Osmoregulation of the Atlantic Stingray (*Dasyatis sabina*) from the Freshwater Lake Jesup of the St. Johns River, Florida

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ABSTRACT

The goals of this study were to (1) measure plasma osmolytes and rectal gland weights of a freshwater (FW) Atlantic stingray (*Dasyatis sabina*) population in the St. Johns River, Florida, and (2) determine how these parameters change after acclimation to seawater (SW). We hypothesized that the FW *D. sabina* may show physiological divergence from marine *D. sabina,* because the FW individuals reproduce and complete their life cycle in the St. Johns River. The FW *D. sabina* hyperregulate their plasma osmolality (621.4 mOsm kg^{-1}), with plasma Na^+, Cl^-, and urea concentrations of 211.9, 207.8, and 195.9 mmol L^{-1}, respectively. FW *D. sabina* were exposed to 100% SW for 8 d, and their hematocrit did not change significantly compared to control animals left in FW. However, plasma osmolality increased significantly (953 mOsm kg^{-1}), with significant increases in plasma concentrations of Na^+, Cl^-, and urea to 319.13, 296.1, and 329.76 mmol L^{-1}, respectively. The plasma of the SW-adapted *D. sabina* was hypo-osmotic and hypoionic to 100% SW. Rectal gland weight to body weight (RGBW) ratios of FW *D. sabina* were about 80% lower than RGBW ratios reported for marine *D. sabina;* the RGBW ratio did not increase significantly after SW acclimation. This may indicate that branchial and renal mechanisms are also involved with ion excretion. We conclude that the FW *D. sabina* are physiologically euryhaline and have not evolved the osmoregulatory strategy of stenohaline FW Potamotrygonid stingrays.

Introduction

The common osmoregulatory strategy employed by euryhaline elasmobranchs in freshwater (FW) is a reduced serum/plasma osmotic pressure relative to marine elasmobranchs (Smith 1931). This is primarily attained by a decrease in serum/plasma urea to levels that are 30%–50% lower than those found in marine elasmobranchs (Smith 1931; Urist 1962; Thorson et al. 1973). The other main organic osmolyte, trimethylamine oxide (TMAO), has not been measured from a euryhaline elasmobranch in FW, but on the basis of studies from elasmobranchs in dilute seawater (SW), TMAO is expected to decrease in similar proportion to urea (Goldstein et al. 1968; Goldstein and Forster 1971; Thorson et al. 1973). Serum/plasma concentrations of Na^+ and Cl^- are also lowered, but not to the same degree as urea (Smith 1931; Urist 1962; Thorson 1967; Thorson et al. 1973). Overall, euryhaline elasmobranchs in FW maintain high concentrations of urea in their plasma (100–250 mmol L^{-1}).

In contrast, stenohaline FW stingrays of the family Potamotrygonidae have completely lost the ability to reabsorb urea and TMAO (Thorson et al. 1967; Thorson 1970; Gerst and Thorson 1977), and they maintain plasma ion concentrations much lower than those of euryhaline elasmobranchs in FW (Thorson et al. 1967). *Potamotrygon* sp. can no longer survive in environments hyperosmotic to their plasma (Griffith et al. 1973; Bittner and Lang 1980) and may represent the ultimate stage of elasmobranch evolution in FW (Thorson et al. 1983).

Since there is no need to excrete excess ions in a FW environment, euryhaline elasmobranchs in FW have a decreased size and function of their salt-secreting rectal gland. Thorson et al. (1978, 1983) reported that FW elasmobranchs have smaller rectal gland to body weight (RGBW) ratios than marine species. This has been correlated with a reduction in the number of tubular structures associated with salt secretion (Oguri 1964; Thorson et al. 1978) and a decreased Na^+,K^+-ATPase activity in the gland (Gerzeli et al. 1976).

