Cougar Journal of Undergraduate Research

Review

Review of osmoregulation mechanisms of vertebrate marine animals, the *Dugong dugon*, *Dermochelys coriacea*, *Pygoscelis adeliae*, and *Rachycentron canadum*, following ion-loading from food consumption

Vaden Edmondson[1,*] and Dennis Kolosov [1]

[1] Department of Biological Sciences, California State University San Marcos
* Correspondence: edmon021@cougars.csusm.edu

Abstract: Earth's oceans readily contain many dissolved ions necessary for biological function; however, the relative concentrations of ions may be troublesome for some organisms to maintain homeostasis. Every cell of every organism has a salinity tolerance threshold that may result in cell dysfunction or death if violated. Organisms are categorized as osmoregulators, that spend energy to maintain a strict internal osmotic range, and osmoconformers, that passively match their internal osmotic concentrations to their environment In marine habitats, ambient salts threaten those whose cells cannot tolerate oceanwater or sufficiently regulate against it. Marine osmoregulators have adapted to survive in high-salinity waters despite the threat of excessive ion-loading though ambient salt uptake. Dietary ion-loading, or excess salt intake, occurs when ion uptake from dietary sources or ambient seawater exceeds net ion loss, resulting in blood plasma osmolality above optimal range. Without access to fresh drinking water, marine animals cannot dilute their cellular osmolality and must have a way to excrete salts at higher concentration to that of surrounding seawater. *Dugong dugon*, *Dermochelys coriacea*, *Pygoscelis adeliae*, and *Rachycentron canadum* are all distantly vertebrate osmoregulators that can survive in high-salinity marine environments by their respective morphologies.

To combat harmful sodium and chloride ion fluctuations within their bodies, each of these marine osmoregulators have specialized organs to excrete salts while retaining water. The kidney is the most basal commonality among all four taxa as it allows the blood to be filtered for salts and wastes to be excreted as urine. Looped nephrons allow for countercurrent multiplication to continually draw water from waste solutions as they pass into the collecting duct, while the un-looped type are only capable of isotonic urine production. While the morphology of the nephron varies in structure and efficiency among these organisms, they all possess the capacity to produce concentrated waste to retain water to combat ion-loading. While fresh drinking water is unavailable, dietary water sources also help to restore water lost in urinary excretion and other biological processes (Rash and Lillywhite, 2019). Metabolic water production accounts for a small amount of net water intake, although the intake of seawater and high salt concentrations exceed metabolic production (Rash and Lillywhite, 2019). Excess sodium and chloride ion-loading is inevitable given any seawater intake as expected of living in marine habitats, but as demonstrated by *Dugong dugon*, *Dermochelys coriacea*, *Pygoscelis adeliae*, and *Rachycentron canadum*, the specialized organs and mechanisms employed by these organisms are all capable of effectively maintaining optimal plasma osmolality despite external conditions.

Ion-loading is a perpetual issue for Dugongs, *Dugong dugon*, because their diet is almost entirely composed of seagrasses, which are particularly rich in sodium ions, and they likely consume ambient ions during feeding (Touchette, 2007). These marine

Citation: Edmondson, V.; Kolosov, D. Review of osmoregulation mechanisms of vertebrate marine animals, the *Dugong dugon*, *Dermochelys coriacea*, *Pygoscelis adeliae*, and *Rachycentron canadum*, following ion-loading from food consumption. *Cougar JUGR* **2022**, *1*.

