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Coupling salinity reduction to aquatic animal well-being and ecosystem representativeness at the Biodôme de Montréal

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Abstract

This paper presents a case study of a locally adapted sustainable strategy of salinity reduction applied to the Saint Lawrence maritime ecosystem at the Biodôme de Montréal. In conformity with the standards of the CAZA (Canadian Aquarium and Zoos Association), this procedure was implemented to reconcile animal well-being, ecosystem representativeness and control of costs under the operational environment of a cold seawater recirculation system featuring the Golfe du Saint Laurent Ecosystem (GSLE) and its associated live collection. A simple methodology to carry out safe salinity reduction procedures of artificial seawater environments (from 28 to 24 Practical Salinity Units) is proposed and detailed. Adapted salinity challenge tests at 14, 21 and 24 were conducted beforehand and simple adapted indicators were used on a selection of key species (thorny skate: *Raja radiate*; little skate: *R. erinacea*; barndoor skate: *R. laevis*; Atlantic cod: *Gadus morhua*; green urchins: *Strongylocentrolus droebachien* and American lobster: *Homarus americanus*) to evaluate the well-being and mortality risks associated with both a lower operational salinity (long-term exposure) and an unavoidable salinity drop (short-term exposure) observed during routine large-scale water renewal operations. Economic gains achieved through reduction in the use of costly synthetic salt formulation were calculated. The savings achieved during three years of operation at 24 PSU have been applied to the improvement of the water quality control management capacities of the GLSE exhibit such as a sulphur-based denitrification unit, additional ozonation and protein skimming capacities.

Background

Providing captive animals with an enriched environment and more than adequately meeting their physical needs has become a priority for zoos and aquariums (Kirkwood 2003). Welfare concerns are now coupled with an increasing need for the sector to affirm its role in conservation, education, research (Hutchins and Thompson 2008) and sustainability (Townsend 2009). To address all of these priorities, adapted and cost-effective strategies must be applied within limited operational budgets. The real challenge lies in finding a range of conditions which will guarantee survival and allow species to show an acceptable range of welfare states (Hutchins, 2006). This challenge becomes more important when dealing with species assemblages that recreate community dynamics and ecosystems.

In aquariums, parameters such as salinity, temperature, pH, nitrates and turbidity are closely monitored to provide adequate captive conditions. The costs of recreating seawater from artificial salts are major. In compliance with its mission statement, the Biodôme de Montréal recently decided to explore a reduction in the salinity level of the main aquatic exhibit to release financial margins with the benefit of tackling water quality issues more efficiently. Changing salinity levels requires understanding of how different species cope with changes in environmental salinity (for a systematic review see Jobling 1996). Aquatic organisms display ecophysiological adaptations to salinity that can be divided into two categories: osmoconformers and osmoregulators. Echinoderms and coelenterates are osmoconformers, as are the majority of polychaetes, bivalve molluscs, and crustaceans. Faced with a salinity reduction, these organisms absorb water and lose salts

until their body fluids are isosmotic with the external environment. Euryhaline osmoconformers eliminate excess liquid by producing isosmotic urine. For osmoconformers, the physiological tolerance of changes in external osmolality is supplemented by behavioural mechanisms to limit undesirable exposure (e.g. burrowing, escaping and closing of shells). The ability of osmoconformers to inhabit estuarine habitats is limited by their tolerance to dilution of their body fluid. Osmoregulators regulate the water content and ionic composition of their tissues and body fluids despite salt concentrations in the external medium. They possess osmosensitive cells and chemoreceptors that trigger mechanisms such as drinking water and reduction of the integument's permeability to salts, or urinary salt excretion and reduction of the integument's permeability at high and low salinity respectively. Osmoregulators can adjust to both high-salt and low-salt environments (Karleskint et al., 2010). Elasmobranchs are osmoconformer fishes that use high body fluid urea and TMAO (N-trimethylamine oxide) levels to reduce osmotic stress (Yancey 2005).