Stingrays of the family Dasyatidae are well known for their ability to inhabit FW environments of Africa, Southeast Asia, Australia, New Guinea, and South America (Thorson and Watson 1975; Taniuchi 1979, 1991; Compagno and Roberts 1982, 1984; Thorson et al. 1983; Compagno and Cook 1995). The only North American stingray species frequently captured in FW is the Atlantic stingray, *Dasyatis sabina* Lesueur. This coastal, euryhaline species ranges from the Chesapeake Bay to Central America (Bigelow and Schroeder 1953) and is known to enter FW rivers on a seasonal basis throughout the Southeastern Atlantic and Gulf of Mexico coasts (Gunter 1938; Sage et al. 1972; Schwartz 1995). *D. sabina* also inhabits the St. Johns River of Florida (McLane 1955; Tagatz 1968), where this species has established populations over 300 km from sea that

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reproduce and complete their life cycle in FW (Johnson and Snelson 1996).

To date, no studies have investigated the osmoregulation of *D. sabina* in FW. De Vlaming and Sage (1973) examined how plasma osmotic parameters of marine *D. sabina* responded to diluted concentrations of SW and found that plasma Na^+ and Cl^- decreased to a greater extent than plasma urea in salinities greater than 350 mOsmol L^{-1} (35% SW). The lowest salinity that De Vlaming and Sage (1973) exposed marine *D. sabina* to was approximately 25% SW, at which level plasma urea concentrations were substantially lower than those of marine individuals.

The goals of this study were to (1) describe the osmoregulation of *D. sabina* from the FW Lake Jesup of the St. Johns River by measuring plasma osmolytes and rectal gland weights, and (2) determine whether these FW *D. sabina* can osmoregulate in hyperosmotic waters by measuring the same parameters from animals in higher salinities. We hypothesized that the FW population may show physiological evidence of differentiation from marine *D. sabina* (i.e., lack of urea reabsorption) since the St. Johns River *D. sabina* reproduce and complete their life cycle in FW (Johnson and Snelson 1996). This represents the first study concerning the osmoregulation of *D. sabina* in the St. Johns River, and the first experimental determination of how a euryhaline elasmobranch in FW osmoregulates in response to a salinity increase.

Material and Methods

Field Studies

All *Dasyatis sabina* were captured from Lake Jesup of the St. Johns River, Florida, using two trotlines (50 hooks each) baited with shrimp. Lake Jesup is a shallow, inland lake (maximum depth ~3 m) located about 270 km from the mouth of the St. Johns River. A total of 15 *D. sabina* of both sexes (disc width = 23–31 cm) were captured during June 1996, October 1996, and February 1997. Immediately after capture, 0.5-mL blood samples were collected via cardiac puncture using 1-mL heparinized (Na^+-heparin) syringes equipped with a 21- or 25-gauge needle. Water samples were also collected from Lake Jesup at these times.

The blood was transported on ice to Gainesville, Florida, where it was centrifuged, and the plasma was frozen at −20°C until analyzed. Plasma samples were analyzed for Na^+ and K^+ (Radiometer/Copenhagen FLM3 flame photometer), Cl^- (Radiometer/Copenhagen CMT10 chloride titrator), Ca^{2+} (Buck Scientific model 210 atomic absorption spectrophotometer), urea (Sigma kit 535), and total osmolality (Wescor 5100B vapor pressure osmometer). Water samples were analyzed for the same components, except for urea. Plasma TMAO measurements were attempted using the method of Wekell and Barnett (1991), but values are not reported because of complications with the technique.

Rectal gland and body weights were measured from 32 *D. sabina* of both sexes (disc width = 21.75–30.5 cm) captured during May and June 1996 to calculate RGBW ratios in milligrams of rectal gland per kilogram of body weight. These ratios have previously been used as a relative indication of rectal gland function (Thorson et al. 1978, 1983).

Acclimation Experiments

An additional 16 *D. sabina* of both sexes (disc width = 23.3–27.5 cm) were captured from Lake Jesup during October and February 1997. Animals were transported to Gainesville, Florida, in aerated 128-quart coolers, where they were transferred to two 100-gallon Rubbermaid tanks (four animals in each tank).

The control group of *D. sabina* was left in FW (0.1‰) for the entire experimental period of 32 d. The experimental animals were left in FW for the first 12 d and then transferred to 50% SW (15‰–16‰) over 2 d. After 8 d in 50% SW the animals were transferred to 100% SW (29‰–30‰) over 2 d, where they remained for the last 8 d of the experiment. Experimental and control animals were starved throughout the entire experimental period to minimize any variation in plasma osmolytes due to differential feeding of animals. Water temperature in the control and experimental tanks ranged from 20° to 23°C; pH was maintained at 7.8–8.2 using FW and marine buffers. Tanks were equipped with biological filtration to maintain NH_3 and NO_2 concentrations at low levels (<1 ppm).