Academic Editor: Dennis Kolosov

vertebrates closely related to manatees, differentiated by living in warm shallow coastal regions that are physically separated from fresh or brackish water sources (Smoll et al., 2020; Rudy et al., 1998). They do not have access to freshwater, so Dugongs can produce urine with significantly higher osmotic concentrations, 1468 mOsm/kg, than surrounding seawater, 1055.5 mOsm/kg, caused by significantly higher excretion concentrations of sodium and potassium ions (Smoll et al., 2020). The deliberate production of highly concentrated urine in the kidneys after drinking seawater is unlikely to be the primary mechanism for water retention due to its high energy cost; however, this mechanism may assist with maintaining homeostasis in addition to water consumed from seagrasses and water production by metabolic oxidation (Smoll et al., 2020). The trifecta of naturally high plasma osmolality, a glucose rich seagrass diet, and long, looped nephrons in the kidney are predicted to be the major factors by which Dugongs effectively remain hydrated after seagrass-induced sodium ion-loading (Smoll et al., 2020). The Dugong kidney is understudied due to their conservation status but is likely to be morphologically similar to that of other fully marine mammals such as Cetaceans; meaning, their kidneys are expected to have a large ratio of medulla to renal cortex and an efficient renin-angiotensin-aldosterone response system (Rudy et al., 1998; Xu et al., 2013). Being that dugongs are endangered, it is unlikely that their internal physiology can be readily studied, so at the moment, educated speculation is the best option.

Leatherback turtles, *Dermochelys coriacea,* are large marine reptiles that live in tropical waters and are known to regularly feed on jellyfish (Davenport, 2017). Adult Leatherbacks attempt to maintain an internal osmotic concentration of 320-370 mOsm/kg, while their jellyfish food source matches seawater at about 980-1050 mOsm/kg (Davenport, 2017). Ion loading is inevitable, both from consumption of ambient seawater during feeding and by consumption of large amounts of jellyfish; however, unlike Dugongs, Leatherbacks

Figure 1. Photomicrograph depicting the basic histological organization and the vascular system of a lobule in the lacrimal gland of *Chelonia mydas*. Branching secretory tubules are small at the periphery of the lobule and increase steadily in size centripetally, where they join the central canal (arrows). At the left a large artery penetrates the lobule and branches to form arterioles that extend along the central canal. From the arterioles capillaries radiate between the secretory tubules to the periphery of the lobule where they empty into venous Sinusoids (Abel and Ellis, 1966).

cannot produce hyperosmotic urine due to the lack of looped nephrons in the kidney (Davenport, 2017; Bodil and Lowell, 1968). Instead, Leatherbacks must excrete excess ions through a set of specialized lachrymal salt glands located near the eyes (Davenport, 2017; Abel and Ellis, 1966). These glands are capable of producing hyperosmotic "tears" that contain up to 1800 mOsm/kg of dissolved sodium and chloride ions and remove bulk ion loads from blood plasma sourced from the stomach (Davenport, 2017; Abel and Ellis, 1966). These glands are morphologically similar to that of a green sea turtle, *Chelonia mydas*, and consist of compound branch tubules intertwined with blood vessels that underlie truncated secretory epithelial cells (Fig.1) (Abel and Ellis, 1966). The structure of the lachrymal gland allows for a large surface to volume ratio between blood plasma and Na-K-Cl cotransporters that actively pump ions into the gland with minimal water content. They are responsible concentrating excess ions in excretory fluid utilizing ATPase pumps to create a basolateral concentration gradient for ion excretion while maintaining plasma volume by effective water-conservation (Abel and Ellis, 1966). Lacrymal glands act as pseudo-kidneys for Leatherback turtles in place of their isosmotic-only reptilian kidneys, which by themselves, would likely not be capable of sustaining osmotic homeostasis.

Adelie Penguins, *Pygoscelis adeliae,* are a species of Antarctic penguin that utilize a combination of the previously described mechanisms. These birds almost exclusively consume sodium and chloride ion-loaded krill at annual nesting sites, so Adelie Penguins chicks will need to be effective at compensating regurgitated krill ion loads from their parents (Janes, 1997). Like most other marine vertebrates, these penguins possess kidneys capable of removing excess ions from the blood; however, much like the Leatherback turtle, they must also utilize salt glands to assist in sodium ion excretion (Janes, 1997; Gutiérrez, 2014). Adelie penguin chicks are found to be capable of producing hyperosmotic urine, about 800 mOsm/kg, relative to plasma osmolality due to the partial expression of looped, mammalian-type nephrons in the kidney. Urine collected from growing chicks were found to contain negative regression of Na+ osmolality as chick mass

