progression of oocyte maturation prior to spawning. Our data suggest temporal and spatial reproductive isolation between river and lake populations.

**IMPACT OF EMBRYO DEVELOPMENT AND ASYNCHRONOUS HATCHING ON MORPHOLOGY OF LARVAL BURBOT (LOTA LOTA)**

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**Abstract:**

Burbot (*Lota lota*) are a native fish to the Great Lakes that spawn in both river and lacustrine environments. Burbot display asynchronous hatching with hatch dates spread out over several weeks. In aquaculture, this trait is overcome by increasing temperature just prior to hatching, but it is unclear what the ramifications of this trait are in the wild. To investigate hatching asynchrony, adult burbot were captured from several river and bay locations within the southern Lake Superior watershed to collect gametes for fertilization into 1:1 male:female families. Families were kept in a laboratory setting at 3°C and imaged throughout their development period to determine differences in the development rates among embryos. Upon hatching, larvae were separated by their hatch date and photographed at hatching and every two days thereafter. Differences in total length, yolk sac area, and yolk sac utilization rate were compared between hatch dates to determine if asynchronous hatching caused differences in morphology, stage, or growth of larvae. Larvae hatched earlier in the asynchronous hatching period had shorter total lengths with larger yolk sacs than those hatched near the end of the period. River spawned larvae will be compared to lake spawned larvae to determine if the differences in location and timing play a role in the development and survival of larval burbot. Our results suggest that asynchronous hatching results in substantial variability in initial larval morphology in this species which may be relevant to larval success in varying environments.

ABSTRACT N° ICBF22-134

## **AUTS2A EXPRESSION DURING NEURODEVELOPMENT IN MEDAKA (ORYZIAS LATIPES)**

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### **Abstract:**

AUTS2 (activator of transcription and developmental regulator, previously named autism susceptibility candidate 2) is involved in a broad range of brain malformations and neurodevelopmental disorders in humans, including autism spectrum disorders, intellectual disability and schizophrenia. Nuclear AUTS2 activates transcription and therefore gene expression in the central nervous system, controls neuronal differentiation and modulates the excitatory/inhibitory balance by limiting the number of excitatory synapses, while cytoplasmic AUTS2 regulates neuronal migration and neuritogenesis. Here we used the RNAscope, a state-of-the-art in situ hybridization method to spatially monitor *auts2a* expression at key early stages of medaka (*Oryzias latipes*) embryonic neurodevelopment (stage 21 and 29). Stage 21 corresponds to brain regionalization, from this stage the three main brain regions (forebrain, midbrain and hindbrain) can be identified. Stage 29 follows neural tube formation, it is part of the late embryonic brain formation where the major brain areas shape and become identifiable. We observed that *auts2a* was expressed in the forebrain (telencephalon) and the hindbrain (myelencephalon) at stage 21 and in the forebrain (telencephalon), midbrain (optic tectum) and hindbrain (cerebellum and rhombencephalon) at stage 29. Our results are consistent with previous studies on zebrafish embryos and mouse embryos. Moreover, *auts2a* was expressed in the telencephalon and the cerebellum, brain areas involved in social behavior, memory, emotion and learning, consistent with the role of AUTS2 (i.e., the human ortholog of *auts2a*) in neurodevelopment and neurodevelopmental disorders.

ABSTRACT N° ICBF22-299

## **EFFECTS OF ENVIRONMENTAL ENRICHMENT ON BEHAVIOUR AND BRAIN ACTIVITY IN FARMED AND MODEL FISH**

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### **Abstract:**

To mitigate the adverse effects of hatchery-rearing on fish, enriching hatchery practices (i.e. environmental enrichment; EE) are used. Notably, EE has been shown to reduce stress and aggression, improve learning capacity, and increase neural plasticity. However, the optimal species-specific enriched environment is not established, and results vary between studies. We aimed at understanding which EE is best for Atlantic salmon and zebrafish by conducting two separate studies. That is, farmed salmon were kept in one of four treatments: stones, bubbles, increased flow (i.e. increased exercise) or control, for five weeks. Brain samples from a subset of fish were collected to quantify markers for neurogenesis. We found a higher number of new-born cells in the forebrain and the cerebellum and are currently quantifying differences between groups. Following EE, a selected number of individuals were transferred to glass aquaria in pairs to quantify their behavioural response to a novel environment. We found treatment-specific differences in feeding and aggression, with fish from stones and bubbles. We are running zebrafish behavioural trials with a plus maze to study conditioned place preference by fish towards 4 distinct EE (stones and plants, bottom stone picture, stone and plant pictures or barren). The aim is to determine which EE fish prefer to later assess their neurobiological state. Results may provide evidence about positive effects of EE on brain plasticity which improves fish behaviour in novel conditions, provide good welfare for fish and increase research quality.

## **THE BIOLOGICAL BACKGROUND FOR DEPRESSION-LIKE SYNDROME**

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### **Abstract:**

In salmon aquaculture farms, there is a percentage of the population that displays depression-like syndrome (DLS). This DLS fish exhibits signs including anorexia, a small size, and a behaviorally inhibited profile. We have previously shown that DLS fish are characterized by chronically elevated levels of cortisol levels and increased serotonin (5-HT) signaling under basal conditions and that they are unable to respond further to stress. In the current experiment, DLS fish were either treated with buspirone, an antagonist, for serotonin regulation, over four weeks or left untreated (control). The fish were euthanized immediately following treatment (basal condition) or after a chronic stress test (stress). Biochemical analysis of monoamines shows that after acute stress, the buspirone fish had increased dopamine (DA) signaling in the amygdala homolog (Dm), preoptic area (POA), and raphe nuclei. Meanwhile, the 5-HT signaling was increased in the raphe nuclei in buspirone, compared to controls. The Dm is associated with emotional learning; the POA is important for stress regulation, and the raphe nuclei is responsible for 5-HT synthesis. These results indicate that buspirone DLS fish are more stress-reactive than control DLS fish. This suggests that 5-HT manipulation, which also affects DA regulation, can potentially reverse the DLS fish profile. This is evidence of a common vertebrate biological background for DLS.

ABSTRACT N° ICBF22-350

## **HOW ARE PARASITES AFFECTING THE REPRODUCTIVE NEUROENDOCRINOLOGY OF THE ARCTIC CHARR?**

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### **Abstract:**

Some parasites alter their host's behavioral and physiological phenotypes to facilitate transfer to the next host in their life cycle. Such phenotype manipulation increases the probability that the intermediate host is eaten by the next host in the parasite's life cycle, a phenomenon known as parasite-increased trophic transmission. Several studies have shown a positive association between the level of infection from the parasite *Diplostomum* sp. and the intensity of the red coloration on the abdomen of arctic charr. This might suggest that the parasite is affecting the fish in a way that makes the fish invest more in reproduction.

To study how parasites affect the reproductive neuroendocrinology and behavior of arctic charr, we collected wild infected charr during the spawning season. Parasites were quantified and the fish were divided into two contrast groups (high and low parasite load). The analysis of immediate early gene markers expression is being conducted to establish neuronal regions of interest. These brain regions will be further analyzed for the expression of target genes associated with reproductive behavior.

We hypothesize that the parasites are targeting structures in the brain which affect the brain-pituitary-gonadal axis. That is, fish with the highest parasite load will show increased gene expression values associated with regulating increased activity in the brain-pituitary-gonadal axis. This might result in a more conspicuous and risk-taking fish, leading to increased predation risk.

## **TROUT INTESTINE: WORKING UNDER THE CLOCK**

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### **Abstract:**

The circadian system in fish involves multiple oscillators that interact with each other. Oscillators synchronize to different internal and external cues, such as the light-dark cycle and food availability, which are the most studied ones. In mammals, a circadian oscillator locates at the gastrointestinal tract (TGI) and drives its daily rhythmic physiology. Information at this respect is scarce in fish, and only few studies report clock genes to display daily rhythms of mRNA abundance. No data exist at this respect in rainbow trout, which lead us to evaluate the rhythmic profile of clock genes in the TGI of this teleost, and the influence of light and food on such rhythms. Four cohorts of trout were subjected to different experimental conditions: 1) Control, consisting on 12L:12D photoperiod, and food being provided at ZT3 (ZT = zeitgeber time; ZT0 = lights on); 2) constant darkness and fasting for 48 h (DD+F); 3) DD and refeeding at CT3 (CT0 = subjective day onset) for the last 72h; 4) 12L:12D and feeding at ZT15. Then, animals were sacrificed every 4 hours and individual samples of foregut were collected for further qPCR analysis of clock genes (*clock1a*, *bmal1*, *per1*, *cry2*, *rev-erb*). Our results reveal daily rhythmic expression of each gen that persist even in the absence of environmental cues (light and food), which perfectly fit with the existence of a circadian oscillator that localises within the intestine of the trout.

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**THE SALINITY AND TEMPERATURE EFFECTS TO THE ULTRASTRUCTURE CHARACTERIZATION OF MITOCHONDRIA-RICH PSEUDOBRANCH TYPE CELLS IN THE PSEUDOBRANCH OF THE EURYHALINE TELEOST BRACKISH MEDAKA (ORYZIAS DANCENA)**

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**Abstract:**

The pseudobranchs of the euryhaline teleost species brackish medaka (*Oryzias dancena*) acclimated to environments of different salinities and temperature were examined using optical and electron microscopy. The medaka pseudobranch was the embedded type, covered with connective tissues and with only one group of mitochondria-rich pseudobranch type cells (PSCs). The PSCs of medaka showed an empty space on the apical region from the hematoxylin and eosin (HE) staining and were periodic acid-Schiff (PAS)-positive. Compared those of freshwater (FW)-acclimated medaka with seawater (SW) specimens, the mitochondria in the PSCs were exhibited with fewer cristae, less electron-dense, and often vacuolated in the hypo-osmotic environment. Moreover, the PSCs were found more tubules to aggregately surround the mitochondria and basolateral membranes in the FW-acclimated medaka. Conversely, the larger and more electron-dense mitochondria in PSCs of their 7 days FW15-acclimated counterparts were concentrated with more derivatives microvilli stretching to the vascular lumen than those of FW28-acclimated medaka. In addition, the empty space on the PSCs apical region disappeared from the HE staining in the cold treatment group. Therefore, salinity and temperature-dependent changes in the ultrastructures of PSCs suggest their multi-potential role in energy metabolism of the embedded type pseudobranchs.

## **ACUTE IMPACT OF THYROID HORMONE ON CA<sup>2+</sup> CYCLING ON SALMON (SALMO SALAR) VENTRICULAR CARDIOMYOCYTES**

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### **Abstract:**

Farmed salmon suffer from a variety of heart pathologies that impair cardiac function and ultimately contribute to cardiac failure and mortality. Thyroid hormones (TH) have well-studied cardioprotective effects in mammals, through indirect and direct, genomic and non-genomic (acute) actions. Mammalian cardiomyocytes acutely exposed to TH have shown increased force of contraction (positive inotropic effect) and increased Ca<sup>2+</sup> extrusion, preventing the heart from Ca<sup>2+</sup> overload. These hormones peak during critical events of salmon life cycle with increased cardiac demand (e.g. smoltification), but their role in cardiac physiology and pathology in Atlantic salmon (*Salmo salar*) remain largely unknown. The aim of the current study was to investigate whether TH have direct acute effects on Ca<sup>2+</sup> dynamics in salmon ventricular cardiomyocyte (svc). Svcs were isolated using a standard enzymatic technique, while Ca<sup>2+</sup> transients were recorded in the presence of different concentrations of the TH 3,3',5-Triiodo-L-thyronine (T3). Stimulation with T3 decreases the time of half removal and increases the Ca<sup>2+</sup> removal rate of the Ca<sup>2+</sup> transient, allowing more time between each contraction. These effects could help the heart to work effectively when the heart rate is elevated. While these findings are somehow in line with observed effects of T3 in mammalian cardiomyocytes, further investigation are required to understand the underlying physiological mechanisms involved. Nonetheless, the results indicate that TH could have cardioprotective role also in salmon, for example in contexts that increase the demand of the heart such as during smoltification.

## **ESSENTIAL AMINO ACIDS ACTIVATION OF CALCIUM SENSING RECEPTOR IN ATLANTIC SALMON**

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### **Abstract:**

The calcium-sensing receptor (CaSR) is known to play a crucial role in sensing amino acids in the mammalian gastrointestinal (GI)-tract. Located in the enteroendocrine cells facing the GI-tract lumen, this receptor is activated by dietary amino acids, and stimulates the release of gut hormones involved in regulation of diverse physiological functions, including nutrient digestion and absorption, allocation of substrates for growth, feed intake, etc. However, little information is available regarding the CaSR role in sensing amino acids in the GI-tract of teleosts, including Atlantic salmon (*Salmo salar* L.), a key commercial species in global aquaculture. Here, we focused on the Atlantic salmon *casr* gene located in the chromosome *ssa11*, which share a high similarity with the human homologue. We found that the *casr* mRNA was more abundant in the proximal part of the intestine compared to the distal area, and very low levels of expression were observed in the stomach of Atlantic salmon postsmolts. To determine the exact essential amino acid preferences and the signaling pathways activated, we have generated a stable Flp-In™-293 cell line expressing Atlantic salmon CaSR. To establish the signaling properties of the receptor, we tested for activity in the Gq, Gi, and extracellular-signal regulated kinase (ERK) signaling pathways. Our preliminary results suggest that salmon CaSR can integrate signals arising from amino acids, activating distinct pathways. The data presented herein identify the CaSR as a molecular target for essential L-amino acids and with different sensitivity.