Studies often indicate growth stimulatory effects at intermediate salinities. For instance, in Atlantic cod *Gadus morhua*, Atlantic halibut*, Hippoglossus hippoglossus*, Atlantic sturgeon *Acipenser oxyrhynchus*, Atlantic wolfish *Anarhichas lupus,* turbot *Scophthalmus maximus,* or winter flounder *Pseudopleuronectes americanus,* growth rates are significantly increased at salinities of 12–24 (Lambert et al. 1994; Imsland et al. 2008; Imsland and Cunnarsson 2010; Niklitschek and Secor 2009; Le François et al. 2004; Manderson et al. 2002 respectively). Le François et al. (2004) linked higher growth in common wolffish (*Anarhichas lupus*) to reduced metabolic costs of ion regulation. Lower salinities can also positively affect food conversion efficiency (*G. morhua*; Lambert et al. 1994) and reduce fish parasitic infections, as in brackish water, the variety of marine and freshwater parasite species is considerably reduced (Möller 1978). Reduced salinity is also considered more appropriate for the use of sulphur-based denitrification, an addition to the water treatment loop considered at the GSLE. Many studies have reported that relatively high salinity restrained the denitrification activity of sulphur-oxidising bacteria *Thiobacillus denitrificans* (https://microbewiki.kenyon.edu/index.php/Thiobacillus_denitrificans). Koenig and Liu (2004) indicated that denitrification rate started to decrease at approximately 70% full strength SW, i.e. ≈24 PSU.

Additionally, salinity reduction can translate into significant operational cost cutbacks. For example, introduction of routine salt supplementation to penguin diets allowed elimination of the costs of building and maintaining saltwater habitats (Mazzaro et al. 2004). This practice is currently in operation for the Arctic and Antarctic ecosystems of the Biodôme de Montréal. Public aquariums routinely keep high salinities for their exhibits while experimental evidence largely suggests that it could be lowered significantly without prejudice for many species commonly found in estuarine environments. This study seeks to test the assumption that salinity reduction is worth considering when dealing with large bodies of artificial seawater, especially where exhibits aim to represent estuarine ecosystems, which are characterised by large fluctuations and a wide range of environmental salinities along their axes.

Environmental disturbances such as salinity reduction can be detected by changes in hormones (e.g. cortisol) or substrate concentrations in plasma (e.g. ionic composition, glucose, lactate etc.), or by changes in erythrocyte parameters (e.g. cell volume or enzyme activities) (Wedemeyer et al. 1990). Disturbances in osmolality and haematocrit values are reliable indicators for teleosts, whereas for crustaceans, change in clotting time is suggested (Fotedar et al. 2006). Additionally, behavioural observations are necessary complements to get a full picture of experienced osmotic stress level (Wedemeyer et al. 1990).

Our contribution depicts an operation inspired by Fabrègas et al. (2011), suggesting a more systematic evaluation of naturalistic and non-naturalistic enclosures in relation to the suitability of environments for animals and their well-being. A case study outlining the methodology involved from species selection to water renewal procedures to the evaluation of physiological effects is presented.

Methods

The Biodôme de Montréal's marine ecosystem is an assemblage that replicates community dynamics of the St Lawrence river maritime estuary. It is a 1620 m^2 exhibit that includes a saltwater marsh and two basins: a 23,000 litre rocky, wave-beaten shore basin containing mostly invertebrates and a 2.5 million litre basin (Fig. 1) housing a 70-species assemblage based on the biogeography study by Mahon et al. (1998). The GSLE represents a very variable ecosystem. Estuaries, as transitional zones, are sites of strong vertical and horizontal salinity gradients (El-Sabh and Silverberg 1990) and the St Lawrence estuary system can be divided into

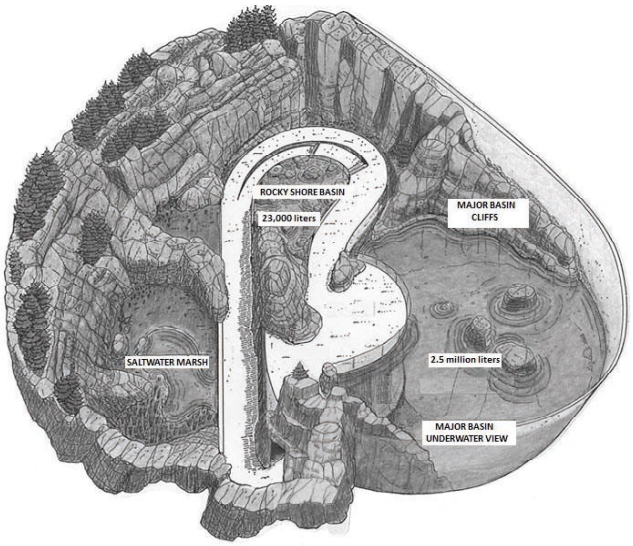

Figure 1. Diagram of the St Lawrence Estuary Ecosystem exhibit at the Biodôme de Montréal.

