carbonic anyhydrase-catalyzed hydration of $CO_2$ and high rates of $H^+$ extrusion.

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### A5.29
### The effects of saltwater acclimation on Na–K–2Cl and Na–K-ATPase distribution, activity and gene expression in salt glands of the estuarine crocodile

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The estuarine crocodile, *Crocodylus porosus* utilises lingual salt glands to excrete excess NaCl accumulated whilst living in saline environments. In avian salt gland models, Na–K-ATPase (NKA) is essential for the maintenance of the electrochemical gradients which facilitate the secretory activity of other ion pumps, while Na–K–2Cl (NKCC) functions to pump $Na^+$ and $K^+$ into the paracellular space, in exchange for $Cl^-$ which is transported into the cell. We have investigated the effects of chronic saltwater acclimation on the distribution, activity and gene expression patterns of both NKA and NKCC in the crocodilian salt gland. Using immunohistochemistry, both NKA and NKCC were localized to the basolateral membranes of salt gland secretory cells in both freshwater (fw) and saltwater (sw) acclimated salt gland tissues. The intensity of staining was slightly, but significantly, higher in tissues from SW-acclimated animals. Western blotting indicated that there was an increase in NKA and NKCC abundances in SW-acclimated tissues. The relative metabolic costs associated with these two pumps were investigated *in vitro*. While there were no differences between fw- and sw-acclimated tissues in the relative costs associated with NKA activity, NKCC activity was almost 50% higher in SW-acclimated tissues. We are currently exploring patterns of NKA and NKCC gene expression in sw- and fw-acclimated animals. This study has demonstrated the presence of NKA and NKCC in the salt glands of *C. porosus* and has shown that the activity and abundance of these transporters can be influenced by prolonged saltwater acclimation.

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### A5.30
### Happy fish? The implications of antidepressant treatment and exposure on toadfish urea excretion

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The most commonly prescribed antidepressants, including fluoxetine (Prozac™), inhibit the reuptake of serotonin (5-HT; 5-hydroxytryptamine) by 5-HT transporters. This inhibition results in an increase in local 5-HT concentrations; thus, the action of fluoxetine is analogous to the physiological action of 5-HT itself. After consumption, fluoxetine and its metabolites are excreted from the body, enter sewage treatment facilities but are not degraded. Consequently, these compounds have reached measurable quantities in surface waters. Many 5-HT sensitive physiological processes have been identified in fish, making them potentially susceptible to environmental fluoxetine contamination. Previous evidence shows that pulsatile urea excretion in the gulf toadfish, *Opsanus beta*, a process that also has behavioral implications, is regulated by 5-HT. To test the effect of fluoxetine on this process, toadfish were treated with fluoxetine either by intravenous injection, intraperitoneally using slow-release coconut oil implants or exposed to fluoxetine through the water. Circulating 5-HT and cortisol, an important stress hormone in teleost fish, as well as nitrogen excretion were monitored in these groups of fish. When treated with fluoxetine by intravenous injection or intraperitoneal implant, toadfish showed a significant increase in the excretion of urea. However, most interestingly is that exposure to environmentally realistic concentrations of fluoxetine in the water has the same effect as internal treatment. The effect of fluoxetine on toadfish urea transporter mRNA expression is currently being investigated. The behavioral as well as ecological impact of these findings will be discussed.

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### A5.31
### *In vitro* evidence for the involvement of Rh proteins and $H^+$ efflux in ammonia excretion across the gills of the freshwater rainbow trout

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The recent identification of Rh proteins in the gills of rainbow trout (Nawata et al., 2007) has opened a new paradigm in the controversial area of the mechanism(s) of branchial ammonia excretion. The present study utilized cultured gill epithelia *in vitro* (double seeded inserts, DSI; Fletcher et al., 2000) of freshwater rainbow trout, exposed to apical freshwater and basolateral plasma-like media, as a model to investigate these issues. Elevated basolateral ammonia and cortisol had synergistic effects on ammonia permeability of DSI. This increase in ammonia permeability could not be explained by changes in paracellular permeability. mRNA expressions of Rhbg, Rhcg2, $H^+$-ATPase and $Na^+/H^+$ exchanger-2 (NHE-2) were up-regulated in DSI exposed to elevated ammonia and cortisol. Bafilomycin (1 μmol $L^{-1}$), phenamil (10 μmol $L^{-1}$) and 5-(*N*,*N*-hexamethylene) amiloride (10 μmol $L^{-1}$) applied to the apical solution significantly inhibited ammonia efflux, indicating that $H^+$-ATPase, $Na^+$ channel and NHE-2 on the apical surface were involved in ammonia excretion. Low apical $Na^+$ treatment caused an increase in ammonia efflux and this increase was not accompanied by changes in paracellular permeability. There was also an increase in Rhcg2 mRNA expression in these low $Na^+$ treated DSI. DSI epithelia treated with elevated ammonia and cortisol exhibited significantly increased $Na^+$ influx, and there were positive correlations between ammonia efflux and $Na^+$ uptake. These data suggest that Rhesus glycoproteins are important for ammonia transport in DSI, and are coupled with $H^+$ excretion mechanisms (NSERC Discovery).

References

Fletcher, M., et al., 2000. J. Exp. Biol. 203, 1523–1537.
Nawata, C.M., et al., 2007. Physiol. Genomics 31, 463–474.