Serial blood samples were taken from unanesthetized control and experimental animals on days 12, 22, and 32 through cardiac puncture using 25-gauge needles attached to 1-mL heparinized (Na^+-heparin) syringes. Approximately 0.5 mL of blood was taken from each animal to measure hematocrit and plasma osmolytes. The blood was centrifuged, and plasma samples were frozen at −20°C until analyzed. Water samples were also obtained on each respective day. Both plasma and water samples were analyzed as described above. In addition, all the animals were killed on day 32 to obtain their rectal gland and body weights.

A potential concern of the blood-sampling technique used in this study was the effect of stress on plasma osmolytes. Previous studies have shown that plasma Na^+, Cl^-, and osmolality of some freshwater teleosts was influenced by stress (see McDonald and Milligan 1997). In the present study, we did not notice any superficial indicators of stress in the captive animals (i.e., changes in body pigmentation, behavioral modifications). In addition, a preliminary study on elasmobranchs found no significant changes in serum Na^+ and Cl^- after exposure to various levels of stress (C. A. Manire, personal communication). Therefore, we believe it is unlikely that any stresses

Table 1: Comparison of plasma osmolytes from FW *Dasyatis sabina* to FW elasmobranchs, FW teleosts, marine *D. sabina,* and Lake Jesup water

Species	Na^+	Cl^-	Urea	K^+	Ca^{2+}	Osmolality
D. sabina (FW)	211.9 ± 2.8	207.8 ± 3.4	195.9 ± 7.9	5.2 ± 0.25	4.3 ± 0.24	621.4 ± 10.8
Carcharhinus leucas (FW)	245 ± 4.1	219 ± 4.9	169 ± 5.4	6.4 ± .30	4.5 ± .15	673.3 ± 12.0
Potamotrygon (FW)	164.0 ± 5.6	151.7 ± 5.0	1.1 ± .1	4.45 ± .25	3.0 ± .4	282.0 ± 16.8
Teleost (FW)	130	125	. . .	2.9	2.1	274
D. sabina (SW)[a]	310.0 ± 5	300.0 ± 4.5	394.5 ± 5.5	6.95 ± .7	3.1 ± .2	1,034 ± 7.5
Lake Jesup water	3.0 ± 1.4	3.7 ± 1.5	. . .	.075 ± .03	1.1 ± .16	38.0 ± .5

Sources. *D. sabina* (FW) and Lake Jesup water, this study; *C. leucas* (FW), Thorson et al. (1973) and Otake (1991); *Potamotrygon* (FW), Griffith et al. (1973); teleost (FW), Evans (1993); and *D. sabina* (SW), De Vlaming and Sage (1973).

Note. All units are mmol L^{-1}, except for osmolality, which is mOsm kg^{-1}. Values are means ± 1 SE (if available).

[a] Values averaged from *D. sabina* in 98% and 100% SW (animals starved for 6 d).

associated with the blood-sampling technique would affect the measured plasma parameters.

Statistical Analyses

All statistical analyses were conducted with *SPSSwin.* To determine whether the salinity treatment affected plasma osmolytes significantly, a one-way repeated-measures ANOVA ($\alpha = 0.05$) was performed comparing control and experimental plasma values. To determine whether plasma osmolytes between the control and experimental groups were different on a given day, a one-way ANOVA ($\alpha = 0.05$) was used. A one-way ANOVA ($\alpha = 0.05$) was also conducted to compare RGBW ratios of control and experimental animals. A two-tailed, unpaired Student's *T*-test ($\alpha = 0.05$) was used to determine whether plasma values of the experimental *D. sabina* were equivalent to the values of the tank water on days when blood samples were taken. Also, a two-tailed, unpaired Student's *T*-test ($\alpha = 0.05$) was used to determine whether the plasma values of the experimental *D. sabina* in 100% SW were equivalent to those from De Vlaming and Sage (1973) for marine *D. sabina.*

Results

Plasma Osmolytes and Rectal Gland Weights of Dasyatis sabina *from Lake Jesup*

Plasma concentrations of Na^+, Cl^-, urea, K^+, and Ca^{2+} from FW *Dasyatis sabina* were greater than the respective water concentrations from Lake Jesup (Table 1). These osmolytes contributed to a plasma osmolality that was 16 times greater than the lake water osmolality (Table 1). The RGBW ratio of the FW *D. sabina* was 49.75 mg kg^{-1}, which is smaller than that published for marine *D. sabina* (see "Discussion").