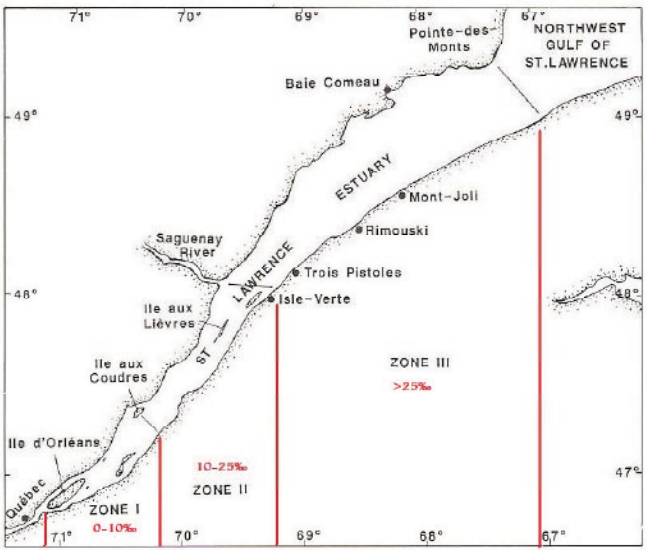

Figure 2. The St Lawrence estuary (Québec, Canada) divided into three zones based on salinity (adapted from de Lafontaine, 1990).

Table 1. Review of salinity tolerance of all species present in the St Lawrence Estuary Ecosystem at the Biodôme de Montréal. Species are listed by taxa.

	Species	Salinity tolerance	Refs
Osteichthyes	Atlantic salmon *Salmo salar*	High adaptability to low salinities	1
	Brook charr, *Salvelinus fontinalis*	Adaptability to low salinities	2
	Arctic charr, *Salvelinus alpinus*	Adaptability to low salinities	3
	Atlantic wolffish, *Anarhichas lupus*	High adaptability to low salinities	4
	Spotted wolffish, *Anarhichas minor*	High adaptability to low salinities	5
	Longhorn sculpin, *Myoxocephalus octodecemspinosus*	Adaptability to low salinities	6
	Atlantic cod, *Gadus morhua*	High adaptability to low salinities	7
	Haddock, *Gadus aeglefinus*	Larval survival at low salinities	8
	Atlantic halibut, *Hippoglossus hippoglossus*	High adaptability to low salinities	9,10
	Winter flounder, *Pseudopleuronectes americanus*	High adaptability to low salinities	11
	Atlantic sturgeon, *Acipenser oxyrhynchus*	High adaptability to low salinities	12
	Striped bass, *Morone saxatilis*	Adaptability to low salinities	13, 14
	Windowpane, *Scophthalmus aquosus*	*S. maximus:* Positive effect of lower salinities on growth	15
	Sea raven, *Hemitripterus americanus*	?	
	Shorthorn sculpin, *Myoxocephalus scorpius*	?	
	Yellowtail flounder, *Limanda ferruginea*	?	
	Cunner, *Tautogolabrus adspersus*	?	
	Atlantic mackerel, *Scomber scombrus*	?	
	Grubby, *Myoxocephalus aenaeus*	?	
	Ocean pout, *Macrozoarces americanus*	?	
	Lumpfish, *Cyclopterus lumpus*	?	
	Pollock, *Pollachius virens*	?	
Chondrichthyes	Winter skate, *Raja ocellata*	Adaptability to low salinities	16
	Little skate, *Raja erinacea*	Adaptability to low salinities	17
	Barndoor skate, *Raja laevis*	?	
	Thorny skate, *Raja radiata*	?	
	Spiny dogfish, *Squallus acanthias*	?	
Crustacea	American lobster, *Homarus americanus*	Low adaptability to salinity reduction	18,19
	Hermit crab, *Gurus sp.*	Adaptability to low salinities	20
	Spider crab, *Hyas araneus*	?	
	Common rock crab, *Cancer irroratus*	Adaptability to low salinities	21
Echinodermata Asteroidea	Purple sea star, *Asterias rubens*	Adaptability to low salinities	22
	Polar sea star, *Leptasterias polaris*	*L. hexactis* tolerant to brief exposures to low salinities	23
	Blood sea star, *Henricia sanguinolenta*	?	
	Purple sun star, *Solaster endeca*	?	
Holothuroidae	Orange-footed cucumber, *Cucumaria frondosa*	Larva tolerant to moderate salinities (24–34)	24
	Scarlet psolus, *Psolus fabricii*	?	
Echinoidae	Green sea urchin, *Strongylocentrolus droebachien*	?	
Gasteropoda	Common periwinkle, *Littorina littorea*	*L.irrorata* adaptability to low salinities Tolerant to variations in salinity	25
	Northern moon snail, *Euspira heros*	?	
	Common limpet, *Patella vulgata*	Larva tolerant to intermediate salinities	26
	Waved whelk, *Buccinum undatum*	?	
Lamellibranchia	Soft-shelled clam, *Mya arenaria*	Adaptability to low salinities	27
	Iceland scallop, *Chlamys islandica*	?	
Anthozoa	Marbled anemone, *Stomphia coccinea*	?	
	Nodular anemone, *Hormatia nodosa*	?	
	Northern red anemone, *Urticina felina*	?	
	Red soft coral, *Gersemia rubiformis*	?	
Polychaeta	Clam worms, *Nereis* spp.	?	