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### A5.32
### Metabolic and osmoregulatory alterations and cell proliferation in gilthead sea bream (*Sparus aurata*) exposed to cadmium

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Contamination of aquatic ecosystems by cadmium (Cd) from industrial and mining activities is an environmental problem. This metal can exert a wide range of pathological effects on fishes. In this work, the impact of Cd was assessed in gilthead sea bream (*Sparus aurata*) acclimated to seawater (SW) and then challenged to hypersaline water (HSW 55‰). Fish were injected intraperitoneally with a sublethal dose of Cd (1.25 mg Cd/kg body wt) in 9‰ NaCl or sham injected with 9‰ NaCl alone. After 7 d half of the injected fish were sampled. The remaining fish were transferred to HSW and sampled 4 d later. Gill and kidney $Na^+$, $K^+$-ATPase (NKA) activities, plasma osmolality and levels of cortisol, osmolites ($Na^+$, $Ca^{++}$ and $Cl^-$), metabolites (glucose, lactate and triglycerides) and protein were measured. Protein level expression of NKA, heat shock proteins (Hsp70, Hsp90) and proliferating cell nuclear antigen (PCNA) were assessed by western blotting. Our results indicate that in *S. aurata* Cd impairs NKA expression but osmoregulation is not compromised in SW or HSW. Increased cell proliferation and expression of Hsp90 likely contribute to the attenuation of the deleterious effects of Cd.

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## A5.33
## Taking a chance; factors affecting between-patch movements of juvenile Atlantic cod, *Gadus morhua*

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Complex habitat provides protection from predators for numerous animal species. Movement between areas of complex habitat may provide access to new resources, but at the cost of increased predation risk. This trade-off has been studied extensively in terrestrial species, however little work has focused on the marine environment. The current study examines several variables which may play a role in the between-patch movement of juvenile Atlantic cod (*Gadus morhua*) and identifies possible mechanisms influencing between-patch movement. We conducted a behavioural study in a 12 m by 3 m raceway tank using artificial eelgrass patches with varying between-patch distances, release densities and predator locations. Results show between-patch distance, average group size and predator location each have significant effects on the tendency of juvenile Atlantic cod to cross gaps in complex habitat.

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## A5.34
## A new experimental system to study toxicological effects of dispersants and dispersed oil on fish juvenile species

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Keywords: Experimental system; Dispersant; Dispersed oil toxicity; Water accommodated fraction; Fish species.

The objective of dispersant (a chemical product) use is to transfer oil from the water surface into the water column. When applied before spills reach the coastline, dispersants will potentially decrease exposure for surface dwelling organisms (e.g., seabirds) and intertidal species (e.g., mangroves, salt marshes), while increasing it for water column (e.g., fish) and benthic species (e.g., corals, oysters). The use of oil dispersants is a controversial countermeasure in the effort to minimize the impact of oil spills. The risk of ecological effects will depend on whether oil dispersion increases or decreases the exposure of aquatic species to the toxic components of oil. Our experimental system is based on a system probates by AFNOR (T 90-349) to determine the acute toxicity of a substance with regard to shrimps (*Palaemonetes varians*). Fish juvenile species were exposed, during 24 h to three pollution types: water accommodated fraction, mechanical dispersion, and chemical dispersion. After, a 24 h post-contamination period was carried out. This post-contamination was performed in open circuit, with water regeneration. Then, acute toxicity of each pollution type on fish species were compared by LC50 with this experimental system. Finally, some parameters such as oxymetry, pH and temperature were measured and maintained to constant values during the experimentation regarding with the basal metabolism of fish. Dispersed oil concentration was measured at the beginning and at the end of the exposure. These experiments suggest that this experimental system is the first to permit stabilisation of dispersed oil concentration, on a 24 h exposure.

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## A5.35
## Gastric proton pump expression in teleost fishes: Nongastric sites of expression in gastric versus agastric fishes

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The gastric proton pump or $H^+/K^+$-ATPase is responsible for stomach luminal acidification in vertebrates but has also been found in non-gastric organs where it possibly has a role in ionoregulation. $H^+/K^+$-ATPase is a heterodimer composed of α and β subunits encoded by the genes ATP4A and ATP4B, respectively. ATP4A has been identified in the stomach of a few teleosts and the gills of an elasmobranch but no data on non-gastric sites of expression exists in teleost fishes. In addition there are some teleosts (e.g. cyprinids, pufferfish) that are also agastric (stomachless). Using RT-PCR for ATP4A and non-homologous antibodies to ATP4A and ATP4B we set out to determine if $H^+/K^+$-ATPase is indeed also expressed in the gills of teleost fishes and whether this expression is dependent on the presence of a functional stomach. The following species were examined: GASTRIC: *Oncorhynchus mykiss*, *Gasterosteus aculeatus*, AGASTRIC: *Tetraodon nigroviridis*, *Cyprinus carpio*, *Misgurnus anguillicaudatus*, *Danio rerio*. In gastric species, branchial and gastric $H^+/K^+$-ATPase was detected. In agastric species, in silico analysis of genome databases (Ensembl.org) and RTPCR of gill and intestine failed to identify ATP4A and β-subunit immunoblotting results were negative. However, immunoblotting analysis of α-subunit indicates the presence immunoreactive bands in gill (80–150 kDa) and intestine (110 kDa) of some but not all species. In gastric fishes α-subunit is ~100 kDa. From these results we can conclude that gastric teleost fishes also expression $H^+/K^+$-ATPase branchially and that there is at least an immunologically related α-subunit of $H^+/K^+$-ATPase in agastric fishes which awaits further characterization.

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## A5.36
## Ammonia tolerance in the zebrafish (*Danio rerio*): Effects of ionicstrength and ontogeny

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