

Osmotic and volaemic effects on drinking rate in elasmobranch fish

W. Gary Anderson^{1,*}, Y. Takei² and N. Hazon^{1,2}

¹*School of Biology, Gatty Marine Laboratory, University of St Andrews, St Andrews, Fife KY16 8LB, Scotland and*

²*Ocean Research Institute, University of Tokyo, Tokyo 1648639, Japan*

*e-mail: wga@st-andrews.ac.uk

Accepted 28 January 2002

Summary

An increase in drinking rate of two species of marine elasmobranch fish, *Scyliorhinus canicula* and *Triakis scyllia*, acclimated to 80% sea water was observed following the introduction of 100% sea water to experimental tanks. The drinking response in both species was found to be maximal within 6 h, and a significant increase was sustained for up to 24 h in *T. scyllia*. Plasma osmolality was significantly increased within 6 h following introduction of 100% sea water, and this increase was principally due to elevated plasma Na⁺ and Cl⁻ concentrations. Administration of 2 mol l⁻¹ mannitol, 75% sucrose and vehicle (elasmobranch Ringer) did not induce a significant increase or decrease in the drinking rate of *S.*

canicula. However, injection of 20% NaCl was found to decrease drinking rate significantly in *S. canicula* 60 min after administration. Controlled haemorrhage of approximately 5.7% of total blood volume in *S. canicula* induced a rapid 36-fold increase in drinking over basal levels. The present study demonstrates a physiological dipsogenesis in response to hypovolaemia in marine elasmobranch fish as part of their overall iso/hyperosmoregulatory strategy.

Key words: elasmobranch, hypovolaemia, hyperosmoraemia, drinking rate, dogfish, *Scyliorhinus canicula*, *Triakis scyllia*.

Introduction

Drinking has long been established as an essential component in the homeostatic control of body fluid and ionic balance. In terrestrial mammals, a 1–2% deficit in cellular fluid volume is sufficient to induce a powerful drinking response, whereas 8–10% of extracellular fluid loss is required to induce dipsogenesis (Fitzsimons, 1998). As a consequence, an increase in plasma osmolality, or hyperosmoraemia, acts as a more acute trigger for drinking in mammals than a reduction in extracellular fluid volume or hypovolaemia (Fitzsimons, 1979). However, in aquatic vertebrates such as teleost fish, hypovolaemia appears to be the main drinking stimulus (Takei, 2000).

In the hypo-osmotic environment of fresh water, osmotic gradients favour the passive entry of water across semi-permeable membranes such as those of the gills in teleost fish, negating a requirement to drink (Maetz, 1970). However, in the hyperosmotic environment of sea water, water is constantly lost and teleost fish drink to maintain ionic and water balance (Oide and Utida, 1968), with excess ions being excreted at the gills and/or kidney (Evans, 1993).

In contrast to marine teleosts, marine elasmobranchs maintain blood plasma osmolality iso-osmotic or slightly hyperosmotic to sea water (Smith, 1931). This elevation in plasma osmolality is achieved through the regulation of three principal components, Na⁺, Cl⁻ and urea, with each component constituting approximately one-quarter to one-third of total plasma osmolality (900–1000 mosmol kg⁻¹) (Smith, 1936).

The toxicity of urea is counteracted by the retention of methylamines, in particular trimethylamine oxide (TMAO) (Yancey and Somero, 1980). As a consequence of their iso/hyperosmoregulatory strategy, marine elasmobranchs experience little osmotic water flux, no risk of dehydration, as experienced by the hyporegulating marine teleosts and, therefore, no apparent reason to drink (Schmidt-Nielsen, 1997).

Although the majority of elasmobranchs are considered stenohaline marine species, numerous reports have demonstrated that these species successfully acclimate to varying degrees of salinity within the laboratory (Burger, 1965; Hazon and Henderson, 1984; Goldstein and Forster, 1971). Furthermore, a number of species, such as the bull shark *Carcharhinus leucas* and the Atlantic stingray *Dasyatis sabina*, inhabit both marine and freshwater environments (Thorson et al., 1973; Piermarini and Evans, 1998). The presence of elasmobranchs in a range of aquatic habitats and the ability of presumed stenohaline marine elasmobranchs to acclimate to dilute sea water indicates that the group as a whole has, to varying degrees, the physiological capacity to survive in changing environmental salinities.