Acclimation of Lake Jesup D. sabina *to Increased Salinity*

No mortality was associated with the acclimation of FW *D. sabina* to 100% SW. Mean hematocrit of stingrays acclimated to 50% and 100% SW (experimental) did not differ significantly from *D. sabina* left in FW for 32 d (control). The control and experimental hematocrit values were 21.5% ± 0.7% and 20.6% ± 0.9%, respectively. In contrast, mean plasma osmolality increased significantly in the experimental *D. sabina* (Fig. 1*A*); as ambient salinity increased to 100% SW, plasma concentrations of Na^+, Cl^-, and urea increased significantly by 61%, 68%, and 58% (Fig. 1*B–D*). Plasma concentrations of K^+ and Ca^{2+} also increased in the experimental *D. sabina* but were not significantly greater than those of the controls until day 32 ($P < 0.05$; experimental plasma $[K^+] = 5.07 \pm 0.16$ mmol L^{-1}, $[Ca^{2+}] = 5.5 \pm 0.1$ mmol L^{-1}). Plasma levels of all osmolytes in the experimental *D. sabina* were significantly different from the tank water ($P < 0.05$; Table 2; Fig. 1*A–C*), except for Na^+ in the 50% SW-adapted *D. sabina* (Table 2; Fig. 1*B*). Rectal gland weights and RGBW ratios of the experimental *D. sabina* (0.0345 ± 0.004 g; 60.4 ± 5.4 mg kg^{-1}) were not significantly different from those of the control animals (0.0345 ± 0.002 g; 70.8 ± 3.25 mg kg^{-1}).

Holding of the animals in captivity without food had a significant effect on most plasma osmolytes. By day 32, a significant decrease in plasma osmolality was noted in the control animals (Table 3). Plasma concentrations of Na^+, Cl^-, K^+, and urea also decreased significantly, but plasma Ca^{2+} levels were not affected during the captivity period (Table 3).

Discussion

Osmoregulation of Dasyatis sabina *in FW*

The plasma osmotic composition of the FW *Dasyatis sabina* is similar to that of other euryhaline elasmobranchs in FW, such