## **GEN-FISH: GENOMIC NETWORK FOR FISH IDENTIFICATION, STRESS AND HEALTH**

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### **Abstract:**

Freshwater fish resources (commercial, recreational and Indigenous fishing sectors; fish (aqua)culture) contribute to Canada's economy both directly (e.g., fish sales, tourism) and indirectly (e.g., "ecosystem services"). Yet, freshwater fish populations are under threat. Furthermore, the logistical difficulties of monitoring the status of fish stocks in Canada's millions of lakes and rivers are compounded by the limitations of conventional (e.g., capture-based) sampling methods, plus, such methods can be harmful to the fish community and their habitat. Through GEN-FISH, a network of over 23 researchers and over 50 partners across Canada, we aim to develop genomic resources to aid in the effective management of Canada's freshwater fish resources. Our project will provide three main deliverables to academics, regulatory agencies, and various stakeholders: 1) Fish Survey Genomics: a standardized environmental DNA (eDNA) toolkit for the detection and quantification of all freshwater fish species in Canada. 2) Fish Stress & Health Genomics: a suite of quantitative gene expression assays to assess fish health and environmental coping capacity. 3) Decision-Guiding Tools: identification of translational pathways that would accelerate the informed uptake of genomics-based tools in the fisheries/aquaculture sectors and develop strategies that maximize broad end-user receptivity. This poster outlines the development of the Stress Transcriptional Profiling Chip (STP-Chip) that will be used to assess fish stress and health for important fishes throughout Canada. The goal is to work with Canadian researchers and freshwater fish stakeholders to help stem the decline in freshwater fish abundance and diversity, initially in Canada, but ultimately globally.

**EFFECTS OF TEMPERATURE AND OXYGEN AVAILABILITY ON BLOOD PARAMETERS AND ERYTHROCYTE AND LEUCOCYTES DISTRIBUTION IN HALOBATRACHUS DIDACTYLUS (BLOCH & SCHNEIDER, 1801).**

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**Abstract:**

Temperature and oxygen are two of the most affected environmental variables in our age of climate. The changes in these variables can have a plethora of effects on marine biota, and understanding them is of outmost importance to address this issue. We simulated climate change scenarios, to evaluate the effects of high temperature, hypoxia and a combination of both, on blood parameters and cell proportions of *Halobatrachus didactylus*.

We used a condition index to estimate the welfare of the fish in each treatment. The two environmental drivers studied showed effects on the number of white blood cells and shape of red blood cells over the course of 30 days of exposure. The most affected parameter was the red and white blood cell count, which increased significantly in hypoxic conditions. Hematocrit increased significantly in hypoxic conditions. Hemoglobin concentration was unaffected by our treatments. Red blood cell counts were higher in high temperature, but oxygen levels showed no effects on this parameter, which leads us to believe hypoxia triggers an increase in white cell numbers. Blood pH was significantly lower in the high temperature normoxic scenario in comparison to all other treatments.

These changes seem to have negatively impacted the condition of the fish, as individuals on the high temperature conditions exhibited much lower condition index values.

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**CHARACTERIZATION OF THE GUT MICROBIOME OF THREE SPECIES OF MEDITERRANEAN SCORPIONFISH (SCORPAENA SCROFA, SCORPAENA NOTATA AND SCORPAENA PORCUS) AND EFFECT OF GEOGRAPHIC LOCATION ON ITS COMPOSITION**

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**Abstract:**

The gut microbiome plays a crucial role in the host's survival and fitness by the regulation of metabolism and homeostasis. In fishes, it is reported to contribute to nutrition, immune system and epithelial development and xenobiotics metabolism. For most of the fish species studied so far, several factors are shown to drive the variation in the composition and structure of these bacterial communities, among all, host species, habitat and diet seems to be the most influential ones. This study is the first to characterize the microbiome of three sympatric predator species of the genus *Scorpaena* (*Scorpaena scrofa*, *Scorpaena notata* and *Scorpaena porcus*) and to analyze the influence of host species and geographical location on the composition of the microbial communities symbiotic to this fish genus.

The gut microbiome of 69 specimens of *Scorpaena* collected in multiple locations along the French Mediterranean coast was analyzed using the 16S rRNA amplicon sequencing technology.

Our results revealed that the microbiome of the three scorpionfish species is linked by the common presence of the genus *Photobacterium*. Moreover, each of the species displays a unique core microbiome characterized by the genus *Vibrio* in *S. scrofa*, *Clostridium senso stricto* in *S. porcus* and *Enterovibrio* in *S. notata*. The effect of geographical location on the composition of the bacterial communities of *S. notata* was also evaluated.

Besides confirming that phylogeny is a relevant factor shaping the composition of the microbiome in fishes, this study lays the foundation for future analyses of the microbiome functionality in different species of scorpionfishes.

## **GROWTH AND BEHAVIOR OF CACHAMA PIARACTUS BRACHYPOMUS UNDER DIFFERENT THERMAL REGIMES**

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### **Abstract:**

This study deals with the effects of different thermal regimes, thermal preference and thermal tolerance on growth performance and stress level, of the cachama *Piaractus brachypomus*, under controlled laboratory conditions. For this purpose, 900 fish ( $0,325 \text{ g} \pm 0,1 \text{ sd}$  and  $2,7 \text{ cm} \pm 0,30 \text{ sd TL}$ ) were used. To assess the optimum growth temperature, three experimental temperatures (T1:26°C, T2:28°C and T3:30°C) were evaluated after 60 days and verified an effect of temperature. Higher values occurred for fish exposed to 30°C, while no significant differences ( $p > 0,05$ ) were found between treatments. Thermal preference was determined using a horizontal temperature gradient tank (19°C, 22°C, 25°C, 28°C, 31°C, 33°C, 36°C y 39°C) with observations every 10 min/4h. Experimental fish showed in a ranged from 33 to 35°C for T2; for T2, between 29°C and 31°C and for T3 between 31°C and 33°C confirming that thermal preference was influenced by prior acclimation temperatures. Thermal tolerance was evaluated too: The temperature was increased or decreased by 1°C/30min', and the temperatures at which an increase in activity, initial disorientation and total disorientation occurred were recorded. The trial was ended when until 50% of fish lost their balance. After that, fish blood glucose concentration was evaluated. The fish of T1, T2 and T3 endured minimum temperatures of 14°C, 15°C and 15°C and maximums of 42°C, 42°C and 43°C respectively. Elevated glucose levels occurred in T1 for TCmax (279 mg/dl) and T3 for TCmin (184 mg/dl), suggesting high stress levels. There is a relationship between the acclimatization temperature of cachama fry and critical temperatures.

ABSTRACT N° ICBF22-309

**VARIATIONS OF N-3 HIGHLY UNSATURATED FATTY ACIDS (N-3 HUFA) CONTENT IN MUSCLE OF SARDINES FED WITH DIFFERENT SIZES AND QUANTITIES OF FOOD**

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**Abstract:**

Small pelagic fish are key components of marine ecosystems, accounting for 25% of worldwide landings. However, a reduction in the size and body condition index of sardines (*Sardina pilchardus*) has been recorded in the Gulf of Lions since 2008 and in the Bay of Biscay since 2012. This phenomenon is thought to be the result of a change in prey quantity and/or quality. Indeed, a recent experiment showed that the growth and body condition of sardines were (i) an immediate response to food quantity (0.3 and 0.6% of the total fish mass in tanks) and pellet size (0.1 and 1.2 mm) and (ii) optimal when sardines consumed large amount of large pellets. Here, we hypothesise that little food (small pellets and/or small quantities) would induce an insufficient supply of n-3 highly unsaturated fatty acids (n-3 HUFA) and constrain the individuals' growth, as n-3 HUFA are essential to vital functions and fitness of fishes. The objective is to investigate food size/quantity effects on n-3 HUFA' thresholds during the growth of sardines, by measuring the retention rate of n-3 HUFA in muscle from sardines previously conditioned for seven months to four different feeding conditions (small or large pellets, in small or large quantities; n=30 individuals/condition). The analysis of fatty acids will give us an additional element of response to the decreases in growth and body condition of sardines exposed to variable dietary conditioning. It will ultimately contribute to better estimate the consequences of prey quantity and quality on the population dynamics.

*15-The future of fishes: how will they cope physiologically with a changing planet?*

ABSTRACT N° ICBF22-265

**EFFECTS OF PARENTAL CO-EXPOSURE TO HEAT WAVE AND COPPER ON ZEBRAFISH (DANIO RERIO) OFFSPRING THERMAL TOLERANCE**

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**Abstract:**

Increased anthropogenic activities have led to global climate change, which is known to increase the occurrence of extreme thermal events, such as heat waves. Heat waves represent a challenge especially for ectotherms, such as fish, as their functions heavily rely on the temperature of their surrounding environment. At the same time the occurrence of toxins, such as trace metals, is increasing in the environment. Toxicity of metals is dependent on temperature increase, which accelerates the fish metabolic rate, which in turn might lead to faster bioaccumulation. Therefore, climate change driven heat waves, combined with metal exposure, might constitute a significant challenge for fish survival via synergistic effect. Changes in the environment not only can shape the individual plasticity within a generation, but this phenotypic adjustment can be transferred to the next generation through transgenerational plasticity. Previous studies have shown that heat exposed parents can produce offspring with enhanced thermal tolerance. However, the effect of added metal toxins to this phenomenon is not known. Because of this knowledge gap, the goal of the current study is to uncover potential transgenerational effects of heat wave and copper exposure in zebrafish offspring whose parents are exposed to a (1) laboratory induced heat wave (33 °C), (2) copper exposure (25 ug/l), and (3) combination of both. To achieve this, larval development was monitored until 5 dpf and thermal tolerance, as well as HSP70 and HIF expression was measured.

*15-The future of fishes: how will they cope physiologically with a changing planet?*

ABSTRACT N° ICBF22-453

**DISRUPTION OF DAILY AND SEASONAL RHYTHMS IN MIGRATING FISH**

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**Abstract:**

Virtually all organisms have adapted to day-night cycles by the evolution of endogenous rhythms that regulate most biological processes. The duration and intensity of light during daily and seasonal rhythms is crucial for maintaining the biological clock's "connection" to the external environment, on which fish rely for the functioning of an array of behavioural and physiological patterns, such as orientation, foraging, breeding and migration.

Light pollution (artificial light at night, ALAN) can disrupt these rhythms, negatively impacting the behaviour and physiology of fish as day-night differences become less discernible, which is especially concerning for migratory fish species. Light pollution may mask the natural cues used for the triggering of and navigation during migration, which can influence energy expenditure and migration success.

Using swimming respirometry in the field and a tilted flume in the lab, we will test swimming patterns and energy consumption of migrating and non-migrating sticklebacks from light and non-light polluted areas. Furthermore, using mesocosm systems, we will monitor activity over time of PIT tagged sticklebacks by running caged experiments, which will be manipulated with varying light conditions. The differing migratory types will be tested to understand if they vary fundamentally in swimming physiology and possibly clock rhythmicity, which could be used as indicators for how they will cope in a light polluted environment.

With our results, we aim to provide an in-depth overview of the impact of light pollution on freshwater ecosystems, allowing for the establishment of policies in order to mitigate negative impacts on the biological clock.

*15-The future of fishes: how will they cope physiologically with a changing planet?*

ABSTRACT N° ICBF22-467

**BURSTED! USING RASPBERRY PI'S AND LASERS TO MEASURE FISH BURST SWIM PERFORMANCE**

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**Abstract:**

Fish swim performance can vary widely both among and within species as well as under different environmental conditions. Therefore, it is imperative to incorporate a suite of physiological performance measures when determining swim performance. The literature is rich with indices of sustained, aerobic swim performance (e.g., critical swimming velocities ( $U_{crit}$ ), tail beat frequency, aerobic scope). However, anaerobic swim performance (i.e., burst swimming) may be an important predictor of ecological performance. Burst swimming is a common method of prey capture or predator evasion, as well as for the passage of hydraulic challenges such as river rapids or fish ladders. Yet it remains uncommonly reported in physiological studies, likely because it is challenging to measure. We address this issue by developing a burst-swim performance chamber using a Raspberry Pi computer, allowing for the accurate monitoring of 25 laser-gates. By repeatedly measuring the fish's velocity as it breaks the laser gates we are able to calculate multiple metrics of burst performance. Additionally, our design allows for controlled, rapidly repeated trials that provide a proxy for exhaustion. The burst tunnel can also be used for controlled tests of burst performance in response to an organism's past rearing histories (e.g., exposure to aquatic contaminants, acclimation temperatures). Our burst tunnel design is flexible to accommodate different size fishes (3g to 300g) and could be easily adapted for deployment in field conditions. We have measured burst performance among several resident California species (e.g., Chinook salmon, green sturgeon, rainbow trout and largemouth bass) and have found burst performance to be temperature-dependent and species-specific suggesting implications for trophic interactions in the wild.