The present study investigated a potential role for drinking in marine elasmobranchs during environmental manipulation and the osmotic/volaemic control behind the drinking response. In the first series of experiments, two species of

marine elasmobranch, the European lesser-spotted dogfish *Scyliorhinus canicula* and the Japanese dogfish *Triakis scyllia*, were subjected to a change in environmental salinity. Drinking rate was monitored in both species acclimated to a reduced environmental salinity of 80 % sea water (SW) and during acute exposure to the hyperosmotic environment of 100 % SW. To determine the physiological trigger for dipsogenesis, a second series of experiments was conducted in which drinking rate was assessed in *S. canicula* acclimated to 100 % SW during administration of a variety of osmotic and volaemic challenges.

Materials and methods

Scyliorhinus canicula (L.) of mixed sex weighing 0.42–0.83 kg were obtained from waters off the west coast of the British Isles and transported to the Gatty Marine Laboratory aquarium, Fife, Scotland. They were maintained in aerated flow-through SW tanks at ambient temperature (16–18 °C) for a minimum of 2 weeks prior to experimentation. *Triakis scyllia* (Muller and Henle) of mixed sex weighing 0.8–1.4 kg were collected in Sagami Bay, Kanagawa, Japan, and maintained at ambient temperature (24–26 °C) in aerated flow-through SW tanks at Misaki Marine Biological Station, University of Tokyo, Japan, for a minimum of 2 weeks before use.

Surgical procedures

All procedures outlined below were carried out by licensed personnel under the guidelines set out by the Animals (Scientific Procedures) Act 1986, UK. Drinking rate was assessed using a preparation similar to that previously described for teleost fish (Takei et al., 1998). Dogfish were anaesthetised using tricaine methanesulphonate (MS-222, 250 p.p.m.) neutralised with sodium bicarbonate (250 p.p.m.). A ventro-lateral incision into the abdominal cavity was made just posterior to the pectoral fin. Retraction of the intestine exposed the stomach, oesophagus and mesenteric artery. The stomach was cannulated using polyethylene tubing (1.5 mm outer diameter; Portex tubing, Hythe, Kent), and a tight ligature was placed around the anterior end of the stomach to prevent fluid from entering *via* the oesophagus. The oesophagus was then cannulated with tubing of similar bore, and the mesenteric and coeliac arteries were cannulated using 0.5 mm outer diameter polyethylene tubing to allow for the administration of reagents and collection of blood samples. The incision was closed with 5-6 gauge silk sutures, and the fish were allowed to recover in individual flow-through aquaria (40 l) at ambient temperature (16–18 °C) for a minimum of 24 h. During times of rest, the oesophageal and stomach cannulae were linked to allow imbibition of water and thus the maintenance of body fluid homeostasis. For the experiments involving *S. canicula*, the oesophageal and stomach cannulae were disconnected, fluid imbibed was collected through the oesophageal cannula into pre-weighed vials, and the volume of fluid was assessed gravimetrically assuming a specific gravity

of 1. The volume drunk was then replaced into the animal *via* the stomach cannula. For the experiments involving *T. scyllia*, the volume of fluid imbibed through the oesophageal cannula was assessed by a drop counter, and the volume of fluid drunk was returned to the fish automatically by a pulse injector synchronised with the drop counter.

Series 1: salinity challenge

In the salinity challenge experiments, *T. scyllia* and *S. canicula* were stepwise acclimated to 80 % salinity in their respective aquaria (755 mosmol kg⁻¹ at the Gatty Marine Laboratory, 772 mosmol kg⁻¹ at Misaki Marine Station) in a manner similar to that described previously (Tierney et al., 1998). Briefly, fish were held in acclimation tanks for a minimum of 3 days, after which the salinity of the water was adjusted to 90 % for the following 3 days, with the final reduction to 80 % occurring 3 days later. Once at 80 % SW, the fish were left to acclimate for a minimum of 2 weeks prior to surgery. Following a minimum of 2 h of assessment of basal drinking rate at 80 % SW, 100 % SW was introduced into the experimental tanks, with a complete water change occurring within 30 min. Drinking rate was then assessed in both species as they acclimated to the increase in environmental salinity.

Blood samples (0.5 ml) were taken from the coeliac arterial cannulae using pre-chilled syringes 0 and 1.5 and 6 h after the introduction to 100 % SW. Each blood sample was centrifuged at 1300 g for 1 min, and the plasma was removed and assessed for plasma osmolality (Roebing Osmometer, Camlab, Cambridge, UK) and for Cl⁻ (Corning 925 Cl⁻ analyser), Na⁺ (Corning 480 flame photometer) and urea (Sigma kit no. 640) levels. An equivalent volume of isotonic Ringer was injected into the fish *via* the mesenteric arterial cannula immediately after a blood sample had been taken.