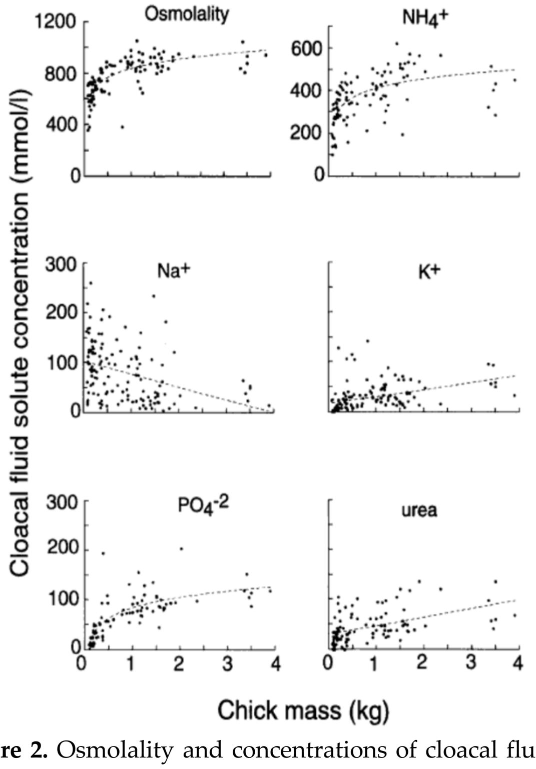

Figure 2. Osmolality and concentrations of cloacal fluid collected from Adelie Penguin chicks of various sizes. All cloacal fluid was spontaneously voided and was collected within 10 minutes of capturing the chick. All regressions are significant (p<0.05) with net osmolality, ammonium, potassium, phosphate, and urea displaying increasing cloacal voiding with increasing chick mass, and sodium ions decreasing with increasing mass. Osmolality units are mOsm/kg (Janes, 1997).

increased, likely caused by salt-gland maturation and sodium ion excretion assisting cloacal excretion. (Fig. 2). Their excretory osmolality can be hypertonic to plasma osmolality but is limited to about twice their maximum plasma osmolality because of the partial expression of un-looped, reptilian-type nephrons (Gutiérrez, 2014). However, similarly to the Leatherback turtle, their lobed salt glands are capable of producing hyperosmotic excretions and are morphologically similar to that of reptiles likely due to common ancestry (Gutiérrez, 2014). The culmination of semi-looped nephrons in the kidney and looped salt glands allow the Adelie penguin to survive excess-ion consumption from food sources and retain safe amounts of water for sufficient hydration. While their salt gland excretion rates have not been measured in great detail, the presence of large, energetically costly salt glands in hatchlings suggest these marine birds' kidneys likely require their assistance to maintain osmotic homeostasis in their environment from an early age (Janes, 1997).

Unlike the previously describes species, Cobia, *Rachycentron canadum*, are a type of marine teleost that can favor ion-loading to tolerate low ambient salinities (Santos et al., 2012). These fish also possess kidneys capable of hyperosmotic urine production; however, Cobia raised in low salinities will produce dilute urine and must combat the loss of ions through their gills because of ion flux by paracellular leak- a symptom of the osmo-respiratory compromise (Santos et al., 2012; Grosell, 2006). In hypoosmotic waters, Cobia fed sodium chloride supplemented foods were found to downregulate NKA pump density in the gill due to ion-loading, likely because energy-conservation was favorable to active transcellular reuptake of sodium ions (Santos et al., 2012). Ion-loading also increased the chloride cell density, ionocytes, on the gills to facilitate the active uptake of chloride ions from the surrounding water caused by paracellular leak (Fig. 3) (Santos et al., 2012). The upregulated expression within the gills demonstrates the ability of this fish to osmoregulate against unfavorable external salinities, especially in the case of preventing unnecessary sodium ion loss through the gill-water interface. While Cobia favor ion-uptake in hypoosmotic waters, the reverse processes are expected to occur in hyperosmotic waters that encourage ion loading; although for future research, this will need to be tested in addition to factoring in effects of skin and gut regulation mechanisms.