ABSTRACT N° ICBF22-470

**CARDIAC RESPONSES OF GILTHEAD SEABREAM SPARUS AURATA TO ACUTE WARMING: A COMPARISON BETWEEN CTMAX AND CTSMAX PROTOCOLS.**

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**Abstract:**

Our study aimed to better understand the role of cardiac performance in tolerance of acute warming in fishes. Sea bream *S. aurata* (n=7) were fitted under anaesthesia with an intraperitoneal heart rate (fH, beats min<sup>-1</sup>) bilogger (Star-Oddi®). After recovery at 25°C, each bream was exposed to two acute warming protocols, both involving stepwise temperature increases every 30 minutes. A critical thermal maximum (CT<sub>max</sub>) while swimming freely in a tank until loss of equilibrium (LOE), and a critical maximum for swimming (CTS<sub>max</sub>) while exercising aerobically in a swim tunnel, with fatigue as endpoint. Each fish also underwent a critical swimming speed (U<sub>crit</sub>) protocol at 25°C, to improve interpretation of warming responses.

The CT<sub>max</sub> was 35.5±0.2°C, significantly higher than CTS<sub>max</sub> at 34.1±0.4°C. The sea bream were very sensitive to warming between 25 and 29°C with a significant increase in fH. Individuals varied widely in cardiac responses and had higher mean fH in the CTS<sub>max</sub> test at some temperatures. Maximum fH did not, however, differ significantly between protocols, being 135±17 bpm in CT<sub>max</sub> and 168±8 bpm in CTS<sub>max</sub>. The maximum fH in CT<sub>max</sub> occurred at 33.7±0.6°C, significantly higher than at 31.9±0.9 °C for CTS<sub>max</sub>. Maximum fH in U<sub>crit</sub> was 150±4 bpm, similar to in CT<sub>max</sub> but slightly and significantly lower than in CTS<sub>max</sub>. The data indicate that both acute warming protocols may challenge the intrinsic cardiac scope of the sea bream, evidence that cardiac performance may be a factor defining tolerance of acute warming in this species.

*15-The future of fishes: how will they cope physiologically with a changing planet?*

ABSTRACT N° ICBF22-531

**THE PHYSIOLOGICAL BASIS FOR ACUTE UPPER THERMAL TOLERANCE IN FISHES**

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**Abstract:**

With climate change projected to increasingly afflict fishes with warming challenges, understanding the impacts on fishes is becoming ever more important. We review the literature on the physiological mechanisms proposed to explain the loss of motor function observed in fishes at their upper thermal limits, from molecular impacts such as denaturation of proteins and lipid membrane fluidity, through forms of mitochondrial insufficiency, to higher levels of biological organisation such as cardiorespiratory collapse and brain dysfunction. Tissue oxygenation has been proposed as an overarching factor for thermal tolerance in fishes. The literature shows that oxygen-limitation can be a central mechanism but not always, and it is currently not clear in which contexts oxygen limitation is the dominating mechanism. The thermally limiting physiological systems differ between species and life stages, and the rate of warming can determine the limiting physiological system and the endpoint temperature of thermal tolerance. We propose a framework linking thermal performance curves and thermal tolerance traits, and conclude that a single silver bullet mechanism determining acute warming tolerance in all species and contexts will not be found. Finally, we propose future avenues of research that can elucidate major patterns of physiological thermal limitations.

## **THE EFFECT OF ORAL MICROPLASTICS APPLICATION WITH EMPHASIS ON RAINBOW TROUT INTESTINAL MICROFLORA**

Aneta Hollerova\*<sup>1,2</sup>, Nikola Hodkovicova<sup>1</sup>, Martin Faldyna<sup>1</sup>, Jana Blahova<sup>2</sup>, Jan Mares<sup>3</sup>, Denisa Medkova<sup>2,3</sup>, Zdenka Svobodova<sup>2</sup>

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### **Abstract:**

The production of plastics is increasing dramatically throughout the world and is considered to be serious threat for the aquatic environment. Microplastics are particles with a size between 20 µm – 5 mm, are able to permeate intestinal endothelium and reach the tissues. Our study is focused on the polystyrene microparticles and their impact on rainbow trout (*Oncorhynchus mykiss*) organism. The experiment will be performed in accordance to the Fish Juvenile Growth Test (OECD 215) while the microparticles will be incorporated into the commercial pellets of experimental fish. The fish will be fed twice a day with amount 2% of their wet weight, three experimental groups will be tested – microplastics in concentration 0.5%, 2% and 5%, control group without addition of microplastics. One group will be consisted from 20 fish pieces and experiment will be performed in duplicate. Whole experiment will be in duration 8 weeks with 14-days long period of acclimatization and 6 weeks of experimental phase. At the end of experimental phase, the effect on hematological, biochemical, histological and immunological indices in time and dose-dependent manner will be evaluated. Main task of our study will be analysis of intestinal microflora with emphasis on *Lactobacillus* spp. and *Brevinema* mutual ratio after microplastics exposure when the DNA will be isolated and submitted for qRT-PCR. Project was funded by the ERDF/ESF “PROFISH” [no. CZ.02.1.01/0.0/0.0/16\_019/0000869].

## **MICROPLASTICS MAY AFFECT THE GENE AND PROTEIN EXPRESSION IN RAINBOW TROUT'S INTESTINE**

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### **Abstract:**

According to several studies, the polyethylene (PE) and polystyrene (PS) are recognised as the main polymer pollutants of the aquatic environment. As plastics in micro sizes are known to be the serious issue for the aquatic biota with yet not completely described consequences, our study aimed to characterise PE and PS effects on the gene and protein expression in intestine. Two separated experiments were conducted with rainbow trout (*Oncorhynchus mykiss*), where the PE ( $46.6 \pm 11.3 \mu\text{m}$ ) and PS ( $52.5 \pm 11.5 \mu\text{m}$ ) were submitted to fish orally in commercial feed pellets for six weeks. Both, PE and PS, were tested in same concentration doses – 0.5%, 2% and 5% of the feed/day. Regards gene expression, the significant changes were found for solute carrier family 9 member A1b (*slc9a1b*), ghrelin/obestatin prepropeptide (*ghrl*) and transferrin-a (*tfa*) gene. Plastics caused gene expression changes mainly in the highest (5%) tested concentration. Both, *slc9a1* and *ghrl*, interact with immune system and cytokine release. Moreover, the *slc9a1b* is involved in pH regulation and sodium proton antiporter activity and the *ghrl* has an appetite-stimulating effect and induce ROS production. Additionally, *tfa* participating in iron ion binding and homeostasis was down-regulated which may cause higher iron concentrations and provoke the ROS release in intestine. As all of altered genes are part of important metabolic processes in the organism, these gene expression changes might have serious consequences. Data will be completed by the results of proteomic analysis hereafter; however, with respect to the results obtained so far, it is clear that microplastics submitted orally may affect the function of the digestive tract, immune system and induce the oxidative stress.

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**BIOMONITORING THE EFFECT OF ACCUMULATING PERFLUORO-CHEMICALS ON PHYSIOLOGICAL RESPONSES OF A BENTHIVOROUS AND A POTADROMOUS FISH SPECIES BY USING A MULTIPLE BIOMARKER APPROACH**

Sophia Schumann<sup>1</sup>, Elisabetta Piva<sup>1</sup>, Serena Dotteschini<sup>1</sup>, Elena Negrato<sup>2</sup>, Marco Bonato<sup>1</sup>, Paola Irato<sup>1</sup>, Andrea Marion<sup>3</sup>, Daniela Bertotto<sup>2</sup>, Gianfranco Santovito\*<sup>1</sup>

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**Abstract:**

Understanding the effects of exposure to non-native environmental substances on fish is essential to evaluate their potential risk for aquatic ecosystems. Among pollutants, perfluoroalkylated substances (PFAS) are incredibly persistent in the environment due to the perfluorinated tail and their anionic head group. Fish are highly affected by pollutants, where the intake focuses on contaminated food sources and epithelial absorption. The stress responses of a sessile and a potamodromous fish species were assessed to evaluate the health impact on riverine fish. *Squalius cephalus* and *Padogobius bonelli* were caught in three different rivers, characterized by high, medium and low concentrations of PFAS in the Veneto region, Italy, where PFAS pollution has recently emerged as an environmental and human health problem. The stress response was studied through the analysis of the hypothalamic-pituitary-internal axis. In particular, the acute stress response was determined by the cortisol level in the blood, whereas the chronic stress was assessed in scales. Furthermore, gene expression of antistress proteins was investigated in the target organs and correlated to cortisol levels and oxidative stress biomarkers such as lipid peroxidation and protein oxidation. (Supported by the European Union Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie Actions, Grant Agreement No. 860800)

## **EFFECTS OF ENDOCRINE DISRUPTORS OCCURRING IN AQUATIC ENVIRONMENT**

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### **Abstract:**

Chemicals, pesticides, pharmacologically active substances and many other types of contaminants have been constantly entering aquatic environment. Such a contamination results in negative effects on both human and aquatic biota. In few past decades, water pollution caused by endocrine disruptors has become a commonly discussed issue. Occurrence of endocrine disruptor residues in aquatic environment is ubiquitous and is mostly result of wastewater discharge and its imperfect purification in wastewater treatment plants. Because of its constantly increasing consumption, hormonal contraception seems to be a major problem concerning endocrine disruptors entering aquatic environment. Endocrine disruptors have been proven to have a potential to affect endocrine system of human and non-target aquatic biota. Thus, endocrine disruptors have the ability to negatively affect development, physiological function, sexual activity and reproduction of non-target fish and other water organisms. Sediment, water, and aquatic biota tissue analysis are examples of frequently used measurements in order to monitor concentrations of endocrine disruptor residues in the environment. Moreover, phospholipoprotein vitellogenin has been included to our study as a biochemical marker of exposure of endocrine disruptors to aquatic organisms. The effects of ethinylestradiol on *Danio rerio* triploid population have been observed in the present study through determination of vitellogenin concentration in body homogenate. All-male *Danio rerio* population was exposed to ethinylestradiol incorporated into feed at the highest tested experimental concentration of 0.1 mg/100g feed for the period of 10 weeks. The concentration of vitellogenin in this group was between 14545 and 48338 µg/ml.

This research was supported by project PROFISH – CZ.02.1.01/0.0/0.0/16\_019/0000869.

## **TOXICITY OF 6PPD-QUINONE AMONG FISHES ACROSS ONTOGENY**

Kerri Lynn Ackerly\*<sup>1</sup>, Kathleen Roark<sup>1</sup>, Andrew Esbaugh<sup>1</sup>, Kristin Nielsen<sup>1</sup>

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### **Abstract:**

Recurrent large-scale fish kills following storms have become a serious issue for salmon populations migrating through highly urbanized watersheds in the Pacific Northwest region of the United States. Recently, these mass die-offs were linked to tire wear particles (TWPs) left on roadways, which are washed into nearby surface waters by stormwater runoff. The lethality of TWPs has been attributed to 6PPD-quinone (a quinone byproduct of the ubiquitous tire antiozonant 6PPD), which has a median lethal concentration of < 1- $\mu$ g/L for juvenile coho salmon. However, there remains a paucity of data describing the differential toxicity of this compound across species and ontogenies, or its sub-lethal effects on developing fish. Moreover, to our knowledge, no toxicity values have been developed for estuarine fish species exposed to 6PPD-quinone. This data gap is particularly noteworthy, as estuaries that receive inflows from highly urbanized watersheds are especially vulnerable to TWP contamination. Therefore, the goals of the present study were to (1) determine the toxicity of 6PPD-quinone to both model (e.g., sheepshead minnow [*Cyprinodon variegatus*]) and non-model (red drum [*Sciaenops ocellatus*], southern flounder [*Paralichthys lethostigma*]) estuarine fishes (2) examine the relative sensitivities of ontological stages within a single species, and (3) derive sub-lethal toxicity values for early life stage fish. Results indicate that the toxicity of 6PPD-quinone varies demonstrably across species and ontology, with toxicity values spanning several orders of magnitude in some instances. Findings of the present study represent a substantial contribution towards advancing our knowledge of this newly identified and pervasive environmental toxicant.