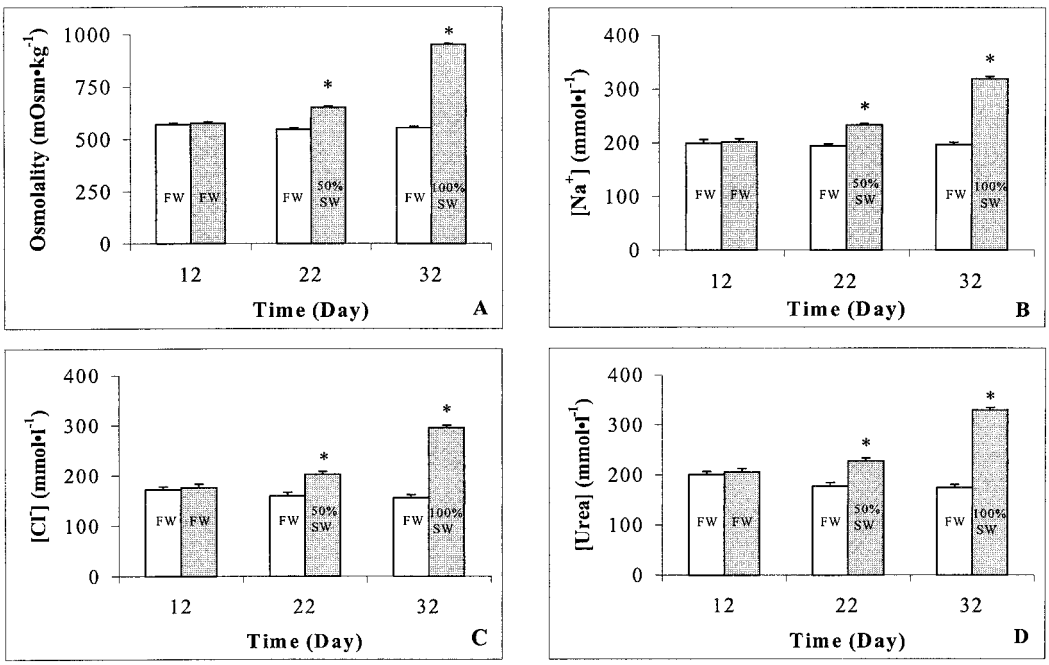

Figure 1. Osmotic composition of plasma from control (*open bars*) and experimental (*shaded bars*) *Dasyatis sabina,* at each sampling period. The control group was left in FW the entire 32 d. For the experimental group, days 12, 22, and 32 represent FW, 50% SW, and 100% SW, respectively. Values are means + 1 SE. Asterisks indicate that plasma from the experimental group was significantly different from that of the control group for that day ($P < 0.05$).

as the bull shark, *Carcharhinus leucas* (Table 1). Compared to marine *D. sabina,* the FW *D. sabina* have a 40% lower plasma osmolality, with plasma concentrations of Na^+, Cl^-, and urea that are approximately 30%, 30%, and 50% of SW values, respectively (Table 1). These values are similar to those of other euryhaline elasmobranchs, in which the reduction of plasma osmolality is primarily through losses of urea, in addition to decreases of Na^+ and Cl^- (Smith 1931; Urist 1962; Thorson et al. 1973).

Compared to FW teleosts and Potamotrygonid stingrays, the Lake Jesup *D. sabina* have substantially higher plasma Na^+, Cl^-, and urea concentrations (Table 1). The osmotic gradient between FW *D. sabina* and Lake Jesup is almost twice that of a typical FW teleost and Potamotrygonid stingray. Therefore, the FW *D. sabina* may have high urine flow rates to maintain water balance, as found in *Pristis microdon* (Smith 1931), especially if the FW *D. sabina* have a relatively high permeability to water, as found in the stenohaline FW *Potamotrygon* sp. (Carrier and Evans 1973).

Besides the osmotic gradient, the *D. sabina* of Lake Jesup maintain large ionic gradients of Na^+ and Cl^-. Since the plasma of FW *D. sabina* is hyperionic to the environment, they would tend to lose Na^+ and Cl^- through diffusion and their urine. However, if *D. sabina* have the same relatively low permeability to ions as *Potamotrygon* sp. (Carrier and Evans 1973), the diffusive losses are likely to be small. Also, the Lake Jesup *D. sabina* have RGBW ratios that are 80% lower than values measured in marine animals (Burger 1972). Gerzeli et al. (1976) demonstrated that atrophied rectal glands from FW *C. leucas* had significantly lower enzymatic activities of Na^+,K^+-ATPase. So, a smaller gland suggests that the FW *D. sabina* secrete relatively few ions through their rectal gland, which would help minimize ion loss.

To balance ion loss in FW, it is necessary to obtain ions from the environment by active branchial uptake (Perry 1997). FW teleosts possess mechanisms associated with active ion uptake in their branchial epithelium, such as H^+-ATPase and Na^+,K^+-ATPase (see Perry 1997). Although marine elasmobranchs have the prerequisite branchial H^+-ATPase and Na^+,K^+-ATPase necessary for ion uptake (Conley and Mallatt 1988; Wilson et al. 1997), few studies have examined branchial ion regulation in FW species. Further work is needed to determine what role the gills of FW elasmobranchs may have in active ion uptake.