Series 2: osmotic and volaemic challenge

Only *S. canicula* was used in series 2. Fish of mixed sex, mean mass 0.64±0.33 kg (mean ± S.E.M., *N*=15), acclimated to 100 % SW and held in a manner similar to that described above were used for the osmotic and volaemic challenge experiments. Surgical procedures were as described above, and all reagents were purchased from Sigma Chemical Company (Poole, Dorset, UK). The osmotic stimuli [2 mol l⁻¹ mannitol, 2.19 mol l⁻¹ sucrose (75 %, w/v) and 3.4 mol l⁻¹ NaCl (20 %, w/v)] were administered at a dose of 1 ml kg⁻¹ body mass to produce a theoretical percentage cellular dehydration of 0.36, 0.33 and 0.47 % respectively. Each treatment was made up in dogfish Ringer (in mmol l⁻¹): NaCl, 240; KCl, 7.0; CaCl₂, 10.0; MgCl₂, 4.9; NaHCO₃, 2.3; Na₂HPO₄·2H₂O, 0.5; Na₂SO₄, 0.5; urea, 360; trimethylamine oxide, 60 (pH 7.6). In addition, controlled haemorrhaging of 2.5 ml of blood from the coeliac arterial cannula was carried out, constituting 5.7±0.29 % (mean ± S.E.M., *N*=6) of total blood volume in the experimental fish (Thorson, 1962). Blood samples from the experimental fish were taken in a manner similar to that described above at 0, 0.5 and 3 h after treatment and analysed for the variables described above.

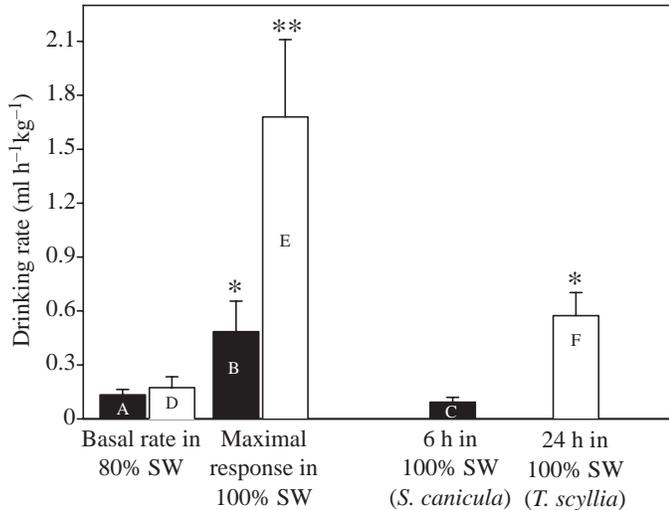


Fig. 1. Basal drinking rates ($\text{ml h}^{-1} \text{kg}^{-1}$) of two species of elasmobranch fish acclimated to 80% sea water (SW) and maximal responses during acclimation to 100% SW. The filled columns represent drinking rates for *Scyliorhinus canicula* and the open columns represent drinking rates for *Triakis scyllia*. Results are expressed as means + S.E.M. ($N \geq 6$ for each group). Column B was significantly greater than column A; and columns E and F were significantly greater than column D (* $P < 0.05$, ** $P < 0.01$) (Student's t -test).

Statistical analyses

In series 1 experiments, both species demonstrated a delay in the drinking response following the introduction of 100% SW. Furthermore, the individual variation in this delay was considerable. Therefore, mean (± 1 S.E.M.) maximal drinking rates for both species ($\text{ml h}^{-1} \text{kg}^{-1}$) were assessed as the maximum volume of fluid imbibed over a single 30 min period during acclimation to 100% SW and compared with basal drinking rate in 80% SW.

For series 2 experiments, values are presented as mean (± 1 S.E.M.) drinking rates ($\text{ml h}^{-1} \text{kg}^{-1}$), which were assessed over 20 min periods following manipulation of the experimental fish. Comparisons were made with basal drinking rates prior to injection of test substances or haemorrhaging of the fish. All osmolalities and body fluid osmolyte concentrations were compared with basal values in each respective experimental group. Statistical assessment of the data was carried out using analysis of variance (ANOVA) followed by Tukey's *post-hoc* test with a Student's t -test to provide a final level of significance.