## **DEEP SEABED MINING AND DEMERSAL FISH**

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### **Abstract:**

Modern society is driving an ever-increasing demand for metals, which have to be extracted from natural deposits at an environmental cost, highly visible as cleared forests, scarred landscape and displaced people. Hence, rich deposits of metals covering a vast area in the Pacific deep sea (Clarion-Clipperton Fracture Zone – CCZ) has become a new potential source and much investment channelled towards developing relevant technologies. The International Seabed Authority (ISA) has issued licences for deep-sea mining in the CZZ. However, our understanding of the environmental impact of deep seabed mining is limited. SMARTEx (Seabed Mining and resilience to experimental impact) is a NERC funded multi-disciplinary project with the aim of adapting well-established ecotoxicological biomarkers to deep-sea fish. The present study was conducted on samples extracted from baited trap caught demersal deep-sea Macouridae from a variety of deep-sea locations. The aim of this preliminary study is to optimise a suite of established ecotoxicological biomarker endpoints to establish a baseline against which fish exposed to mining plumes can be compared. The generation of oxidative radicals can be a direct result of exposure to a toxic substance or as a metabolic response to generic stress. We will measure tissue damage by lipid peroxidation (using a TBARS assay), upregulation of antioxidant enzyme systems (Superoxide dismutase-SOD, catalase-CAT, Glutathione-GSH etc), and subsequent DNA damage (Comet assay). The work is very much on going and we would welcome the opportunity to introduce the project as a poster.

## **INTESTINAL MICROBIOME COMPOSITION: POTENTIAL BIOMARKER OF CONTAMINATION IN TWO EMBLEMATIC SPECIES OF THE NORTH-WESTERN MEDITERRANEAN SEA**

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### **Abstract:**

Environmental pollutants have become an increasingly common health hazard in the last several decades. Despite increasing awareness, one potentially major consequence of exposure to cocktail of contaminants has been largely neglected: the impact of the disruption of the symbiosis between host and the diversity and structure of the gut microbiota. This latter performs important functions for the health of fish, participating in the regulation of many physiological functions. Moreover, a growing literature indicates that these microbial communities may participate in biotransformation and thus constitute a compartment to add to the original biomarker schematic. Knowing the microbial composition and evaluating the possible effects caused by anthropogenic pollution in the gut microbiota of fish populations might represent an important step in defining microbial biomarkers for environmental contamination. This study evaluated the impact of environmental contamination on the gut microbiome of two highly prized fish in the Mediterranean Sea: European sardines (*Sardina pilchardus*) and Gilthead seabreams (*Sparus aurata*). In this survey, the V3-4 region of the 16S rRNA gene was used to characterize and compare the microbiome between the different contamination clusters of the two species in the Gulf of Lions. Significant changes in the richness and diversity of the gut microbiota were observed in response to different level of environmental contamination. These findings support the utility of the microbiota as a pre-clinical tool for identifying exposures to cocktail of pollutants in the wild and so must be considered to fully evaluate the toxicity of environmental contaminants.

ABSTRACT N° ICBF22-449

**EFFECTS OF CHRONIC EXPOSURE TO BISPHENOL S ON SOCIAL BEHAVIOURS AND ANXIETY RESPONSES IN OFFSPRING: NEUROPEPTIDE SIGNALLING DYSFUNCTION AND OXIDATIVE STRESS IN THE BRAIN OF OFFSPRING**

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**Abstract:**

Recent studies show that bisphenol S (BPS) induces multiple adverse effects in exposed organisms; however, the maternal effects of BPS exposure remain poorly understood. In this study, we expose adult female zebrafish to environmentally relevant concentrations of BPS (0, 1, 10, 30 µg/L) and 1 µg/L of 17-estradiol (E2) as a positive control for 60 days. Females were then paired with control males and their offspring were collected and raised in control water for 6 months. The result showed that maternal exposure to BPS can alter social behaviour and anxiety response in a dose-specific manner in male offspring. Social cohesion and group preferences were significantly decreased by maternal exposure to 1 and 10 µg/L BPS, respectively. Additionally, maternal exposure to 1 and 30 µg/L BPS and E2 decreased offspring stress responses during the novel tank test. The impaired behaviours were correlated with elevated arginine-vasotocin (AVT) level as well as with the altered expression of genes involved in AVT signalling pathway (AVT, avpr1aa) and enzymatic antioxidant genes (cat and Mn-sod) in the brain. Collectively, these results propose that maternal exposure to environmentally relevant concentrations of BPS alters social behaviour in zebrafish offspring, which is likely mediated by oxidative stress and disruption of neuropeptide signalling pathways in the brain.

## **ATLANTIC SALMON HEART DEVELOPMENT IN A CHANGING ENVIRONMENT**

Vilde Arntzen Engdal\*<sup>1</sup>, Marco Vindas<sup>1</sup>, Kjetil Hodne<sup>1</sup>, Simen Rød Sandve<sup>1</sup>, Ida Beitnes Johansen<sup>1</sup>

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### **Abstract:**

Anadromous salmonid species go through dramatic physiological changes during their life in order to survive in both fresh- and saltwater. Specifically, the smoltification process prepares the salmon for a habitat change to saltwater which is triggered by growth rate and environmental change of light and temperature. However, in salmon aquaculture, light, temperature and growth rate are manipulated to produce smolt at a faster rate than in the wild. Intriguingly, few studies have focused on the repercussions of this manipulation on physiological parameters, such as cardiovascular function, compared with wild salmon. We compared the heart morphology of wild salmon, simulated natural produced salmon and “fast” aquaculture produced salmon. We found that producing “fast” smolt results in a rounder and shorter ventricle with a greater ventricle-bulbous angle than smolts developed under simulated natural conditions and wild smolts. These morphological differences may have functional consequences, where rounder hearts and misaligned bulbus may result in poorer heart function than the normal pyramidal heart in wild salmon. We fear that a changing climate may alter heart physiology. Therefore, we aim to describe the heart development during the Atlantic salmon life cycle. We will also investigate how wild salmon hearts develop and differ in different locations in Norway where light and temperature vary.

ABSTRACT N° ICBF22-386

## **THE CORONARY VASCULATURE FROM THE WORLD'S OLDEST LIVING VERTEBRATE, THE GREENLAND SHARK**

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### **Abstract:**

The Greenland shark (GS) (*Somniosus microcephalus*) is an elusive arctic predator that has captivated the scientific community since the discovery of its extreme longevity. With an estimated maximum age of 392 +/- 120 years, and individuals reaching sexual maturity at 150 years, the GS is the longest living vertebrate. The physiology of the GS is largely unknown, so how key systems such as the cardiovascular system support such longevity remains to be determined. In humans coronary artery disease is highly prevalent with age, so understanding how the GS coronary circulation is maintained for so long could give new insights for human aging. To further our understanding of cardiac longevity in the GS, we used histology to study the coronary vasculature in paraffin-fixed heart samples from 14 sharks (estimated age range 30-220 years). Coronary vessel density, lumen diameter, vessel wall thickness and the ratios of collagen, muscle and elastin content of vessels were compared across the different ventricle layers (epicardium, compacta and spongiosa). Vessels in the epicardium were the largest (diameter 210 +/- 62um, n=14) and had the highest collagen content (collagen thickness 16 +/- 4um, n=14), with the compacta and spongiosa showing similar values for both. No age-related changes were observed for any of the parameters measured and we found no evidence of coronary artery disease in any specimen. Our study is the first to describe the coronary vasculature of the GS and conclude that GS vessels are similar in structure to vessels from mammals and other shark species. Maintenance of vascular morphology at old age may be important for cardiac longevity in this species. The GS is still classed as data deficient and a vulnerable species; by increasing our understanding of its general physiology we can better protect this remarkable species.

ABSTRACT N° ICBF22-471

## **THE CARDIAC TRANSCRIPTOME OF THE WORLD'S LONGEST LIVING VERTEBRATE**

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<sup>4</sup>University of Massachusetts Dartmouth, Dartmouth, United States

### **Abstract:**

The Greenland shark (*Somniosus microcephalus*) is the world's longest-lived vertebrate, with an estimated lifespan of at least 272 years. However, the mechanisms facilitating its extreme longevity remain unknown. Here, we de novo generate the first transcriptome from the ventricular tissue of 6 Greenland sharks (approximate age range 110-219 years) and use this to investigate both age-related and specific-species patterns in gene expression. The assembly process generated 149,000 transcripts with an N50 of 1,202bp, and a BUSCO score of 76.2%, making it comparable in quality to that of other published squaliform elasmobranch transcriptomes. Age-related analysis revealed the expression of 205 genes changed with age (83 increasing, 122 decreasing). Hypernetwork modelling was used to identify a subset of the age-related genes that shared many connections to the rest of the transcriptome, which implies functionality. These genes were mapped to the initiation of transcription and translation. To put these findings into broader perspective we then re-analysed published RNA-seq datasets from cardiac tissue of the white shark (*Carcharodon carcharias*), short fin mako (*Isurus oxyrinchus*), small-spotted catshark (*Scyliorhinus canicula*), great hammerhead (*Sphyrna mokarran*) and yellow ray (*Urobatis jamaicensis*) and identified consistent differences between the Greenland shark and all the other elasmobranch species in the expression of genes relating to mRNA translation and protein targeting to the endoplasmic reticulum. Together, these analyses suggest that the production of proteins, from DNA transcription and translation through to protein synthesis and localisation, is an interesting avenue of exploration in the study of the world's longest-lived vertebrate, the Greenland shark.

**TO CHASE, TO FEED, TO SWIM, THAT IS THE QUESTION: DIFFERENT APPROACHES YIELD DIFFERENT ESTIMATES OF "MAXIMUM" METABOLIC RATE**

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**Abstract:**

Metabolic rate is an essential feature of animal physiology and ecology. The rate of aerobic metabolism, as determined by oxygen consumption rate (MO<sub>2</sub>), is influenced by a variety of factors, including body size, temperature, and activity levels. Maximum aerobic metabolic rate (MMR) reflects the physiological capacity of an animal for oxygen extraction and utilization. As such, MMR is argued to be an important feature of an animal's life history. In fishes, MMR is frequently estimated as the peak MO<sub>2</sub> immediately following an exhaustive chase, although several studies indicate that this value may underestimate MMR. Instead, MMR may be attained during sustained activity or following ingestion of a large meal. In this study, we used intermittent-flow respirometry to quantify MO<sub>2</sub> by the Gulf killifish, *Fundulus grandis*, after chasing, after feeding, or during maximum aerobic swimming. MMR estimates after chasing were significantly lower than those obtained during swimming ( $P = 0.001$ ); MMR estimates after feeding were marginally ( $P = 0.06$ ) higher than those obtained after chasing and significantly lower than those during swimming ( $P = 0.02$ ). Although MMR estimates obtained by the three techniques were repeatable over two trials ( $r = 0.74$ ), these estimates were poorly correlated across methods ( $r = 0.55$ ). The results demonstrate that MO<sub>2</sub> after an exhaustive chase or during digestion underestimate MMR in this species, and, importantly, such estimates may be poor predictors of inter-individual variation in maximum aerobic metabolism.

ABSTRACT N° ICBF22-547

## **HYPOMETABOLIC RESPONSES TO CHRONIC HYPOXIA: A ROLE FOR MEMBRANE CHOLESTEROL**

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### **Abstract:**

Chronic hypoxia is a state of oxygen limitation common to many aquatic environments. Champions of hypoxia tolerance like the goldfish use metabolic suppression as an essential strategy to cope with low O<sub>2</sub>. Environmental factors have an impact on the lipid composition of membranes that, in turn, modulates metabolism. We have examined the physiological processes used by goldfish to survive in hypoxia and propose a novel mechanism - the modulation of membrane cholesterol - to reduce ATP use and production. We found that goldfish undergo extensive changes in membrane cholesterol metabolism at both the biochemical and molecular (mRNA transcripts involved in synthesis and degradation pathways) level upon prolonged in vivo exposure to low O<sub>2</sub>. These changes may be linked to promoting the downregulation of Na<sup>+</sup>/K<sup>+</sup>-ATPase (major ATP consumer), mitochondrial respiration capacity, and energy metabolism. A common membrane signal regulating the joint inhibition of ion pumps and channels could be an exquisite way to preserve the balance between ATP supply and demand in hypometabolic states. Membrane remodeling, together with other more traditional molecular mechanisms, appear to work in concert to achieve deep metabolic suppression.

**ONTOGENETIC CHANGES IN BLOOD OSMOLALITY DURING THE POST-EMBRYONIC DEVELOPMENT OF ZEBRAFISH (DANIO RERIO)**

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**Abstract:**

The zebrafish *Danio rerio* is a teleost model species widely used in developmental genetics, biomedical studies, toxicology, and drug screening. Despite the interest of this species in research, little is known about its blood osmolality, which is a key parameter for diverse experiments. In this study, we measured blood osmolality using nano-osmometry at different stages of zebrafish post-embryonic development. We found that blood osmolality is close to 240 mOsm.kg<sup>-1</sup> in early larvae. It progressively increases to ca 270 mOsm.kg<sup>-1</sup> during the larval development before reaching ca 300 mOsm.kg<sup>-1</sup> after metamorphosis in juveniles and later in adults. These ontogenetic changes in blood osmolality illustrate the physiological changes in osmoregulation associated with post-embryonic development including metamorphosis. These values are of practical interest for adjusting the osmolality of fixatives and cell and tissue culture media for research using zebrafish as a model.