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distinct zones related to topographical and salinity differences (Fig. 2). The operational salinity of the GSLE was fixed for many years at ≈28–29 PSU i.e. at the higher limit of the natural salinity range, the temperature is fixed at 9–10° C and a natural photoperiod is in effect. Numerous aquatic species commonly found in this habitat display a high level of adaptability to salinity concentrations and fluctuations (see Chapter 5 in Kaiser et al. 2011).

Several studies indicate adaptability and positive effects of reduced salinity on growth of many species (e.g. Salmonidae, Anarhichadidae, Gadidae and Pleuronectidae), but for others, information is scarce. A review of the literature aimed at identifying the salinity tolerance of the organisms currently found in the GSLE was undertaken (see Table 1). Salinity challenge tests were designed to evaluate the salinity tolerance of representative species before any large-scale operations were considered. Species included were chosen according to 1) their abundance; 2) the level of existing knowledge on their salinity tolerance and 3) their conservation status. Three species of skates were included: thorny skate *Raja radiata*, little skate *Raja erinacea* and barndoor skate *Raja laevis*. Skates, in terms of abundance and conservation status were considered key species. The limited osmoregulator, the American lobster, *Homarus americanus* and the osmoconformer, the green urchin, *Strongylocentrolus droebachien*, were also included. Atlantic cod *Gadus morhua*, an osmoregulator, was chosen as a control species since its SW adaptability is well documented.

Specimens/species included were randomly distributed amongst four 500 L tanks operated at 14, 21, 24 and 28 PSU in a recirculating experimental rearing unit held at 9–10° C. All procedures were conducted under the supervision of members of the Biodôme de Montréal staff: a research scientist, a veterinarian and the water quality analyst with the technical assistance of the GSLE aquarists. The experimental tanks successively held *R. radiata*, *R. erinacea, R. laevis* (*n*=5 per species per salinity = 15 specimens of each species: size range 300–600g), juvenile *G. morhua* (*n*=20 per salinity = 60 specimens of 300–400g), *H. americanus* (*n*=10 per salinity = 30 specimens of 500–600g) and *S. droebachien* (*n*=40 per salinity = 120 specimens of 40–60g). Water parameters (oxygen, temperature and salinity) were monitored daily with a multi-parameter meter (Hanna HI 9828) and all organisms were kept in tanks at 28 PSU and thereafter held an additional two weeks at 24 PSU (suggested new operating salinity). Forty-eight hours prior to the challenge tests, feeding ceased and direct transfer into the experimental tanks at 24 (control), 21 and 14 PSU was done. Blood, haemolymph and coelomic fluid of *G. morhua, H. americanus* and *S. droebachien* respectively were collected for initial osmolality measurements (T0). Direct transfer at lower salinities was preferred over gradual transfer to ensure that the physiological limits of the organisms were clearly challenged during our trials. All animal manipulations were previously approved by the in-house Animal Care Committee and in compliance with guidelines for the use of animal in research (Association for the Study of Animal Behaviour).