Results

Series 1: salinity challenge

Increases in drinking rate in both species following the introduction of 100% SW were not immediate; indeed, there was considerable variation in the delay before both the onset of increased drinking rate and the observation of a maximal drinking response. The delay ranged between 2 and 3 h for *S.*

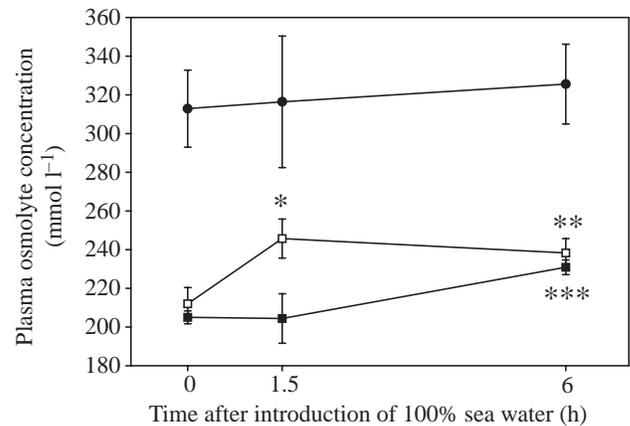


Fig. 2. Plasma concentrations (mmol l^{-1}) of Na^+ (□), Cl^- (■) and urea (●) during acclimation from 80% to 100% sea water in *Scyliorhinus canicula*. Statistical comparisons were made with time zero (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) (Student's t -test). Plasma urea concentration did not change significantly during the acclimation period. Results are expressed as means \pm S.E.M. ($N \geq 6$ for each group).

canicula and 3 and 6 h for *T. scyllia*. Consequently, only maximal drinking rates rather than drinking rate at a specific time point during acclimation are reported (Fig. 1). Nonetheless, basal drinking rates in both species acclimated to 80% SW were of similar magnitude ($0.1\text{--}0.15 \text{ ml h}^{-1} \text{kg}^{-1}$), although the maximal response observed in *T. scyllia* was nearly 3.5 times greater than that observed in *S. canicula*. Furthermore, this significantly higher drinking rate was sustained for up to 24 h in *T. scyllia* during acclimation to 100% SW, whereas the increase was transient in *S. canicula*, in which the rate returned to basal levels within 6 h (Fig. 1).

For both species, plasma osmolality was not significantly higher than basal values until 6 h after 100% SW had been introduced into the holding tanks (Table 1). A significant increase in plasma Na^+ concentration was observed at 1.5 and 6 h after the introduction of 100% SW (Fig. 2). However, plasma Cl^- concentration did not match plasma Na^+ concentration until 6 h after the introduction of 100% SW. This delay in increase in plasma Cl^- concentration in the present

Table 1. Plasma osmolality of two species of elasmobranch fish during acclimation from 80% to 100% sea water

	Plasma osmolality (mosmol kg^{-1})			
	0h	1.5h	6h	24h
<i>T. scyllia</i> (mOsm kg^{-1})	862 \pm 9.3	873 \pm 6	907 \pm 8*	967.7 \pm 7***
<i>S. canicula</i> (mOsm kg^{-1})	831 \pm 22.8	854 \pm 15	930 \pm 15***	–

During acclimation seawater osmolality for *Triakis scyllia* rose from 772 to 970 mosmol kg^{-1} ; for *Scyliorhinus canicula*, it rose from 755 to 950 mosmol kg^{-1} .

Results are expressed as means \pm S.E.M.

Asterisks indicate a significant difference from the 0h value (* $P < 0.05$, *** $P < 0.001$; Student's t -test; $N \geq 6$ for each group).