## **THYROID HORMONE ROLE IN ATLANTIC SALMON (*SALMO SALAR L.*) INTESTINE MATURATION AND SEAWATER ADAPTATION**

Vilma Duarte\*<sup>1</sup>, Juan Fuentes<sup>1</sup>, Marco António Campinho<sup>2</sup>, Stephen D. McCormick<sup>3</sup>

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### **Abstract:**

Salmonids undergo an important post-natal developmental transition termed smoltification where they prepare for migration from freshwater (FW) to seawater (SW). Smoltification encompasses several physiological, endocrine, and morphological changes enabling SW adaptation. The gills and the intestine as the main osmoregulatory organs play a pivotal role in SW tolerance in Atlantic salmon. The gill has been extensively studied, however little about the intestinal development during SW transfer. The intestine has the function to digest and absorb nutrients, but also osmoregulatory capacity enabling water ingestion from intestinal fluid processing and net water absorption.

Thyroid hormone (T3) regulation of mammalian post-natal intestinal development is well established. T3 is also known to be involved in smoltification and here we pursue the hypothesis that T3 may be involved in Atlantic salmon intestinal development prior to and during SW adaptation. T3 status was manipulated in FW parr via feeding for 4 weeks with commercial feed with T3 (20 mg/kg feed), methimazole (MMI; 5000 mg/kg feed) or T3+MMI (20 mg/kg feed + 5000 mg/kg feed). Fish were sampled at the end of FW period and transferred to 25 ppt SW for 2 days. In both time points, blood and anterior and posterior intestine were sampled.

MMI treatment resulted in reduced plasma T3 levels. Analysis on intestinal expression of T3 cellular signaling genes (receptors, deiodinases and transporters) together with intestinal morphological changes are used to determine the role of T3 in SW adaptation of Atlantic salmon.

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**EFFECT OF GROWTH HORMONE ON BK CHANNEL EXPRESSION IN GILLS FROM ATLANTIC SALMON (IN-VITRO).**

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**Abstract:**

Smoltification or Parr-smolt transformation engages several endocrine signaling systems. The light–brain–pituitary axis is stimulated by size- or growth-related threshold, resulting in higher levels of growth hormone, cortisol, and thyroid hormones. The BK potassium channel is one of the most ubiquitously expressed channels in vertebrates and it is expressed in the gills of Atlantic salmon. The expression of the BK channel decreases during smoltification, but the hormonal mechanisms that control this repression are not known so far. As a first approach for the study, we carried out in vitro experiments and evaluated the effect of different hormones on the expression of the potassium channel in Atlantic salmon gills.

We detected BK channel mRNA in this in-vitro model of gills from Atlantic salmon with real-time PCR. In addition, we were able to conduct a time-course curve to evaluate the effect of BK channel and NKA-alpha1a and NKA-alpha1b subunit expression in this in-vitro model of gills up to one day of stimulation.

Acknowledgments: Fondecyt Regular 1180957 ; VIDCA-UACH and Fondecyt Regular 1190857

## **THE ROLE OF CORTISOL IN THE INTESTINAL BICARBONATE SECRETION OF ATLANTIC SALMON**

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<sup>4</sup>University of Massachusetts - Amherst, Amherst, United States

### **Abstract:**

Intestinal bicarbonate secretion (BCS) is a central process in the hypo-osmoregulatory mechanisms of seawater fish. The study of this process is especially interesting in the Atlantic salmon (*Salmo salar*) since they undergo preparatory changes for seawater entry during smoltification. However, little is known about BCS and its hormonal control in this species. Therefore, we investigated the regulatory role of cortisol in intestinal BCS and other osmoregulatory responses in Atlantic salmon. Atlantic salmon post-smolts in freshwater (FW) were given intraperitoneal implants with 0 g g<sup>-1</sup> or 40 g g<sup>-1</sup> cortisol. Twelve days after injection, half of the fish were exposed to 30 ppt seawater (SW) for 48 h. Anterior and posterior intestine were functionally characterized in Ussing chambers, and plasma and intestinal fluid were analyzed. Plasma osmolality and chloride concentration significantly increased in the control group after 48 h in SW. Cortisol caused a reduction of the transepithelial electrical potential and transepithelial resistance in FW post-smolts. The *in vitro* measurements of BCS were not affected by cortisol, but the mass of intestinal carbonate aggregates collected in the intestine of SW fish was significantly lower in the cortisol group. Our results indicate that cortisol has either a direct or indirect effect on the accumulation of carbonate aggregates in the gut of the Atlantic salmon after SW exposure.

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## **OSMOTICALLY SENSITIVE TRANSCRIPTION FACTORS IN MOZAMBIQUE TILAPIA PROLACTIN CELLS**

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### **Abstract:**

In euryhaline fish, prolactin (PRL) plays a key role in freshwater acclimation. Consistent with its role in maintaining ion balance, PRL release is directly stimulated by a fall in extracellular osmolality. Recently, we identified several putative transcription factors (TFs) and TF modules predicted to bind promoter regions of the two prl genes, prl177 and prl188, in Mozambique tilapia, *Oreochromis mossambicus*. We investigated the osmotic sensitivity of these TFs in PRL cells incubated at 20, 26 and 32 °C from fish acclimated to either fresh water (FW) or seawater (SW). Specifically, we characterized the responses of TF transcripts in dispersed PRL cells under a range of medium osmolalities (280, 300, 330, 355 and 420 mOsm/kg) for 6 h. TF activation in the rostral pars distalis, which is comprised of >95% PRL cells, of FW- and SW-acclimated fish was also measured. TFs that were previously found to be abundantly expressed in tilapia PRL cells, including pit1 and stat3, were inversely related to extracellular osmolality in SW-acclimated fish, but not in FW-acclimated fish. Patterns of TF activation in response to changes in environmental salinity were similar to those of transcriptional responses. The hyposmotic effect on stat1 was disrupted at 20 °C. These results indicate possible linkages between osmotic cues and the transcriptional regulation of prls, and their modulation by temperature and salinity acclimation history. [Supported by HATCH (#HAW02051-H), NOAA (#NA18OAR4170347), NIH (1R21DK111775-01) and NSF (IOS-1755016) to A.P.S.]

**ISOTOCIN SIGNALING MEDIATES OCEAN ACIDIFICATION-ENHANCED EPITHELIAL H<sup>+</sup> SECRETION IN MARINE MEDAKA**

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<sup>1</sup>Institute of Cellular and Organism Biology, Academia Sinica, Taipei, Taiwan

**Abstract:**

A major facet of global climate change is the acidification of the ocean induced by elevated atmospheric CO<sub>2</sub>. Fish are known to defend their extracellular pH by increasing blood bicarbonate buffering and stimulating H<sup>+</sup> secretion from ion-regulatory epithelial tissues in response to hypercapnia. Acid-base regulation is tightly regulated by the endocrine system in various vertebrate species, however, hormonal control of acid-base adjustment in marine fish upon exposure to acidified seawater remains largely undescribed. Here, by using marine medaka as a model, we identified isotocin, a neurohypophysial hormone, as a prominent factor involved in the regulation of epithelial H<sup>+</sup> secretion in marine fish under ocean acidification. The transcript levels of one particular isotocin receptor, ITRb, were found upregulation in marine medaka acclimated to hypercapnia, and knockdown of ITRb impaired both basal and hypercapnia-induced H<sup>+</sup> secretion in the yolk-sac skin. This effect was accompanied by a reduction of cAMP level, indicating that ITRb may act via cAMP signaling. Moreover, a transmembrane-associated adenylyl cyclase, ADCY5, was upregulated under hypercapnia, and was co-expressed with ITRb in ionocytes, suggesting that isotocin-mediated mechanisms of acid-base regulation may involve ADCY5. These findings revealed an endocrine mechanism by which marine teleosts may undergo physiological adaptation to climate change.

## **LOCAL GILL ENERGY SUPPLY TO COPE WITH HYPERSALINITY IN EUROPEAN SEA BASS *D. LABRAX***

Quentin Robert\*<sup>1</sup>, Sophie Hermet<sup>1</sup>, Romain Gros<sup>1</sup>, Tsung-Han Lee<sup>2</sup>, Catherine Lorin-Nebel<sup>1</sup>

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<sup>2</sup>National Chung Hsing University, Taichung, Taiwan

### **Abstract:**

European sea bass are euryhaline fish inhabiting environments with high salinity fluctuations as lagoons that can become extremely salty during the summer season. Climate change induced hypersalinity is becoming more and more frequent in the Mediterranean area and requires rapid and efficient mechanisms in fish to face dehydration and diffusive ion invasions. To cope with hypersalinity, fish actively secrete ions across the gills using Na<sup>+</sup>/K<sup>+</sup>-ATPase, which is an energy-consuming pump and is known to be activated upon hypersalinity challenge. Branchial glycogen stores could be involved in local energy supply to support energy-dependent transepithelial ion transport in fish. Glycogen phosphorylase (GP) is an enzyme involved in catabolizing glycogen into glucose and glucose-6-phosphate. GP is rapidly activated following a transfer from fresh- to seawater in another teleost species which suggests that GP activity might be important in fish that have to cope with salinity changes in their habitats. Following salinity transfer, a raise in GP activity at the gill level could indicate local glucose supply for ion transport. In this study, we challenged *D. labrax* to either seawater at 36 ppt or hypersaline water at 55 ppt and sampled their blood and their gills after short- and long-term salinity transfer to determine if hypersalinity affects the number and localization of glycogen-rich cells. Glycogen phosphorylase activity levels have also been measured as well as blood parameters (blood osmolality, glucose, cortisol) to determine overall hydro-mineral homeostasis and stress status.

## **GILL AMMONIA EXCRETION IN HIGHLY ALKALINE WATERS: THE ROLE OF EXTRACELLULAR CALCIUM IN BRANCHIAL PERMEABILITY**

Patricia Ferreira\*<sup>1</sup>, Xena Montoya<sup>1</sup>, Jonathan Wilson<sup>1</sup>

<sup>1</sup>Wilfrid Laurier University, Waterloo, Canada

### **Abstract:**

Increasing water hardness or divalent cation concentrations (eg calcium) has been shown to increase the survival of fish transferred to high pH water. Ammonia is the main nitrogenous waste product in teleosts, which is excreted predominantly across the branchial epithelium. However, it is neurotoxic if allowed to accumulate. Exposure to high pH waters inhibits ammonia excretion by decreasing the ammonia partial pressure gradient (PNH<sub>3</sub>) leading to ammonia accumulation in the body that can be lethal to the fish. Previous studies have presented evidence that the role of divalent cations, mainly Ca<sup>2+</sup>, may be in enhancing and recovering ammonia excretion rates in fish exposed to highly alkaline waters.

Nevertheless, the exact mechanisms behind this recovery remains unexplained. It has also been proposed that water hardness might modulate the paracellular permeability of the gills. It is hypothesized that high Ca<sup>2+</sup> concentration in the water will direct the upregulation of claudins in the branchial tight junctions, thus decreasing paracellular permeability. This decrease in permeability is likely to induce compensatory transcellular ionic uptake mechanisms and aid the restoration of the PNH<sub>3</sub>. Claudin-14 has been shown to actively modulate paracellular Ca<sup>2+</sup> transport. We expect to detect changes in Cldn14 protein expression levels in the gill of *Astyanax mexicanus* exposed to soft and hard alkaline waters (pH 10).

ABSTRACT N° ICBF22-204

## **DO YOU NEED A BIG NOSE TO HAVE A GOOD SENSE OF SMELL? SNOUT MORPHOLOGY AND OLFACTION IN SHARKS**

Stefano Aicardi\*<sup>1</sup>, Alessio Longo<sup>1</sup>, Sara Ferrando<sup>1</sup>

<sup>1</sup>University of Genoa, Genoa, Italy

### **Abstract:**

The first step of the olfactory process is the transport of odorants from the environment to the sensory surface. In shark, the water flows into the olfactory chamber because of sea water currents or because of the forward swimming movements. Also, in sedentary elasmobranchs, the proximity of the nostrils and the mouth can couple the buccopharyngeal pump to the nasal irrigation. Taken together, the shape of snout, mouth, and nostrils, besides reflecting the lifestyle and the phylogeny, could be linked to the olfactory capability of a species. Here, the shape of the head of more than 450 shark species, in ventral view which allows to see the nostrils and the mouth's rim, was analyzed by geometric morphometrics, with the aim of delineating different morphospaces. For the analyzed species, available data regarding phylogeny, tail morphology (that's also linked to swimming rate), and olfactory organ shape were correlated with the morphospaces based on the head/nostrils/mouth. Our results show that the head shape is influenced by phylogeny, at least at lower taxonomic levels (e.g. genus, family). The overall analyses show that it is not possible to explain the head shape just using phylogeny, or other anatomical features, even though clearly linked to functional aspects. On the contrary, the weight of each character in the correlation with the head shape – that's likely to affect the waterflow in the olfactory chamber - seems to be different in different shark taxa.