Survival was monitored over 100 hours and blood, haemolymph, and coelomic fluid samples were collected at 4, 10, 12, 24, 36, 48 and 100 hours post-transfer (≥ 5 specimens per sampling; limited numbers of specimens of cod and lobster required us to sample the same animals on two and three occasions respectively during the sampling period of 100h). Cod were anaesthetised prior to blood sampling (Metomidate 10 mg L^{-1}). Body fluids were collected using 2 ml syringes previously heparinised, transferred in microtube, kept on ice until centrifuged at 10,000g for 5 minutes at 4° C (IEC MultiRF, Thermo IEC). Plasma (*G. morhua*) was extracted and stored at -80° C until analysed for osmolality measurements. For *S. droebachien*, coelomic fluid was collected directly in the cavity, transferred in microtubes and stored at -80° C until analysed. For *H. americanus*, haemolymph was extracted at the base of the front claws. For skates, no blood sampling was planned as it was judged a risky procedure (cardiac puncture with documented high mortality rate). After the monitoring phase, as an additional safety measure, all Rajidae spp. and *H. americanus* resumed feeding and were kept at the experimental salinities for two and four additional weeks respectively to further evaluate possibly undetected detrimental effects.

Survival was carefully monitored for all species. Behaviour and activity observations were monitored to detect possible decreases or increases in the habitual activity level or changes in behaviour. In the case of the sea urchin, mortality was detected through the absence of a return within an hour to the ventral position after positioning on the dorsal surface. However, as we found out later but did not use, Kashenko (2006) applied a more sensitive scale of assessment that considered movements of the whole organisms and organs (podia, pedicillaria, spines).

Osmolality was measured on thawed plasma and haemolymph samples. Osmolality measurements (units: mOsmol/kg) were obtained using a vapour pressure osmometer (Wescor Vapro 5520). Haematocrit (Hct) or Packed Cell Volume (PCV) was only measured in Atlantic cod blood using an Hct centrifuge (3 min @ 12000 rpm). Haemolymph clotting time was measured at different times in the challenge test on *H. americanus* but results were inconclusive (not shown). Vigour indices have been developed and used in several studies dealing with invertebrates (Fotedar et al. 2006; Barrento et al. 2009). A count of tail movements of *H. americanus* individually taken out of the water during a fixed duration was done and no differences were noted between salinities (20–26 tail flaps per minute were counted) (results not shown).

All statistical analysis was performed using Systat 13 statistical software (Systat Software, Inc., Chigaco, IL). The non-parametric Kruskal–Wallis test was used to compare osmolality between the different salinities. Where the KW yielded a significant value, pairwise comparisons were done with the Conover–Inman post-hoc test. The probability level of determining significance was $p \leq 0.05$. Results are presented as means ± SE.

Results

All the Rajidae spp. and *G. morhua* survived in tanks after direct transfer to salinities of 24, 21 and 14 PSU throughout the challenge test (100h) with no behavioural indications of distress. Neither mortality nor pronounced modifications in behaviour occurred during an additional 14 days of exposure at the three experimental salinities. Prior to transfer, Atlantic cod average haematocrit values were ±40%, indicating some level of stress in all groups but were nevertheless in the normal range for the species according to Larsson et al. (1976: 30–40%). Mean haematocrit values

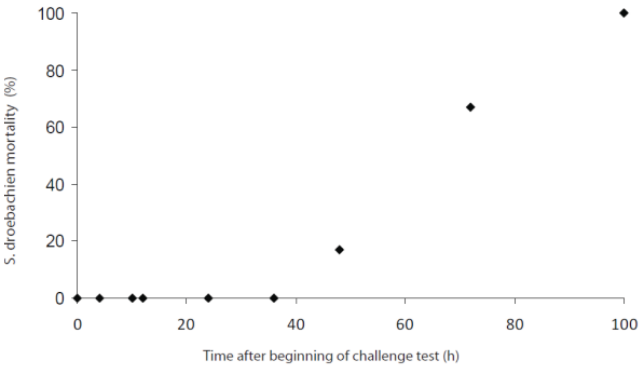

Figure 3. Mortality of *S. droebachien* throughout the salinity challenge test after 100 hours of exposure at 14 PSU.