Besides the gills, mechanisms for ion uptake are also found in the esophagus and intestine of some euryhaline teleosts in FW (Trischitta et al. 1992; Cataldi et al. 1993; Vilella et al. 1995). *D. sabina* held captive for 32 d without food in FW had significantly lower plasma concentrations of Na^+ and Cl^- than *D. sabina* sampled from Lake Jesup (Table 3). This may indicate that the FW *D. sabina* obtain a portion of their ions from their food; however, the effects of captivity cannot be ruled out as a potential cause of ion loss. It has been suggested that stresses associated with relatively short-term captivity (up to several hours) can lead to electrolyte disturbances in fish (see McDon-

Table 2: Ambient osmotic conditions for the experimental *Dasyatis sabina*

	Na^+	Cl^-	K^+	Ca^{2+}	Osmolality
FW (day 12)	1.25 ± .35	1.25 ± .04	<.1	.95 ± .08	42.17 ± 3.06
50% SW (day 22)	230.75 ± 2.47	270.88 ± 20.68	4.9 ± .14	5.93 ± .45	496.33 ± 16.97
100% SW (day 32)	461.00 ± 4.24	565.27 ± 7.16	9.83 ± .25	11.33 ± .80	995.33 ± .47

Note. All units are mmol L^{-1}, except for osmolality, which is mOsm kg^{-1}. Values are means ± 1 SE.

ald and Milligan 1997), but we are unaware of any studies that have determined the effects of chronic captivity (i.e., 30 d) on plasma osmoregulatory parameters. In contrast to plasma concentrations of Na^+ and Cl^-, Ca^{2+} levels were not affected by the captivity period. This may indicate that FW *D. sabina* have an extremely low turnover rate of Ca^{2+} or that they are able to obtain all their Ca^{2+} via active branchial uptake. Further experiments concerning the effects of starvation and captivity on plasma osmolytes would be helpful to determine what role intestinal uptake of Na^+ and Cl^- has on the overall ion balance in these fish.

Although plasma urea concentrations of FW *D. sabina* are lower than those of marine individuals, the urea values are higher than those found in Potamotrygonid stingrays and FW teleosts (Table 1). Since Lake Jesup *D. sabina* complete their life cycle in FW, there is no osmoregulatory advantage to retaining the excess urea. In fact, if more urea were excreted, the plasma osmolality would further decrease, leading to a lower osmotic gradient and energetic cost of osmoregulation. This basal retention of urea by FW *D. sabina* may be a consequence of their kidney anatomy (see below).

Although the renal counter current reabsorptive mechanisms of urea in elasmobranchs are still not completely understood, Lacy and Reale (1995) noted a correlation between the presence of tubular bundles in renal tubules of elasmobranch kidneys and the renal reabsorption of urea. *Potamotrygon* sp., which no longer reabsorb urea, do not possess tubular bundles in their kidneys, while *D. sabina* kidneys still have the bundles (Lacy and Reale 1995). It is possible the FW *D. sabina* and other euryhaline elasmobranchs in FW have a limited ability to excrete urea because of their ancestral renal tubule morphology, which still has the mechanisms for urea reabsorption.

Acclimation of Lake Jesup D. sabina *to SW*

De Vlaming and Sage (1973) suggested that marine *D. sabina* could regulate their blood volume in dilute SW because their hematocrit was equivalent to marine values after 6 d in the dilute medium. In this study, hematocrits of Lake Jesup *D. sabina* acclimated to 100% SW (experimental) were not significantly different from those of *D. sabina* left in FW (control). Therefore, we suggest that the FW *D. sabina* can regulate their blood volume in higher salinities. If the FW *D. sabina* could not regulate blood volume in SW, hematocrit would be expected to increase significantly in the experimental group, because they would lose a portion of their plasma through dehydration.

By day 32 (100% SW), the plasma osmolality of the experimental *D. sabina* was significantly lower ($P < 0.05$) than the values reported for marine *D. sabina* by De Vlaming and Sage (1973; Table 1; Fig. 1*A*). In addition, plasma from experimental *D. sabina* was hypo-osmotic to SW (Fig. 1*A;* Table 2), as opposed to an expected hyperosmotic plasma in SW. Since plasma concentrations of Na^+ and Cl^- in the experimental group increased to values similar to those of marine *D. sabina,* these osmolytes were not responsible for the deficit. Plasma urea concentrations, however, were significantly lower ($P < 0.05$) than those found in marine *D. sabina* (De Vlaming and Sage 1973). The effects of captivity, especially starvation, may account for the lower plasma concentrations of urea (Table 3).