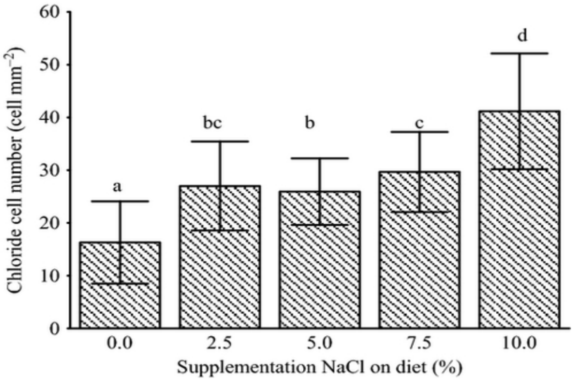

Figure 3. Chloride cell density in gills of juvenile cobia reared at salinity 5 g/L and fed on different dietary NaCl levels for 40 days. Data are means ± standard deviation plotting supplementation percentage of NaCl against chloride cell number. No supplementation had the lowest chloride cell content representing its own group (a) and 10% supplementation belonged to the highest group(d), with 2.5% and 5.0% occupying the second highest level (b) and 5.0% and 7.5% occupying the third (c). Group assignments a-d indicate significant differences (p<0.001) among treatments after one-way ANOVA followed by the Duncan's multiple-range test (Santos et al., 2012).

Each of these marine vertebrates possessed kidneys to filter blood and excrete ions through urine; however, the concentrations of urine varied among species relative to plasma osmolality. Dugongs were capable of producing highly concentrated urine to excrete excess dietary ions, similar to the capacities of Adelie penguin and Cobia. The Leatherback turtle lacks looped nephrons and can only produce isosmotic urine, so it must excrete excess ions through lachrymal salt glands. The Adelie penguin also has salt glands to compensate for their less-efficient kidneys, but both are capable of preventing hyperosmolarity in the animals' plasma. Cobia, having gills, can respond to favor ion-loading in hypoosmotic environments by the higher expression of ionocytes in the primary filament, which is a truly unique physiological response compared to the other mentioned marine species (Table 1). Regardless of the mechanism, all animals were capable of regulating their internal osmolality to create internal conditions favorable to their biological functions. Life is as resilient as the ocean is vast, and the mechanisms developed by these species to regulate dietary ion loading are all fundamental to afford a comfortable life in the water.

Table 1. Mechanisms of ion regulation plasma osmolality, and ion loading study conditions utilized by species.

Species	Common Name	Nephron type	Non-kidney Osmoregultor-Organs	Non-Kidney Mechanism of Action Mentioned in Study	Approximate Plasma Osmolality (mOsm/kg ± SE)	Food Source Type	Salt Content of Food Source	Duration of Ion Loading
Dugong dugon	Dugong	Looped/ Mammalian-Type	NA	NA	339.6 ± 1.8	Marine Grasses: *Halophila ovali* , *Zostera muelleri* , *Halodule uninervis* , and *Halodule pinifolia* .	1800 ± 1.8 mOsm/kg ± SE	4 months[9]
Dermochelys coriacea	Leatherback turtle	Un-looped/ Reptilian-Type	Lacrymal Salt Glands	Na+/K+ Exchange Transporter and paracellular Na+ leak	350 ± 25	Medusa-form Jellyfish: *Rhizostoma pulmo and* Cyanea Capillata	1000 mOsm/kg	13.5 hours[3]
Pygoscelis adeliae	Adelie penguin	Looped/ Mammalian-Type and Un-looped/ Reptilian-Type	Nasal Salt Glands	Na+/K+ Exchange Transporter and paracellular Na+ leak	326 ± 9.9	Antarctic Krill: *Euphausia superba*	96-425 mmol/kg	~10 minutes[4]
Rachycentron canadum	Cobia	Un-looped/ Reptilian Type	Gills	Gill: Na+/K+ Exchange Transporter and Chloride Ionocyte Skin: Paracellular leak	Not Measured	Repelletized (5 mm diameter) commercial diet (NRD 2/5; INVE)	0.0%, 2.5%, 5.0%, 7.5% and 10.0% by weight-dry weight salt (NaCl – 99%, Artificial Supplementation	40 days[8]