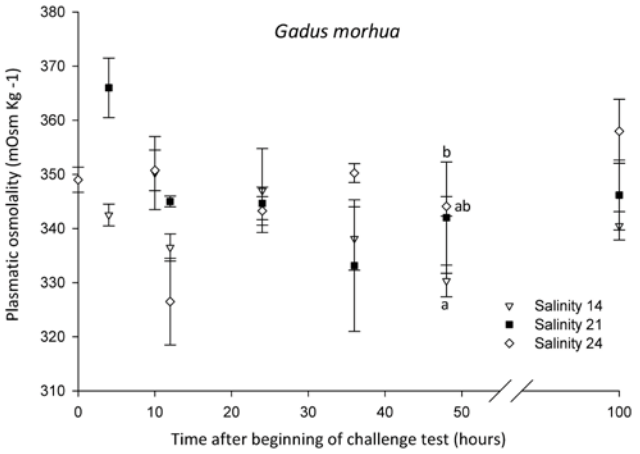

Figure 4. *G. morhua* mean plasmatic osmolality (mOsm/Kg) throughout the total duration of the salinity challenge test at the three experimental salinities. White dots (○) 24 PSU. Triangles (△) 21 PSU. Squares (■) 14 PSU. Error bars show standard deviation. Corresponding measured osmolality at the three experimental environmental salinities can be found in Table 2.

measured during the challenge tests (8 sampling times on n=5 fish) at 24, 21 and 14 PSU were 21.88± 14.33, 23.83±12.74 and 19.13±13.09% respectively, indicating lower values than reported for Atlantic cod held at full salinity (Lie et al. 1990; Larsson et al. 1976), in accordance with the principle of hemodilutions following exposure to low salinities (Wedemeyer et al. 1990). Hematocrit values stabilised 40h post-transfer at the different experimental salinities. All *H. americanus* survived at 24, 21 and 14 PSU throughout the challenge test duration and over a period of 14 days with no indications of reduced vigour (tail flaps). *S. droebachien* survived in tanks with salinities of 24 and 21 PSU throughout the challenge test (100h). However, survival rate decreased over time at 14 PSU until it reached 0% after 100h (Fig. 3). Mortality began occurring after 48h post-transfer suggesting that *S. droebachien* experienced difficulties adapting to low salinities.

At time 0, Atlantic cod displayed a general mean osmolality of 349.00±4.82 mOsmol/kg. During the course of the trial, the only time that significant differences were observed occurred at 48h (Fig. 4). At this sampling time, the cod held at 14 PSU presented an osmolality value of 330.30±6.52 mOsmol/kg significantly reduced in comparison to 24 PSU with an osmolality of 344.10±4.42 mOsmol/k (p = 0.018). At time 100h post-transfer at the different salinities, osmolality were equivalent between salinities and back at initial values, with and average osmolality of 349.19±12.59 mOsmol/kg, indicating a successful acclimation to the different salinities by the osmoregulating species, Atlantic cod. Mean coelomic fluid osmolality levels of *S. droebachien* in the range of 14, 21 and 24 PSU led to 100% mortality of the specimens exposed to the lowest salinity (14 PSU) after 100 hours (Fig. 5), indicating poor hypo-osmotic tolerance of urchins. After 24h and thereafter, osmolality between salinities was always significantly different (p<0.01). Coelomic fluid osmolality measurements after 48 h gave values of 391.5±3.44, 567.83±4.01 and 646.61 ±0.81 mOsmol/kg at 14, 21 and 24 PSU respectively (p<0.01). Sampling was stopped after ≈48 hours in the 14 PSU group to limit manipulation stress of the surviving animals until the end of the trial. Final values reached 567.33±2.02 and 646.17±0.65 mOsmol/kg at 21 and 24 PSU respectively (p=0.02). Mean haemolymph osmolality of *H. americanus* revealed clear differentiation of the profiles at 14, 21 and 24 PSU (Fig. 6) that correspond roughly to the osmolality of the external environment (see Table 2). First indications of osmolality adjustments was noticeable from the first sampling time (4 hours) with 643.5 ± 4.19, 691.63 ± 15.06 and 700.78 ± 27.28 mOsmol/kg at 14, 21 and 24 PSU respectively (Fig. 3b) though statistically not significant. After 10 and 12 hours, osmolality was significantly lower at 14 than 24 PSU (p= 0.02 in both cases). This tendency was amplified with time and after 24 hours, osmolality was significantly different between all three salinities (p< 0.02 in all cases). At 100 h, osmolality of *H. americanus* was 493.36 ± 37.29, 628.8 ± 24.53 and 704.38 ± 18.55 mOsmol/kg at 14, 21 and 24 PSU respectively and significantly different from each other (p<0.001). No mortality occurred during the 100 h of monitoring or after 14 additional days.