**MATERNAL EFFECTS OF TRAINING ON MAXIMAL SWIMMING PERFORMANCE AND CRITICAL THERMAL TOLERANCE OF JUVENILE BROWN TROUT (SALMO TRUTTA)**

Ulla Saarinen\*<sup>1</sup>, Katja Anttila<sup>1</sup>, Luca Pettinau<sup>1</sup>

<sup>1</sup>University of Turku, Turku, Finland

**Abstract:**

Heatwaves cause a notable threat to the ectothermic animals; whose body temperature depends entirely on the ambient temperature. It is predicted that heatwaves will become more protracted, intense and frequent due to climate change. Prolonged high temperatures negatively affect several aspects of fish physiology and biology, for example they may cause internal oxygen deficiency which consequently causes complications in the swimming performance.

Although heatwaves are great concern regarding all aquatic life, a particular concern are farmed fish and their stocked offspring. Farmed fish have weaker heart function and over-all compromised physiological performance as well as a considerably lower swimming capacity compared to their wild conspecifics. Therefore, their ability to tolerate heatwaves might be also compromised. Moreover, the main strategy for the conservation of wild stocks is the release of hatchery-produced fish to nature. However, the stocked offspring of these farmed fish have extremely low changes to survive till maturity.

The aim of this study was to explore whether the offspring of swim trained hatchery brown trout (*Salmo trutta*), which cardiac function has improved via training, have inherited beneficial traits that could improve their swimming capacity and plasticity to adjust to heatwaves. For this purpose, we exposed the offspring (two different life-stages: fry and parr) of trained and non-trained dams to a 3 week heatwave-period, and measured their critical swimming speed (UCRIT) and critical thermal maximum (CTMAX) after the exposure.

## **EFFECTS OF ANNUAL THERMAL REGIME ON GROWTH TRAJECTORIES OF NATIVE AGE-0 BROOK TROUT**

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<sup>1</sup>Northern Michigan University, Marquette, MI, United States

### **Abstract:**

The rapidly increasing temperatures of aquatic systems associated with climate change represent a novel threat to populations of brook trout (*Salvelinus fontinalis*), and much uncertainty remains about the potential of these populations to acclimate, adapt, or compensate for such changes. Brook trout are considered stenothermal and are restricted to watersheds with adequate thermal refugia to withstand warm summer temperatures while providing appropriate winter incubation and rearing temperatures. With the onset of climate change, the long-term success of the species will depend on its ability to cope with, and adapt to, rapidly changing thermal conditions. The current study evaluated growth patterns of wild young-of-the-year brook trout in two tributaries in close proximity with dramatically different annual thermal regimes. Using a common garden design, we then evaluated the effect of variable incubation temperatures on egg size, developmental phenology, yolk sac allocation, hatch timing, and size for the same populations at hatch under laboratory conditions. The results of the field work demonstrated wide variability between populations, individuals, and cohorts. Laboratory studies suggested that population differences were largely mitigated by the effect of the environment, and that the incubation conditions appeared to have a strong moderating effect on population differences in early development. The results suggest evidence for plasticity in the developmental response of brook trout, offering resiliency to the effects of climate change.

ABSTRACT N° ICBF22-307

## **THE EFFECT OF TEMPERATURE AND BODY SIZE ON HEART RATE IN DIPLOID AND TRIPLOID ATLANTIC SALMON.**

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<sup>1</sup>Star-Oddi Ltd, Gardabaer,

<sup>2</sup>Marine and Freshwater institute of Iceland (MFRI), Grindavik, Iceland

### **Abstract:**

Triploidization is a controversial measure that has been used to induce sterility in salmon farming to prevent the negative effects of precocious sexual maturation, such as growth depression, reduced flesh quality and genetic introgression. The present study compared surgical recovery, growth performance and heart rate of farmed diploids (2n), triploids (3n) and all-female triploids (3nx) in three separate growth trials over a wide range of temperature (2.6 – 17.3°C) at different life-stages (150 g – 12.5 kg), using two types of implantable heart rate and temperature loggers. Surgical recovery time was strongly temperature dependent. Mortalities were only observed for the smallest fish (mean weight 160 g) at the lowest temperature (2.6°C) and tagging was otherwise not seen to affect growth performance. Long-term growth performance over the 27-month study period was best in the 3nx group, and the final mean weights were 7526, 8738, and 9435 g in the 2n, 3n, and 3nx groups, respectively. The 3nx group had the lowest mean heart rate overall but differences in size-adjusted heart rate across ploidies were not significant. A polynomial relationship between temperature and heart rate, and a logarithmic relationship between body size and heart rate for all ploidies, were used to construct a heart rate model for Atlantic salmon, predicting heart rate based on the input of body weight (< 1 g) and temperature (0 – 16°C) under a 24:0 photoperiod.

ABSTRACT N° ICBF22-325

**GROWTH AND POPULATION DYNAMICS OF THE COMMON MEAGRE  
ARGYROSOMUS REGIUS (TELEOSTEI: SCIANIDAE) OFF THE MOROCCAN  
SAHARAN ATLANTIC COASTAL WATERS**

Mohammed Znari<sup>1, 2</sup>, Hanane Aharroy<sup>3</sup>, Mohamed Naimi<sup>2, 4</sup>, Jamal Bensbai<sup>3</sup>, Abdelaziz Mounir<sup>1, 2</sup>, Hayat Elmghazli<sup>1, 2</sup>

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**Abstract:**

Common Meagre, *Argyrosomus regius*, represents one of the fish of great economic interest of the Moroccan Saharan Atlantic zone. The objective of this study was to assess the parameters of linear growth, mortality, and the state of exploitation of this species in the Dakhla fishing area. Individual age was estimated by scalimetry and monthly length-frequency data were analyzed by FiSAT II software for the evaluation of the population parameters which include the asymptotic length (L, cm), the growth rate (K). The asymptotic length was 213 and the growth rate (K, year<sup>-1</sup>) was 0.075 while the growth performance index (GPI) was 5.53. Corrected for body size by an auximetric analysis, the growth rate was similar to that of other congeneric species but much lower than other Sciaenids. Total mortality (Z) estimated by the capture of converted length curve was 0.56 year<sup>-1</sup>, fishing mortality (F) 0.4 year<sup>-1</sup>, and natural mortality (M) of 0.16 year<sup>-1</sup>. The exploitation level (E) of *A. regius* was then 0.71, a level (E > 0.50), which is higher than the optimal exploitation efficiency, indicates that the fishing pressure on *A. regius* is very high. Thus, the status of the stock in the Moroccan south Atlantic zone seems to be over-exploited. This would require fishing regulatory measures to ensure the sustainability of the resource.

ABSTRACT N° ICBF22-489

**THE THERMAL TOLERANCE, METABOLIC CAPACITY, AND STRESS RESPONSE OF LUMPFISH (CYCLOPTERUS LUMPUS) REARED UNDER DIFFERENT TEMPERATURE REGIMES**

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<sup>1</sup>Memorial University, St. John's, Canada

**Abstract:**

The use of lumpfish as a “cleaner fish” in Atlantic salmon aquaculture is increasing. However, during the summer months, there have been large-scale mortalities of lumpfish at some North Atlantic salmon sea-cage sites. Thus, the metabolic physiology and upper thermal limits of lumpfish are being investigated, with the goal of better understanding their physiology and mitigating the effects of high summer water temperatures on their welfare. Initially, we investigated to what extent aerobic scope of 6°C-acclimated lumpfish differed when given a critical swim speed (Ucrit) test vs. a critical thermal maximum (CTMax) test (at 2°C h<sup>-1</sup>) vs. being chased to exhaustion. The Ucrit and CTMax of these 45-75 g lumpfish were 2.36±0.08 body lengths s<sup>-1</sup> and 20.6±0.3 °C respectively, and the aerobic scope of fish given these tests was similar (216.6±8.1 vs. 189.0±9.7 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>, p=0.16), and much greater than fish given a chase (131.7±8.1 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>). More recently, we have been examining if changing temperature regimes during embryonic development (incubation) and larval/juvenile rearing influence the lumpfish's metabolic capacity, upper thermal tolerance (CTMax and incremental thermal maximum, ITMax) and stress physiology. The specific incubation/rearing temperature combinations are 6°C/9°C (normal production temperatures), 8.5°C/9°C, 6-11°C/9°C, 8.5°C/9-11°C and 6-11°C/9-11°C, with a range of temperatures indicating that fish were exposed to stochastic temperature changes. This research will provide valuable information on whether lumpfish upper thermal tolerance and metabolic capacity are plastic (i.e., whether their thermal tolerance/welfare in sea-cages might be improved by altering rearing protocols).

## **LARVAL FISH DEVELOPMENT FOLLOWING EXPOSURE TO ULTRAVIOLET RADIATION AT DIFFERENT REARING TEMPERATURES**

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<sup>1</sup>University of North Texas, Denton, United States

### **Abstract:**

Anthropogenic input of greenhouse gases into the atmosphere have led to an increase in sea surface temperatures. Coping with multiple stressors can exacerbate the negative impacts as the ability to tolerate one stressor can reduce the ability to cope with a secondary stressor. Larval fish are extremely vulnerable to UV radiation due to their lack of pigmentation. Developmental UV radiation has previously been shown to increase metabolic rate, cause morphological deformities, and alter behavior. Rearing temperature greatly influences the rate of development and can lead to differences in ability to tolerate secondary stressors. To date, few studies have investigated the combined effects of UV and rearing temperature on larval fish, and those that have focused primarily on UV B radiation. The aim of this study is to examine the effects of UV radiation, with a focus on UV A, at different rearing temperatures on the development and performance of larval fish. Following exposures, larvae will be assessed for morphological deformities, yolk sac depletion, and metabolic rate. Based on previous work, we expect to see increases in morphological deformities, yolk sac depletion, and metabolic rate when co-exposed to UV and higher rearing temperatures. These changes could have significant implications for early life stage survival.

**EFFECTS OF OESTRADIOL-17 INDUCED SEX REVERSAL ON SEX-BIASED GENE EXPRESSION IN SNAKESKIN GOURAMI (TRICHOPODUS PECTORALIS)**

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<sup>1</sup>Suranaree University of Technology, Nakhon Ratchasima, Thailand

**Abstract:**

This study aimed to investigate the effects of oestradiol-17 (E2)-treated sex reversal by oral administration on sex-biased gene expression in snakeskin Gourami (*Trichopodus pectoralis*). The experimental treatment included dietary E2 supplementation at 0 (control) and 200 (E2-200) mg kg<sup>-1</sup> diet. Fry (7 days post hatching; dph) were fed experimental diets for 90 days. Subsequently, experimental fish were cultured and fed with commercial diet through adult stage. Control male and female and sex reversed female were sampling for determination of expression of several gonadal genes involved in sex differentiation and gonadal development including bHLH, cyp19a1, daz, dead-end, esrb, esrrg, gnrhr, gpa, gsg1l, hsd17, mospd, nanos-2, p53, piwi-2, rerg, rps6ka, tgf-beta, VgR except for ar, nanos-1 and piwi-1. The results showed that compared to ovaries, upregulation in testes was found in esrb, gsg1l, gpa, gnrhr, mospd, nanos-2, rerg and rps6ka. However, the expression of bHLH, cyp19a1, daz, dead-end, esrrg, hsd17, p53, piwi-2, tgf-beta and VgR were higher in ovaries. Similar expression patterns were found in the ovary of control female and E2-200 sex-reversed fish. These results suggested that at molecular level, E2-sex reversed female would exhibit similar gonadal development to female fish.

## **IN VIVO MR IMAGING AS A MONITORING TOOL FOR GONADAL GROWTH IN FISH**

Nicole Vogt\*<sup>1</sup>, Daniela Storch<sup>1</sup>, Felizitas C. Wermter<sup>2</sup>, Christian Bock<sup>1</sup>

<sup>1</sup>Alfred-Wegener-Institut, Bremerhaven,

<sup>2</sup>University of Bremen, Bremen, Germany

### **Abstract:**

An in vivo approach of magnetic resonance imaging (MRI) will be presented to follow the gonadal development of unanaesthetised female and male fish. Individual fish were placed in the MR scanner that was continuously perfused with aerated seawater. A series of specialized 3D MRI techniques were used to visualise the morphology of various organs, including the gonads. The resolution of the MR images allowed for specific sex determination, calculation of specific organ volumes, and determination of egg sizes. Further, we adapted the specific RARE (rapid acquisition with relaxation enhancement) and MSME (multi slice multi echo) methods to determine T1 and T2 values, which might be used to distinguish male and female polar cod even when they are in a non-reproductive state. The first screening procedure was carried out on a set of reproductive Polar cod (*Boreogadus saida*) every two to four weeks. Gonad maturation was followed from November until shortly before spawning in February. The presented techniques have great potential for various applications such as monitoring restricted or delayed development or sex determination under different environmental conditions.