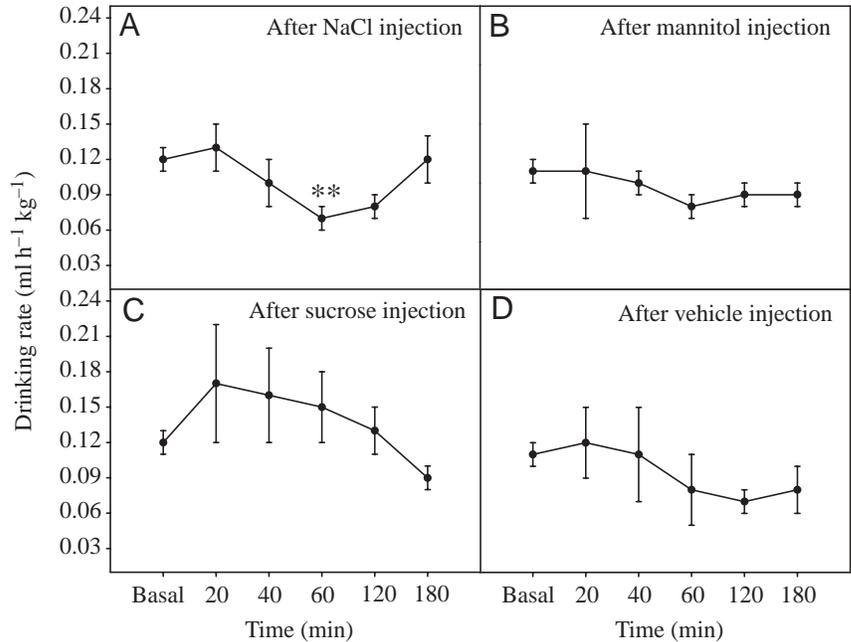


Fig. 3. Drinking rates ($\text{ml h}^{-1} \text{kg}^{-1}$) in *Scyliorhinus canicula* following administration of 1 ml kg^{-1} body mass of (A) 20% NaCl ($N=9$), (B) 2 mol l^{-1} mannitol ($N=7$), (C) 75% sucrose ($N=7$) and (D) vehicle ($N=4$). Results are expressed as means \pm S.E.M. Drinking rate was significantly reduced (** $P < 0.01$) below basal levels 60 min post-administration of 20% NaCl (Student's t -test).

study is difficult to explain. Plasma urea levels in *S. canicula* 6 h after the introduction of 100% SW did not show a significant change from the concentration observed in fish acclimated to 80% SW (Fig. 2), demonstrating a delay in the increase in plasma urea levels during acclimation in comparison with plasma Na^+ and Cl^- levels.

Series 2: osmotic and volaemic challenge

Drinking rates in *S. canicula* treated with 2 mol l^{-1} mannitol (1 ml kg^{-1} body mass), 75% sucrose (1 ml kg^{-1} body mass) and vehicle (1 ml kg^{-1} body mass) were not significantly different from basal values assessed just prior to the administration of

the test substances (Fig. 3B–D). However, following injection of 20% NaCl (1 ml kg^{-1} body mass), drinking rates were found to be significantly lower than basal values 60 min post-injection (Fig. 3A). With the exception of plasma Na^+ concentration, which was significantly lower 30 min after injection of 75% sucrose (Fig. 4C), plasma osmolality and plasma osmolyte concentrations did not differ from basal values following administration of the test substances (Table 2; Fig. 4).

Controlled haemorrhaging of 2.5 ml from the experimental fish produced a rapid and profound dipsogenic response: drinking rate was 36 times greater than basal levels within

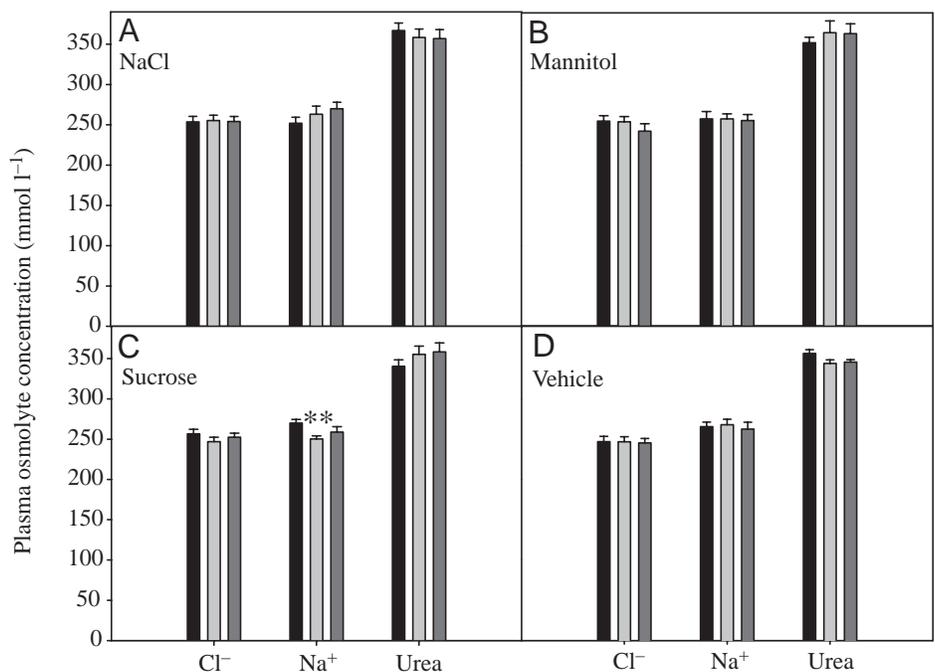


Fig. 4. Plasma concentrations (mmol l^{-1}) of Cl^- , Na^+ and urea in *Scyliorhinus canicula* at 0h (black columns), 0.5h (light grey columns) and 3h (dark grey columns) following administration of 1 ml kg^{-1} body mass of (A) 20% NaCl ($N=9$), (B) 2 mol l^{-1} mannitol ($N=7$), (C) 75% sucrose ($N=7$) and (D) vehicle ($N=4$). Results are expressed as means \pm S.E.M. Plasma sodium concentration was significantly (** $P < 0.01$) reduced relative to the 0h value 30 min after administration of 75% sucrose (Student's t -test).