Our challenge tests indicate that both fish species, Rajidae and *G. morhua*, and the crustacean representative, *H. americanus*, included in our trial displayed good tolerance to salinities over the range of 14–24 PSU based on survival results. The echinoderm representative, *S. droebachien*, proved unable to adapt to a salinity of 14 PSU after less than 48h. American lobster and green urchins are osmoconformer species that both displayed a reduction in osmolality at the lower salinities, but this occurred to a lesser extent in the case of *S. droebachien*, indicating different levels of adaptability to reduced salinities. The Atlantic cod, on the other hand, displayed complete recovery, re-establishing normal levels of osmolality after less than 100h post-transfer.

Implementation of results-based salinity management

After the challenge tests, a water change of the main basin (3400m^3) was carried out gradually over a period of one week to reach 24 PSU. At each water change, water parameters (temperature, oxygen, pressure, salinity and pH) were recorded every 5 min with a multi-parameter meter (see Fig. 7). Over the first three days, sequential dilution events were performed. Each event resulted in a 5–6% dilution of total salinity over 12 hours where the lowest salinity level was recorded (17 PSU). Ten percent (10%) of the total basin's water capacity was then emptied over a period of 24h. Salinity level in the basin was gradually increased from 17 to 24 by adding 28 bags of salt (1000kg/bag) over a period of 24h. At the end, SW at 24 was prepared and added to complete the previously removed 10% volume.

A salinity of 24 PSU is now considered as a safe operational salinity. The lower limit was fixed at 14 and should not be exceeded under any circumstances during salinity reduction procedures. Accordingly, we are considering renewing water more frequently but in smaller percentages (10% bi-monthly instead of 30% twice a year). However, we plan to turn the rocky shore exhibit (Le Littoral Rocheux; 22 m^3), part of the global water volume, into a fully autonomous recirculating system at higher levels of salinity to house the more vulnerable organisms such as anemones and urchins.

Table 2. Osmolality (mOsm · Kg^{-1}) of the seawater measured at the four experimental salinities (14, 21, 24 and 28 PSU).

Salinity (PSU)	Osmolality (mOsm · Kg^{-1})
14	420
21	616
24	712
28	840

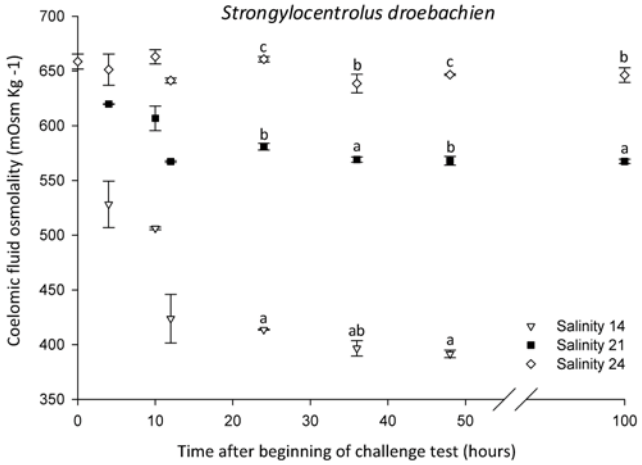

Figure 5. *S. droebachien* mean coelemic fluid osmolality (mOsm/Kg) throughout the total duration of the salinity challenge test at the three experimental salinities. White dots (○) 24 PSU. Triangles (△) 21 PSU. Squares (■) 14 PSU. Error bars show standard deviation. Corresponding measured osmolality at the three experimental environmental salinities can be found in Table 2.