References

1. Abel, John H, & Ellis, Richard A. (1966). Histochemical and electron microscopic observations on the salt secreting lacrymal glands of marine turtles. American Journal of Anatomy, 118(2), 337-357. https://doi.org/10.1002/aja.1001180203.
2. Bodil Schmidt-Nielsen, & Lowell E. Davis. (1968). Fluid Transport and Tubular Intercellular Spaces in Reptilian Kidneys. Science (American Association for the Advancement of Science), 159(3819), 1105-1108. https://doi.org/10.1126/science.159.3819.1105.
3. Davenport, John. (2017). Crying a river: how much salt-laden jelly can a leatherback turtle really eat? Journal of Experimental Biology, 220(Pt 9), 1737-1744. https://doi.org/10.1242/jeb.155150.
4. Donald N. Janes. (1997). Osmoregulation by Ad√©lie Penguin Chicks on the Antarctic Peninsula. The Auk, 114(3), 488-495. https://doi.org/10.2307/4089249.
5. Grosell, Martin. (2006). Intestinal anion exchange in marine fish osmoregulation. Journal of Experimental Biology, 209(Pt 15), 2813-2827. https://doi.org/10.1242/jeb.02345.
6. Guti√©rrez, J. S. (2014). Living in environments with contrasting salinities: a review of physiological and behavioural responses in waterbirds. Ardeola, 61(2), 233-256.
7. Rash, R. & Lillywhite, H. B. (2019). Drinking behaviors and water balance in marine vertebrates. Marine Biology, 166(10), 1-21. https://doi.org/10.1007/s00227-019-3567-4
8. Rudy M. Ortiz, Graham A. J. Worthy, & Duncan S. MacKenzie. (1998). Osmoregulation in Wild and Captive West Indian Manatees (Trichechus manatus). Physiological Zoology, 71(4), 449-457. https://doi.org/10.1086/515427.
9. Santos, R.A., Bianchini, A., Jorge, M.B., Romano, L.A., Sampaio, L.A. and Tesser, M.B. (2014), Cobia Rachycentron canadum L. reared in low‚Äêsalinity water: does dietary sodium chloride affect growth and osmoregulation?. Aquaculture Research, 45: 728-735. https://doi-org.ezproxy.csusm.edu/10.1111/are.12017
10. Smoll, Laetitia I, Beard, Lyn A, & Lanyon, Janet M. (2020). Osmoregulation and electrolyte balance in a fully marine mammal, the dugong (Dugong dugon). Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology, 190(1), 139-148. https://doi.org/10.1007/s00360-019-01250-8.
11. Touchette, Brant W. (2007). Seagrass-salinity interactions: Physiological mechanisms used by submersed marine angiosperms for a life at sea. Journal of Experimental Marine Biology and Ecology, 350(1), 194-215. https://doi.org/10.1016/j.jembe.2007.05.037.
12. Xu, Shixia, Yang, Yunxia, Zhou, Xuming, Xu, Junxiao, Zhou, Kaiya, & Yang, Guang. (2013). Adaptive evolution of the osmoregulation-related genes in cetaceans during secondary aquatic adaptation. BMC Evolutionary Biology, 13(1), 189-189. https://doi.org/10.1186/1471-2148-13-189.