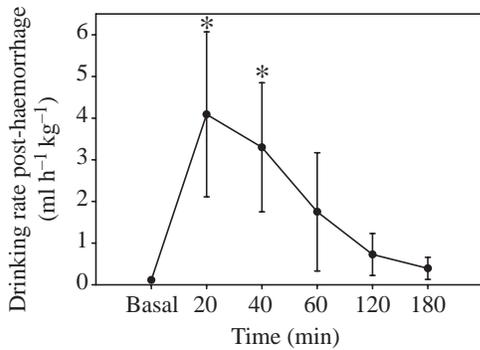


Fig. 5. Drinking rate (ml h⁻¹ kg⁻¹) in *Scyliorhinus canicula* following controlled haemorrhage of approximately 5.7% of total blood volume (N=6). Results are expressed as means ± S.E.M. Drinking rate was significantly (*P<0.05) increased above the basal level for up to 40 min post-haemorrhage (Student's *t*-test).

20 min of haemorrhaging (Fig. 5). This significant response was sustained for a total of 40 min and returned to basal levels within 2 h of haemorrhaging. Plasma concentrations of Na⁺, Cl⁻ and urea and plasma osmolality did not differ from basal concentrations after haemorrhaging of the fish (Table 2; Fig. 6).

Discussion

The present study has demonstrated that, under the appropriate environmental conditions, marine elasmobranchs will drink the environmental medium as part of their overall iso/hyposmoregulatory strategy. The adoption of an iso/hyposmoregulatory strategy demands an increase in plasma osmolality during acclimation from the dilute 80% SW to the more concentrated medium of 100% SW. The increase in plasma osmolality in both *T. scyllia* and *S. canicula* within 6 h of the introduction to 100% SW was attributable to a concomitant increase in plasma Na⁺ and Cl⁻, but not urea, concentration. It appears therefore, from this and other studies, that in response to increased environmental salinity Na⁺ and

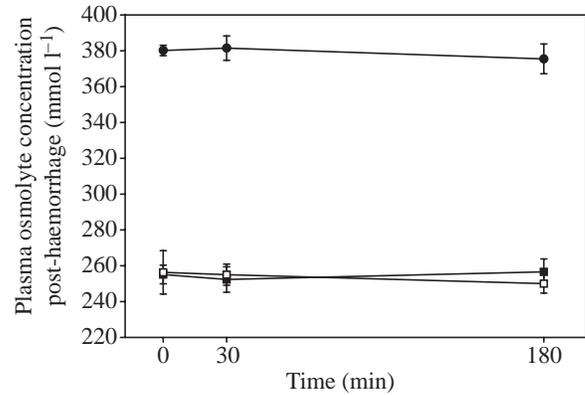


Fig. 6. Plasma concentrations (mmol l⁻¹) of Na⁺ (□), Cl⁻ (■) and urea (●) following controlled haemorrhage of approximately 5.7% of total blood volume in *Scyliorhinus canicula* (N=6). Results are expressed as means + S.E.M. Statistical comparisons were made with time zero. Plasma, Na⁺, Cl⁻ and urea concentrations did not change significantly (Student's *t*-test).

Cl⁻ concentrations are increased in the first instance followed by an increase in plasma urea concentration through decreased urea excretion and/or increased hepatic urea production (Goldstein and Forster, 1971; Armour et al., 1993).

Differences in drinking rate with respect to environmental salinity have previously been reported in *S. canicula* using a radio-labelled tracer technique. Basal values were significantly lower and higher in media more dilute and more concentrated, respectively, than the control of 100% SW (Hazon et al., 1999). The drinking responses observed in the present study were maximal between 2–3 and 3–6 h after the introduction of 100% SW in *S. canicula* and *T. scyllia*, respectively, and in general occurred prior to the observed increase in plasma osmolality at 6 h. The delay in maximal drinking response contrasts with the rapid and predictable responses observed during transfer from a dilute to a concentrated environment for a variety of teleosts such as the Japanese eel *Anguilla japonica* (Hirano, 1974; Takei et al., 1988), the flounder *Platichthys flesus* (Carrick and Balment, 1983) and the rainbow trout *Onchorhynchus mykiss* (Shehadeh and Gordon, 1969).