**EVIDENCE OF INCIPIENT SEX DEVELOPMENT IN CYP19A1 METHYLATION PATTERNS IN A CICHLID FISH**

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<sup>2</sup>Reed College, Portland,

<sup>3</sup>University of Rochester, Rochester, United States

**Abstract:**

For *Pelvicachromis pulcher*, an African cichlid in which early pH exposure influences both sex and alternative male morph, we sequence both copies of aromatase (*cyp19a1*), a key gene for sex determination. We analyze gene expression and epigenetic state, comparing gonad and brain tissue from females, alternative male morphs, and fry. Relative to brain, we find elevated expression of the A-copy in the ovaries but not testes. Methylation analysis suggests strong epigenetic regulation, with one region specifying sex and another specifying tissue. We find elevated brain expression of the B-copy with no sex or male morph differences. B-copy methylation follows that of the A-copy rather than corresponding to B-copy expression. In 30-day old fry, we see elevated B-copy expression in the head, but we do not see the expected elevated A-copy expression in the trunk which would reflect ovarian development. Interestingly, the A-copy epialleles that distinguish ovaries from testes are among the most explanatory patterns for variation among fry, suggesting epigenetic marking of sex prior to differentiation and thus laying the groundwork for mechanistic studies of epigenetic regulation of sex and morph differentiation.

**HOMEBOX GENES IN THE REGULATION OF FEEDING OF GOLDFISH  
CARASSIUS AURATUS**

Helene Volkoff<sup>1</sup>, Kelsey Vinnicombe<sup>1</sup>

<sup>1</sup>Memorial University of Newfoundland, St John's, Canada

**Abstract:**

Homeobox genes play an important role in embryonic development and have been shown to regulate feeding by acting as transcription factors of appetite regulators. Examples of these genes are a brain-specific homeobox transcription factor (BSX), NK2 homeobox 1 (NKX2.1) and the Iroquois homeobox 3 (IRX3). Very little is known about the role of these genes in the regulation of feeding and nutrient homeostasis in fish. In this study, we assessed the effects of fasting and cholecystokinin injections on their brain expression in goldfish. Our results suggest that, in goldfish as in mammals, central BSX, NKX2.1 and IRX3 are present in regions of the brain regulating feeding, and that they are sensitive to nutrient status and interact with appetite-regulating peptides.

## **MEASUREMENT OF CIRCULATING INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-2B IN RAINBOW TROUT**

Ayaka Izutsu\*<sup>1</sup>, Shiori Habara<sup>1</sup>, Daiji Tadokoro<sup>2</sup>, Munetaka Shimizu<sup>3</sup>

<sup>1</sup>Graduate School of Environmental Science, Hakodate,

<sup>2</sup>FRD Japan, Co., Kisarazu,

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### **Abstract:**

Insulin-like growth factor binding proteins (IGFBPs) stabilize levels of IGF-1 in the circulation and modulate growth in vertebrates. Three IGFBPs, IGFBP-2b, -1a, and -1b, are consistently detected in salmon circulation and useful as indices of positive or negative growth. However, their applicability to rainbow trout (*Oncorhynchus mykiss*) is still unclear. We examined the responses to fasting and refeeding of circulating IGFBPs and their relationships with growth in rainbow trout. Ligand blotting using digoxigenin-labeled human IGF-1 was used to detect and semi-quantify the bands of IGFBPs in trout serum. Ligand blotting revealed that IGFBP-1a and -1b were hardly detected even in the fasted fish, suggesting they may not serve as negative growth indices in rainbow trout. In contrast, the band intensity of serum IGFBP-2b had a positive relationship with individual growth rate, indicating that its quantification is useful to monitor growth status in this species. In order to establish a time-resolved fluoroimmunoassay (TR-FIA) for IGFBP-2b, we produced a recombinant trout (rt) IGFBP-2b with fusion partners (thioredoxin, Trx, and His-tag) using a bacterial expression system and used it for europium (Eu)-labelling. Eu-Trx.His.rtlIGFBP-2b showed a cross-reactivity with an antiserum against purified Chinook salmon IGFBP-2b and its binding was displaced by adding non-labeled protein and trout serum, suggesting that these components are useful to establish the TR-FIA.

## **SELECTED WAVELENGTHS OF LIGHT STIMULATE THE GROWTH ENDOCRINE AXIS IN THE MALABAR GROUPE**

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### **Abstract:**

Growth performance of fish is influenced by cues in environmental factors, in which light conditions including duration of exposure (photoperiod), intensity of exposure (illumination), and quality (light spectrum) play a crucial role in maximizing growth performance. The present study aimed to clarify how selected light spectrum is perceived and transduced as an internal signal to stimulate growth performance of the Malabar grouper *Epinephelus malabaricus*, which is an important aquaculture species in Asian countries. Four opsins (RH1, RH2, SWS2, and LWS) could be found by searching the database of photoreceptors. Reverse transcription polymerase chain reaction (PCR) revealed that these opsins were expressed not only in the eyes but also in the brain. When fish were reared under conditions of long-day (LD = 14:10) with red (peak at 632nm), green (519 nm), and blue (465 nm) LED and natural lights, specific growth rate (SGR) was higher in fish exposed to blue LED light. Rearing fish under blue LED light resulted in high transcript levels of rhodopsin (RH1) in the eyes and of short-wavelength sensitive (SWS2) in the brain, suggesting that selected light spectrum activates photoreceptors in light perceptive organs. The transcript levels of growth hormone and insulin-like growth factor (IGF-I) increased in the pituitary and liver of fish reared under blue LED light, respectively. Moreover, similar increases in the transcript levels of neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) in the brain of fish reared under blue LED light. It is concluded that blue light perceived by visual opsins in the eyes and/or the brain activates the growth endocrine axis and its interaction with NPY, and that, consequently, high growth performance is obtained in fish reared under a suitable range of selected light spectrum.

## **THE EMERGING ROLE OF LONG NON-CODING RNAs IN DEVELOPMENT AND FUNCTION OF GILTHEAD SEA BREAM (*SPARUS AURATA*) FAST SKELETAL MUSCLE**

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### **Abstract:**

Long non-coding RNAs (lncRNAs) are a group of ncRNAs that can modulate gene expression at transcriptional and translational level. While several lncRNAs have been characterized in humans and model species, their role in fish is still largely unknown. In this study, previously published transcriptomic data were used to identify lncRNAs expressed in gilthead sea bream white skeletal muscle. The cellular localization of lncRNAs, as well as their target mRNAs and microRNAs were predicted. The transcriptional profile of lncRNAs was analyzed in different ontogenetic stages, in response to a nutritional challenge, during muscle regeneration and throughout a myocyte cell culture. Two hundred and ninety lncRNAs were identified and seven lncRNAs appeared to be potentially important for muscle development based on transcriptomic differences between juveniles and adults. Our data suggested that the downregulation of most of the studied lncRNAs might be related to increased myoblast proliferation, while their upregulation could be necessary for differentiation. Although with these data it was not possible to propose a formal mechanism to explain their effect, the bioinformatic analysis suggested two possible mechanisms. First, the lncRNAs may act as sponges of myoblast proliferation inducers microRNAs such as miR-206, miR-208, and miR-133 (minimal free energy < 25.0 kcal). Secondly, lncRNA20194 had a strong predicted interaction towards the myod1 mRNA (ndG = 0.17) that, based on the positive correlation between the two genes, might promote its function. Our study represents the first characterization of lncRNAs in gilthead sea bream fast skeletal muscle and provides evidence regarding their involvement in muscle development.

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## **INTERACTION BETWEEN THE EFFECTS OF SUSTAINED EXERCISE AND DIET COMPOSITION ON REDOX STATUS IN GILTHEAD SEA BREAM (SPARUS AURATA)**

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### **Abstract:**

Physical exercise and a balanced diet present substantial health benefits and could improve fish production. Nevertheless, the redox balance can be affected by training regimen, dietary macronutrient ratio and their interaction. In this study, we conjointly evaluated the effects of diet composition and exercise on oxidative stress status in liver, white and red muscle in gilthead sea bream juveniles. Fish were maintained for 6 weeks under voluntary or sustained swimming (2.5 BL/s) and fed with a high-protein or high-lipid commercial diets. The high-lipid diet increased reduced thiols and the biochemical redox markers associated with lipid and protein peroxidation in the different tissues. Exercise increased these markers in liver but reduced lipid peroxidation in white muscle. Regarding oxidative stress, chaperones and apoptosis-related gene expression, the fish under voluntary swimming and fed with the high-lipid diet generally showed the highest mRNA levels, whereas the group fed with the high-protein diet, the lowest values. Nevertheless, in fish subjected to exercise, those differences were partially equalized. Fish fed with the high-lipid diet prioritized the superoxide dismutase and catalase activities, and the high-protein fed groups, the glutathione-associated enzymes. Exercise also modified enzyme activity, but in a tissue-dependent manner. Overall, the redox balance in gilthead sea bream juveniles is modulated by diet composition and physical activity although the response will partly depend on the interaction between these factors and the tissue studied. It is suggested that the application of sustained exercise could be used in fish production to improve the redox status, particularly for high-lipid diets. Funded by MINECO AGL2015-70679-R and RTI2018-100757-B-I00.

ABSTRACT N° ICBF22-377

**IMPROVING THE AEROBIC PERFORMANCE OF SKELETAL MUSCLE IN EUROPEAN SEA BASS: SUSTAINED EXERCISE TO BOOST UP MITOCHONDRIAL FUNCTION**

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**Abstract:**

Sustained swimming induces beneficial effects on growth and energy metabolism in diverse fish species. However, the anatomical, behavioral, and physiological differences among species points out that there is not any universal workout that guarantees an optimal response to exercise. The objective of this study was to assess the metabolic response of the European sea bass to a continuous and moderate exercise exposure. An exercise trial with 600 sea bass yearlings (3-5g body weight) was carried out distributing in two groups (Control: voluntary swimming; exercised; sustained swimming at 1.5 body lengths·s<sup>-1</sup>). After 6 weeks, growth parameters were not affected by sustained swimming, but an increased synthetic capacity (increased RNA/DNA ratio) and more efficient use of proteins (decreased N15) was observed in white muscle. The mitochondrial proteins in white muscle were not affected at gene expression level by exercise, but an increase in the UCP3 protein amount as well as increased COX/CS ratio at protein and activity level points out to an enhanced oxidative capacity of this tissue. Interestingly, the red muscle of exercised fish presented transcriptional downregulation of tFAM, PGC1, CS and TMEM20a. However, CS presented a high increase at protein level, indicating a negative feedback regulation of the transcription of such gene and increased lipid oxidation in this tissue. Such metabolic adaptation to sustained exercise was then reflected in an enhanced maximal oxygen consumption rate and in the aerobic scope during tunnel swim stress test in comparison to non-trained fish. Supported by MICIUN (AGL2015-70679-R and RTI2018-100757-B-I00). M.P-A grant (BES-2016-078697).

ABSTRACT N° ICBF22-382

**PLASTICITY OF MUSCLE MITOCHONDRIA IN GILTHEAD SEA BREAM:  
COMBINED EFFECTS OF SUSTAINED SWIMMING AND HIGH-  
CARBOHYDRATE DIET**

Isabel García-Pérez<sup>1</sup>, Lucía Aragón-Serrano<sup>1</sup>, Adrià Robles-Briones<sup>1</sup>, Albert Sánchez-Moya<sup>1</sup>, Miquel Perelló-Amorós<sup>1</sup>, Jaume Fernández-Borràs<sup>1</sup>, Joaquim Gutiérrez<sup>1</sup>, Josefina Blasco<sup>1</sup>

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**Abstract:**

In gilthead sea bream fed a high-lipid diet, sustained exercise induces tissue specific mitochondrial adaptations towards more efficient lipid use while promoting a protein sparing effect. However, there is no evidence about this beneficial response in fish fed high carbohydrate diets. This study aims to examine the combined effects of swimming activity [voluntary swimming; exercise] and diet composition [high-protein diet; high-carbohydrate diet] on growth performance, aerobic capacity and mitochondrial energy metabolism in gilthead sea bream juveniles. After 6 weeks, fish fed the high-protein diet and subjected to exercise presented higher body weight and lower hepatosomatic and mesenteric fat indexes. In white muscle, the high-carbohydrate diet induced an increase in glycogen while decreasing the lipids. Exercise and diet did not have a significant impact on the energetic cost of swimming in an extenuating swimming test, but in the recovery phase, the exercised fish seemed to consume more oxygen. The gene expression of energy metabolism and mitochondrial biogenesis markers in red muscle was clearly affected by diet composition. The groups fed the high-carbohydrate diet showed an upregulation of *pgc1*, *ucp2*, *ucp3*, *cpt1a*, *cpt1b* and *sirt1*, suggesting an imbalance between energy demand and supply. Exercise increased the transcription of the mitochondrial fusion inducer *mit1*, indicating an optimization of energy balance in this group. The gene expression of these mitochondrial proteins in white muscle and liver will be presented and discussed to obtain an overall vision of the mitochondrial metabolic status of the fish in these experimental conditions.