A study by Haywood (1973) found that starvation of marine

Table 3: The effect of the 32-d captivity period on plasma osmolytes of control *Dasyatis sabina*

	Na^+	Cl^-	Urea	K^+	Ca^{2+}	Osmolality
Field *D. sabina* ($n = 4$)	220.38 ± 7.65	206.1 ± 9.91	229.01 ± 20.16	4.56 ± .48	4.95 ± .31	662.08 ± 28.53
Control *D. sabina* ($n = 8$)	198.77 ± 4.15*	156.01 ± 6.86*	174.59 ± 6.05*	2.99 ± .18*	4.62 ± .17	554.96 ± 14.42*

Note. All units are mmol L^{-1}, except for osmolality, which is mOsm kg^{-1}. Values are means ± 1 SE. The field *D. sabina* represent animals sampled from Lake Jesup on the same day the control group was transported to the lab. It is assumed that the plasma composition of the field *D. sabina* represents the plasma osmotic makeup of the control group before transportation.

* The plasma from the control group was significantly different from that of the field *D. sabina* ($P < 0.05$).

dogfish (*Poroderma africanum*) for 32 d induced hypo-osmoregulation. This was attributed to a 100 mmol L^{-1} loss of serum urea; serum Cl^- was not affected by starvation (Haywood 1973). In the present study, *D. sabina* were starved for 32 d, whereas those from De Vlaming and Sage (1973) were only starved for 6 d. In the control *D. sabina,* which were held captive for 32 d without food, significant losses of plasma Na^+, Cl^-, and urea were observed (Table 3). In SW, however, ion loss would not occur, because the medium is hyperionic to the plasma. Urea production is based on the catabolism of amino acids, and without food, available amino acids would probably be limited. So, whereas other factors associated with captivity cannot be ruled out, it appears that starvation may have a significant negative effect on the urea production of these animals.

As previously noted, the plasma Na^+ and Cl^- concentrations of the FW *D. sabina* acclimated to SW were equivalent to those reported by De Vlaming and Sage (1973) for marine *D. sabina.* This would suggest that the experimental group has an ability to excrete ions similar to that of marine *D. sabina;* hence, both groups would be expected to have similar-sized rectal glands. In this study, however, the rectal gland weights and RGBW ratios of the experimental *D. sabina* did not increase significantly after exposure to SW. This may indicate that the experimental group's smaller rectal glands increase their biochemical potential (i.e., enzyme activity and/or expression) to excrete the ion load, and an increase in size may not be noticed until the animals were exposed to SW for a longer time period. It is also possible that the gills and kidneys have a supplemental role in ion excretion until the rectal gland reaches its full size, but further biochemical and histological studies are required before this can be determined.

Evolutionary and Ecological Implications

To date, no study has found any major differences in the biology of FW and marine populations of the Atlantic stingray (Johnson and Snelson 1996; Amesbury and Snelson 1997). This study did not find any major physiological differences between Lake Jesup *D. sabina* and marine individuals. Even though the Lake Jesup *D. sabina* reproduce and complete their life cycle in FW (Johnson and Snelson 1996), they have not evolved the FW osmoregulatory strategy seen in the Potamotrygonid rays. This is probably a result of their relatively recent occupancy (<1 million years) of the St. Johns River and/or lack of appropriate genetic isolation. In contrast, Potamotrygonid rays are thought to have been derived from a Dasyatid ancestor (Lovejoy 1997) and have been isolated in the Amazon River Basin for over 65 million years (Thorson et al. 1983).

An ecological implication of the results from this study is that the FW *D. sabina* may enter estuarine and marine environments. Since these animals complete their life cycle in the St. Johns River and can be as far as 300 km from the ocean, it is unlikely that they make consistent migrations to SW. However, the results from the present study suggest that the FW *D. sabina* still have the osmoregulatory mechanisms to survive in higher salinities. It is possible that some FW individuals migrate to the mouth of the St. Johns River, similar to the *C. leucas* of Lake Nicaragua that live in FW for extended periods of time but predominantly return to brackish water to reproduce (Thorson 1976). An extensive tag-and-release study on the FW *D. sabina* would help determine whether they are an ecologically euryhaline population.

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