The differences in maximal responses observed in the present study, with that of *T. scyllia* being nearly 3.5 times greater than that of *S. canicula*, may be reflected in the ambient temperatures of the two aquaria. During experimentation, water temperature was 24–26 °C at Misaki Marine Biological Station and 16–18 °C at the Gatty Marine Laboratory. Interestingly, a temperature-related variation in drinking rate has previously been reported for the flounder and Japanese eel (Carroll et al., 1994; Takei and Tsukada, 2001). The differences between the two species used in the present study may also reflect a difference in their osmoregulatory capacity. Indeed, the elasmobranchs *Heterodontus portusjacksoni* and *Trygonoptera testacea* both inhabit similar estuarine environments in New South Wales, Australia. However, *T. testacea* was recently reported to have greater control of salt

Table 2. Plasma osmolality of *Scyliorhinus canicula* following administration of 1 ml kg⁻¹ body mass of 20% NaCl, 2 mol l⁻¹ mannitol, 75% sucrose or vehicle or after haemorrhage of 2.5 ml of blood

Treatment	Plasma osmolality (mosmol kg ⁻¹)		
	0h	0.5h	3h
20% NaCl	895.0±14.9	913.6±9.7	905.6±8.6
2 mol l ⁻¹ mannitol	903.0±8.2	902.3±11.0	899.0±12.3
75% sucrose	901.9±12.9	907.9±9.1	915.4±6.2
Vehicle	902.5±8.2	901.0±8.0	907.3±10.2
2.5 ml haemorrhage	919.9±13.8	923.6±13.9	923.9±12.4

Results are expressed as means ± S.E.M.

There were no significant changes (Student's *t*-test) (N≥6 for each group).

and water balance in dilute environments, indicating a greater osmoregulatory capacity than *H. portusjacksoni* (Cooper and Morris, 1998). Despite the differences between the two species observed from salinity challenge experiments in the present study, it is clear that both have a physiological requirement to drink the external medium under the appropriate environmental conditions.

Drinking studies in mammals have demonstrated that intravascular administration of hypertonic osmotically active solutions, causing hyperosmoraemia and thus drawing water from the cellular compartment, induces a profound dipsogenesis (Fitzsimons, 1979, 1998). Similar experiments in teleost fish have produced conflicting results. Intra-arterial administration of hypertonic solutions of NaCl and sucrose were shown to inhibit drinking in freshwater-acclimated *Anguilla japonica* (Takei et al., 1988). However, the same study demonstrated a concomitant increase in plasma angiotensin II concentration, which seemed paradoxical given the potent dipsogenic action of angiotensin II in these fish (Takei et al., 1979). The cause of the inhibition was elucidated by Kaiya and Takei (1996): following injection of hypertonic mannitol and NaCl solutions, there was a transient increase in plasma levels of atrial (ANP) and ventricular (VNP) natriuretic peptide. ANP is known to act as an anti-dipsogen in eels and mammals (Brenner et al., 1990; Tsuchida and Takei, 1998); indeed, the dose of ANP required to induce anti-dipsogenesis in eels was reported to be 100 times less than the dipsogenic dose required for angiotensin II (Takei, 2000). These reports could in part explain the inhibition of drinking, at least in eels, following intra-arterial administration of hypertonic solutions. This agrees with a non-specific effect of hyperosmoraemia leading to hypervolaemia acting as a stimulus for the release of circulating natriuretic peptides in teleost fish (Kaiya and Takei, 1996; Smith et al., 1991; Westenfelder et al., 1988).

In the present study, no significant change in drinking rate was observed following intra-arterial administration of hypertonic mannitol and sucrose, suggesting the lack of a hypervolaemic effect on drinking in elasmobranchs. However, injection of hypertonic NaCl induced a significant decrease in drinking rate within 60 min of injection, which may suggest a specific but perhaps transient effect of NaCl on the drinking response of *S. canicula*.

The only natriuretic peptide in elasmobranchs appears to be C-type natriuretic peptide (CNP) (Suzuki et al., 1994; Kawakoshi et al., 2001), so it is reasonable to assume that CNP may act in elasmobranchs in a manner similar to ANP in teleost fish. Recent investigations using homologous CNP have demonstrated CNP inhibition of an angiotensin-II-induced drinking response in *S. canicula*, and the dose of CNP required to induce this anti-dipsogenesis was 50 times lower than the dipsogenic dose required for angiotensin II (Anderson et al., 2001a). However, intravascular administration of homologous CNP in *A. japonica* had no effect on drinking rate (Tsukada and Takei, 2001), but cerebral ventricular administration of CNP in mammals has been shown to induce drinking (Samson et al., 1991).