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ABSTRACT N° ICBF22-288

**COMPARATIVE TRANSCRIPTOMIC ANALYSIS REVEALS CONTRASTING FEATURES MEDIATED BY GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS IN RAINBOW TROUT.**

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**Abstract:**

Cortisol is an essential regulator of neuroendocrine stress responses in teleost. Cortisol exerts its effects through the activation of glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), however the role of both receptors in skeletal muscle is unknown. To understand in a comprehensive and global manner how GR and MR modulates the skeletal muscle transcriptomic response, we performed an RNA-seq analysis. Juvenile rainbow trout (*Oncorhynchus mykiss*) were intraperitoneally injected with physiological doses of cortisol (1 mg/kg). We also include a pre-treatment with mifepristone (GR antagonist) and spironolacton (MR antagonist). cDNA libraries were constructed from the skeletal muscle of rainbow trout groups: vehicle, cortisol, spironolactone, mifepristone, mifepristone/cortisol and spironolacton/cortisol groups. The expression analysis revealed that 1271 and 979 transcripts were differentially expressed mediated by GR and MR, respectively. In GR group, BP were significantly enriched in ubiquitin-dependent protein catabolic process, myofibril assembly, and autophagy. In MR group, BP were significantly enriched in mitotic nuclear division, nuclear division, and striated muscle cell development. These results suggest that GR and MR have a differential participation in fish muscle growth. Funded by FONDAP 15110027 and FONDECYT 1201498

ABSTRACT N° ICBF22-327

**LONGEVITY-RELATED SIRTUIN EXPRESSIONS AND METABOLIC RESPONSES IN TILAPIA AFTER PHENOLIC COMPOUND RESVERATROL SUPPLY UNDER COLD STRESS**

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**Abstract:**

Temperature is one of the critical abiotic factors, as it determines the distribution, behaviors and physiological responses of animals. Tilapia is a common aquaculture teleost of high ecological and commercial importance in the world; however, there are several serious problems in related industries. One of the most concerns is the winter cold front that always resulted in large-scale disease, death and consequent economic loss.

Resveratrol (3,4',5-trihydroxy-trans-stilbene, RSV) is a phenolic compound that was first isolated from *Veratrum grandiflorum*. It is demonstrated that RSV could serve as a cellular longevity molecule and is able to active sirtuins (SIRTs), a highly conserved family of NAD-dependent deacetylases, based on mammalian studies. However, clear systematic characterizations of RSV-induced biological functions in fish are still in absence. In this study, RSV supplement was found to significantly increase transcript expressions of several sirt paralogues and anti-oxidation molecules. In addition, the intact metabolic performances of tilapia would not be affected after additional RSV feeding under regular 27°C and 15°C cold conditions. Consequently, RSV-induced sirtuin expressions in tilapia may benefit to improve aquaculture practice under winter cold front stress.

ABSTRACT N° ICBF22-379

**DYNAMICS OF SEROTONERGIC RESPONSE IN BRAIN OF COHO SALMON AT IN VIVO AND TISSUE CULTURE TO DIFFERENT DOSES OF OXYTETRACYCLINE AND DIFFERENT EXPOSURE TIMES**

Jose Luis Muñoz Perez<sup>1</sup>, Danixa Martinez<sup>2</sup>, Daniela Nualart<sup>2</sup>, Oscar Mardones<sup>1</sup>, Francisco Morera<sup>2</sup>, Luis Vargas-Chacoff<sup>2, 3</sup>

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**Abstract:**

From economic and sanitary points of view, diseases are considered the most limiting factor in salmon production. In Chile the most frequently used antibiotics drug is florfenicol, followed by oxytetracycline (OTC). But these antibiotics, like any commercial pharmaceutical drug, have many side effects, also known as adverse effects. Side effects of antibiotics have been described in fish, associated with antioxidant and behavioral responses, but there are few antecedents in farmed fish, using recommended doses for treatment and at different post-treatment times. In this work the effects of OTC on serotonergic response in telencephalon and hypothalamus of Coho salmon were investigated following the administration in vivo (i.p. injections) and brain tissue culture (in vitro), of regular doses used of OTC at different times. Serotonin (5HT) levels were significantly affected, both in vivo and in vitro experiments, by both doses of OTC, showing in general a decrease in serotonin levels in the first hours and days of treatment, but at day 5 post-treatment, levels are similar to the control group. The qRT-PCR expression levels of the genes associated with the serotonergic system (TPH1, TPH2, SERT, 5HT1A, 5HT1A receptors) are directly associated with those found in the 5HT levels, varying significantly in relation to the OTC treatments and post-treatment time. These data provide important and useful information for salmonid aquaculture, such as optimizing antibiotic doses and understanding the side effects of antibiotic treatments on farmed fish. Acknowledgments: This work was funded by Fondecyt Regular N°1190857

ABSTRACT N° ICBF22-438

## **OXIDATIVE STRESS RESPONSE OF GILL-CELLS PRIMARY CULTURE IN ATLANTIC SALMON (*SALMO SALAR*) CHALLENGED WITH OXYTETRACYCLINE**

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### **Abstract:**

In Chile, the aquaculture industry represents an important economic activity; however, especially when from 1981 to date, salmon farming has been affected by various bacterial diseases, the use of antibiotics (as Oxytetracycline "OTC") to control these diseases has become necessary and thus routine. But the excessive amounts of OTC are causing side effects in fish. The aim of this study was to determinate how the gills cells are being affected by OTC in *Salmo salar*. Branchial tissue culture was performed and in periods of 0.5, 1, 3, 6, 12 and 24 h, the enzymatic activity and mRNA expression of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (Gr) and super oxydismutase (SOD), in response to two doses of OTC: 0.25 (low) and 3 µL/ml (high). The results indicated that the enzymatic activity CAT, GPX, Gr and SOD shows varied response patterns depending on the time and dose of OTC used and as a difference with respect to mRNA expression. The Na-K-ATPase(alpha) mRNA expression response (as an osmotic marker) increased mRNA levels at 3 and 6 hours in both doses. These results suggest an oxidative response of the gills to OTC exposure, and constitute significant information on the amount of OTC used in aquaculture and how to improve the optimal dose of drugs, fish health and, consequently, environmental health. Acknowledgments: Fondecyt project 1180957 and 1190857, Fondap Ideal Center 15150003, ANID-Millennium Science Initiative Program-Center code "ICN2021\_002".

ABSTRACT N° ICBF22-463

## **LOSS OF THE GLUCOCORTICOID RECEPTOR ACCELERATES OVARIAN AGEING IN ZEBRAFISH**

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### **Abstract:**

Reproductive decline in mid-adult females is an established phenotype of the ageing process. Although stress and glucocorticoids (GCs) have been shown to affect reproductive ageing, little is known about the mechanisms involved. During stress, elevated GC levels activate the glucocorticoid receptor (GR), a ubiquitously expressed ligand-bound transcription factor, to elicit physiological changes to restore homeostasis. Here, we tested the hypothesis that GC-GR signalling is essential for maintaining ovarian development during stress. To test this, we used zebrafish with a ubiquitous GR knockout (GRKO), which are inherently hypercortisolemic, to delineate the role of high cortisol and GR signalling on ovarian development. The loss of GR led to premature ovarian ageing, including a high frequency of typical and atypical follicular atresia in vitellogenic oocytes, yolk liquefaction and large inflammatory infiltrates. The reduction in oocyte quality was associated with a decline in ovarian *tert* expression in the adult GRKO fish compared to the early adult GRKO and adult wild-type zebrafish. Accelerated ovarian ageing in the GRKO fish was also associated with lower breeding success, fecundity, and egg fertilization rate while the resulting progeny had reduced survival and delayed somitogenesis. Given that GRKO fish still have a high-affinity corticosteroid receptor, the mineralocorticoid receptor (MR), we postulate that the balance of GR and MR signalling is a key regulator of the reproductive lifespan in zebrafish.

ABSTRACT N° ICBF22-550

## **DAILY RHYTHMS OF SOCIAL AND ANXIETY-LIKE BEHAVIOUR IN THE ZEBRAFISH DANIO RERIO**

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### **Abstract:**

Fish have often to face challenges and adverse conditions that vary during the day such as predation risk, food availability, competition and even disturbance from human activities. These factors may act as stressors for fish and typically result in physiological consequences endangering their welfare and survival. Hence, we expect that fish behaviour might plastically vary throughout the day to adaptively cope with these challenges. We investigated this hypothesis in the zebrafish *Danio rerio*, a model species for both circadian and behavioural research. To this end, 245 adult zebrafish were initially conditioned for two months to a 12h:12h light:dark photoperiod to ensure synchronisation to the daily rhythmicity. Then, fish were exposed in groups of 5 subjects to a novel environment at different times of the day (every 4h during a 24h cycle) and the behaviour of each group was recorded with an automatic tracking system. The results showed a significant daily rhythmicity for locomotion activity, as expected for a diurnal species. Moreover, we found that anxiety like-behaviours varied throughout the day showing that fish were significantly more stressed when they were exposed to a novel environment at night. Besides, sociability scores were higher during the day but a control test revealed that this rhythmicity was due in part to absence of visual cues at night. This research provides relevant information on fish behavioural rhythmicity which may also be useful to improve fish welfare under captive conditions.

**SOCIAL DYNAMICS OBSCURE THE EFFECT OF TEMPERATURE ON AIR BREATHING IN CORYDORAS CATFISH**

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**Abstract:**

In some fishes, the ability to breathe air has evolved to overcome constraints in hypoxic environments but comes at a cost of increased predation. To reduce this risk, some species perform group air breathing. Temperature may also affect the frequency of air breathing in fishes, but this topic has received relatively little research attention. This study examined how acclimation temperature and acute exposure to hypoxia affected the air-breathing behaviour of a social catfish, the bronze corydoras *Corydoras aeneus*, and aimed to determine whether individual oxygen demand influenced the behaviour of entire groups. Groups of seven fish were observed in an arena to measure air-breathing frequency of individuals and consequent group air-breathing behaviour, under three oxygen concentrations (100%, 60% and 20% air saturation) and two acclimation temperatures (25 and 30°C). Intermittent flow respirometry was used to estimate oxygen demand of individuals. Increasingly severe hypoxia increased air breathing at the individual and group levels. Although there were minimal differences in air-breathing frequency among individuals in response to an increase in temperature, the effect of temperature that did exist manifested as an increase in group air-breathing frequency at 30°C. Groups that were more socially cohesive during routine activity took more breaths but, in most cases, air breathing among individuals was not temporally clustered. There was no association between an individual's oxygen demand and its air-breathing frequency in a group. For *C.aeneus*, although air-breathing frequency is influenced by hypoxia, behavioural variation among groups could explain the small overall effect of temperature on group air-breathing frequency.

**ARAPAIMA GIGAS MAINTAINS GAS EXCHANGE SEPARATION IN SEVERE AQUATIC HYPOXIA BUT DOES NOT SUFFER BRANCHIAL OXYGEN LOSS**

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**Abstract:**

The South American *Arapaima gigas* is an air-breathing fish with substantially reduced gills and therefore completely reliant on access to the air phase. This adaptation is associated with the frequently recurring near anoxic aquatic hypoxia experienced in its habitat. In normoxic water, *A. gigas* covers 70-80% of its O<sub>2</sub> requirement from the air but excretes 60-90% of its CO<sub>2</sub> production to the water. This pattern of gas exchange, thought to be typical of air-breathing fish, is hypothesized to lead to loss of O<sub>2</sub> taken up in the swim-bladder to the water, when aquatic hypoxia is sufficiently deep. On the basis that such loss must be considered costly, we hypothesized that the partitioning of CO<sub>2</sub> would shift to the air phase during severe aquatic hypoxia allowing for reduced branchial water exposure and hence minimized branchial O<sub>2</sub> loss. By adapting a respirometer designed to measure aquatic MO<sub>2</sub>/MCO<sub>2</sub> in insects, we were able to run intermittent closed respirometry on both water and air phase for both of these gasses. In contrast to our prediction, we found that partitioning of CO<sub>2</sub> excretion changed little between normoxia and severe hypoxia (83% vs 77% aquatic excretion respectively) and at the same time were able to quantify branchial O<sub>2</sub> loss in hypoxia to be below our detection limit equivalent to 2.6% of the standard metabolic rate. The morphological adaptations necessary for this interesting gas exchange separation likely involve flow separation through the heart and shunting of separate blood streams past the greatly reduced gills.