Dropping by the order of four units in salinity enabled us to generate savings of roughly 12,000 Canadian dollars annually. The savings achieved after three years at the new salinity were invested in the acquisition of more salts for more frequent water renewals, the development of a sulphur-based denitrification pilot-scale unit (see van Rijn et al. 2006) and the integration of a protein skimmer/ozonation units into the rocky shore ecosystem exhibit. We suggest the following principles should be considered before conducting similar large-scale salinity reduction operations:

1. Verify salinity tolerance and lethal limits for each species in the exhibit in the existing literature.
2. Carry out targeted salinity challenge tests on representative and vulnerable species, making sure that all osmoregulatory strategies are represented in the test groups.
3. Identify a new and safe salinity level for all species, and a lower lethal limit or duration that should not be passed during water changes.
4. Prior to proceeding to salinity reduction operations, identify and remove all gravid females and all other vulnerable developmental stages.
5. Proceed to salinity reduction by gradual dilutions, constantly monitoring salinity levels and water quality.
6. For all new species added to the exhibit, a salinity challenge test should be carried out prior to their insertion to identify/verify salinity tolerance levels.

Salinity reduction at the Maritime Aquarium in Norwalk, CT was applied to certain tanks containing pollack, lumpfish, and American lobster to limit/reduce parasitic infections by ciliated protozoa [J. Schneider, personal communication]. Other institutions such as the Aquarium du Québec have also manifested an interest in this kind of practice. These recommendations could guide them to achieve safe and sustainable salinity reduction practices and reward investment redistribution.

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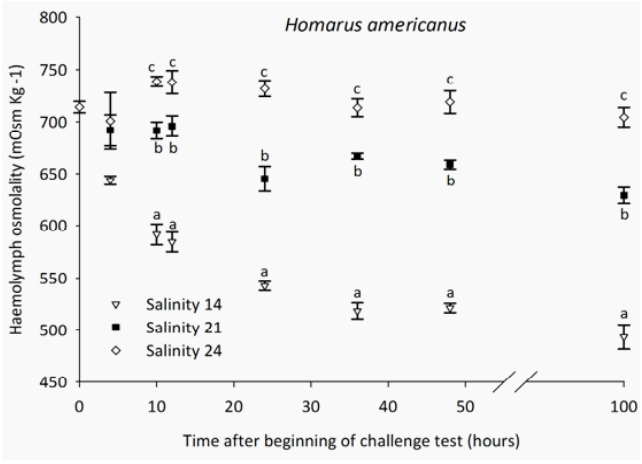

Figure 6. *H. americanus* mean haemolymph osmolality throughout the total duration of the salinity challenge test at the tree experimental salinities. White dots (○) 24 PSU. Triangles (△) 21 PSU. Squares (■) 14 PSU. Error bars show standard deviation. Corresponding measured osmolality at the three experimental environmental salinities can be found in Table 2.

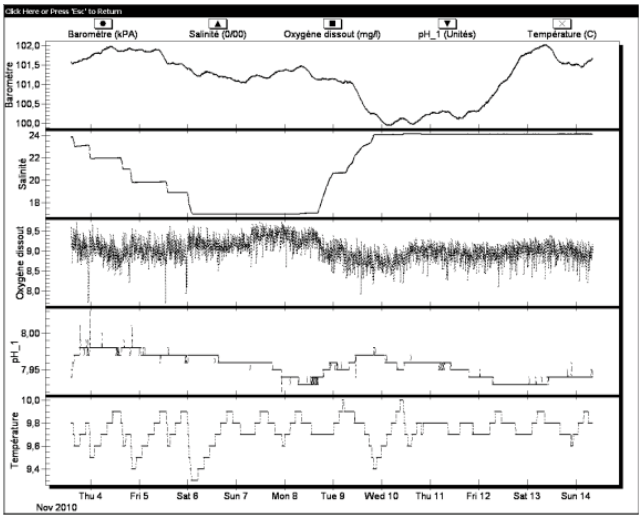

Figure 7. Parameters registered during the 35–40% renewal operations for final 24 PSU seawater (barometric pressure kPA), salinity (‰), dissolved oxygen (mg/l), pH and temperature (°C).

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