Hypervolaemia is a potent stimulus for rectal gland secretion in elasmobranchs (Solomon et al., 1985) and is also considered to be the predominant stimulus for CNP secretion into the circulation (Loretz and Pollina, 2000). Interestingly, the rectal gland acts as a specific route of excretion of NaCl in elasmobranchs, and CNP is known to stimulate rectal gland secretion *in vitro* in both *S. canicula* and the spiny dogfish *Squalus acanthias* (Solomon et al., 1992; Anderson et al., 1995). It is evident that the inhibitory mechanisms of drinking in both teleosts and elasmobranchs are very complex and largely unknown. Clearly, the potential action of hypervolaemia and, in particular, of the administration of hypertonic NaCl on the drinking rate of elasmobranchs requires further research and must be considered alongside the stimulatory role of CNP on rectal gland function.

The present study has demonstrated that the increase in drinking rate in elasmobranchs acclimating to increased environmental salinity appears to be mediated by extracellular dehydration or hypovolaemia. Controlled haemorrhage of 5.7% of blood volume caused a significant and immediate increase in drinking rate in *S. canicula*. In mammals, an 8–10% deficit in extracellular fluid volume is required to induce dipsogenesis (Fitzsimons, 1998), and in freshwater-acclimated *A. japonica* a reduction of as much as 30% of total blood volume was required before drinking began (Hirano, 1974). It appears, therefore, that *S. canicula* was particularly sensitive to a reduction in blood volume. The increased drinking in the haemorrhaged *S. canicula* was almost immediate in comparison with the delayed drinking response observed in the fish presented with a salinity challenge. The delay in the drinking response was probably due to a more gradual hypovolaemia as the fish acclimated to the increased environmental salinity, indicating that a threshold of extracellular fluid loss during acclimation to the hyperosmotic environment of 100% SW may have to be reached prior to the initiation of drinking.

Undoubtedly, passive movement of NaCl and water across the gill membranes will occur as elasmobranchs acclimate to the more concentrated environment of 100% SW. This study is the first to demonstrate that a drinking response may contribute to the observed independent increase in plasma Na⁺ and Cl⁻ concentrations during acclimation to increased environmental salinities. The reduction in blood volume during acclimation to increased environmental salinity, initiating a drinking response, is similar to the response observed in teleost fish (Hirano, 1974; Takei et al., 1988), in which it appears that angiotensin II is the primary modulator of the drinking response (Takei et al., 1988). Sequencing of homologous elasmobranch angiotensin I (Takei et al., 1993) and subsequent identification of angiotensin-II-like receptors in *T. scyllia* (Tierney et al., 1997) has indicated that angiotensin II may also play an important role in elasmobranch osmoregulation. Kobayashi et al. (1983) reported a lack of drinking in elasmobranch fish following administration of angiotensin II. However, that study used a less-sensitive dye-dilution technique than that used in the present study and may not have

detected the low basal drinking rates in seawater-acclimated elasmobranch fish reported in this and other studies (Hazon et al., 1999).

Recent investigations have demonstrated a potent dipsogenic response to homologous angiotensin I that can be blocked by the co-administration of the angiotensin converting enzyme inhibitor captopril and, furthermore, both *S. canicula* and *T. scyllia* responded to the administration of homologous angiotensin II with a dose-dependent increase in drinking rate (Anderson et al., 2001b). These results, combined with the present study, indicate that the renin–angiotensin system may play an important role in the regulation of drinking during acclimation to increased environmental salinities.

In summary, the present study has demonstrated a drinking mechanism in the more primitive marine elasmobranch fishes that were considered not to have the physiological requirement, indeed capacity, to drink for osmoregulatory purposes. This drinking response is initiated during acclimation to hyperosmotic environments and is triggered primarily by hypovolaemia in a manner similar to that described previously for teleost fish.

The studies on *S. canicula* were carried out by W.G.A. with support from NERC grant GR3/11420 awarded to N.H. The studies on *T. scyllia* were carried out by N.H. as a Visiting Research Professor with support from the Ocean Research Institute, University of Tokyo, Japan. The authors would like to thank the Misaki Marine Biological Station, University of Tokyo, for the collection and maintenance of *T. scyllia*.

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