RECENT RESEARCH CONTRIBUTIONS
(Directly supervised Honours & Masters Students indicated with *)

1. Marshall, W.S., C.G. Ossum and E.K. Hoffmann. Hypotonic shock mediation by p38 MAPK, JNK, PKC, FAK, OSR1 and SPAK in osmosensing chloride secreting cells of killifish opercular epithelium. J. exp. Biol. 2005 *in press*. This collaborative work with James Chair Professor Else Hoffmann has revealed the complex cascade of activator enzymes, kinases, that connect mechanical stretch of the cell membrane with the regulatory volume decrease response of the cell, through a cotransporter in the cell membrane sodium, potassium, 2-chloride cotransporter (NKCC). Mechano-sensors occupy central positions in control of blood pressure, thirst regulation and now ion balance. The reviewers called it an important paper; I think it is one our best works to date.

2. Marshall, W.S., E.M. Lynch* and R.F. Cozzi. Redistribution of immunofluorescence of CFTR anion channel and NKCC cotransporter in chloride cells during adaptation of the killifish *Fundulus heteroclitus* to sea water. *J. exp. Biol*. **205**: 1265-1273 (2002). Immunocytochemistry and confocal microscopy (funded by CFI) was used here to trace where in the ion transporting cells were the main Cl transporting proteins CFTR and NKCC. The paper clearly showed changes in transporter distribution that coincide with the appearance of Cl transepithelial transport at 24h-48h after transfer to sea water. This slow redistribution is not simple fusion of membrane vesicles but rather appears to involve *de novo* expression of transporters and insertion in the plasma membrane as well as removal of the old transporter, a real "retooling" of existing chloride cells.

3. Marshall, W.S., J.A. Howard*, R.R.F. Cozzi, and E.M. Lynch* NaCl and fluid secretion by the intestine of the teleost *Fundulus heteroclitus*: Involvement of CFTR. *J. exp. Biol.* **205**:745-758 (2002).
This is the first demonstration that intestinal secretion (i.e. secretory diarrhea) can happen in teleosts. Along with this, we provided a logical explanation (patches of enterocytes with apical CFTR and basolateral NKCC) supported by immunocytochemistry and confocal microscopy. This aligns teleost intestinal function with that of mammals, in spite of the lack in teleosts of the classical "Crypts of Lieberkuhn". Really intriguing is that secreted fluid by seawater fish has as high salt content as seawater, thus SW fish wouldn't suffer severe dehydration during bouts of intestinal secretion.

4. Marshall, W.S., T.R. Emberley* S.E. Bryson and S.D. McCormick.. Time course of salinity adaptation in a strongly euryhaline estuarine teleost *Fundulus heteroclitus*: A multivariable approach. *J. exp. Biol*. **202**:1535-1544 (1999).
This paper used eight sampling times (one hour to 4 weeks) and twelve measured variables, molecular through physiological, to build for the first time a clear picture of the *sequence* and *relationships between* seawater adaptational events in an estuarine teleost.

Important time lines of events (*e.g.* cortisol peaks at 1 hour and kfCFTR expression increases at 8h+) were established which led to many new interesting hypotheses being generated. This paper has received many positive comments from referees, the editor himself and readers. We believe measurement of many variables in a time course to be a much more powerful approach than the study of fully acclimated animals.

5. Marshall, W.S., R.M. Duquesnay*, J.M. Gillis*, S.E. Bryson and C.M. Liedtke. Neural modulation of salt secretion in teleost opercular epithelium by α_2-adrenergic receptors and inositol 1,4,5-trisphosphate. *J. exp. Biol* **201**:1959-1965 (1998).
This "elegant" (referees word) paper was completed mostly by honours students, used a unique nerve-epithelium in vitro preparation and revealed that salt secretion is under neural control by the autonomic nervous system. It strengthened the conclusion of Ca^{2+} mediation by detecting a peak in IP_3. It supports the novel idea that changes in salinity experienced by estuarine fish are NOT necessarily stressful, rather that they can be handled with same facility as are many "higher" autonomic functions, such as sweating in primates.

6. Marshall, W.S., S.E. Bryson, A. Midelfart and W.F. Hamilton. Low conductance anion channel activated by cAMP in teleost Cl- secreting cells. *Am. J. Physiol*. **268**:R963-R969 (1995). This paper reveals presence of the anion channel CFTR in fish. It has finally allowed molecular descriptions of chloride cell operation and is unique in its cellular regulation, making the work of importance to Cystic Fibrosis research. It paved the way for kfCFTR sequencing (see Singer et al 1998, below).

FULL LIST OF RESEARCH CONTRIBUTIONS

FULL PAPERS IN REFEREED JOURNALS

1. **Marshall, W.S**.: Effects of hypophysectomy and ovine prolactin on the epithelial mucus-secreting cells of the pacific staghorn sculpin, *Leptocottus armatus* (teleostei: cottidae). Can. J. Zool. 54:1604-1609 (1976).
2. **Marshall, W.S.**: Transepithelial potential and short-circuit current across the isolated skin of *Gillichthys mirabilis* (teleostei: gobiidae), acclimated to 5% and 100% sea water. J. Comp. Physiol. 114:157-165 (1977).
3. **Marshall, W.S.**: On the involvement of mucus secretion in teleost osmoregulation. Can. J. Zool. 56:1088-1091 (1978).
4. **Marshall, W.S.** Effects of salinity acclimation, ovine prolactin, and cortisol on the mucus cells of *Leptocottus armatus* (teleostei:cottidae). Gen. Comp. Endocrinol. 37:358-368 (1979).
5. **Marshall, W.S.** and Bern, H.A. Teleostean urophysis: urotensin II and ion transport across the isolated skin of a marine teleost. Science 204:519-521 (1979).
6. **Marshall, W.S.** and Nishioka, R.S. Relation of mitochondria-rich chloride cells to anion transport by marine teleost skin. J. Exp. Zool., 214:147-156 (1980).

7. **Marshall, W.S.** and Bern, H.A. Active chloride transport by the skin of a marine teleost is stimulated by urotensin I and inhibited by urotensin II. Gen. Comp. Endocrinol., 43:484-491 (1981).
8. Bern, H.A., C.A. Bisbee, N.L. Collie, J.K. Foskett, B. Hughes, C.A. Loretz and **W.S. Marshall**. Failure of ovine prolactin to elicit rapid effects on osmoregulatory surfaces. Gen. Comp. Endocrinol. 44:128-130 (1981).
9. **Marshall, W.S.** and S.D. Klyce. Cell finder speeds impalements with microelectrodes. Pflugers Archiv 391:258-259 (1981).
10. **Marshall, W.S.** Sodium dependency of active chloride transport across isolated fish skin (*Gillichthys mirabilis*). J. Physiol. Lond. 319:165-178 (1981).
11. **Marshall, W.S.** Active transport of Rb^+ across the skin of the teleost *Gillichthys mirabilis*. Am. J. Physiol. 241:F482-F486 (1981).
12. Klyce, S.D. and **W.S. Marshall**. Effects of Ag^+ on ion transport by the rabbit corneal epithelium. J. Memb. Biol. 66:133-145 (1982).
13. Klyce, S.D., K.A. Palkama, A.H. Neufeld, M. Harkonen, **W.S. Marshall**, S. Hutaniitty, and K.P. Mann. Neural serotonin stimulates chloride transport in the rabbit corneal epithelium. Invest. Ophthalmol. Vis. Sci. 23:181-192 (1982).
14. **Marshall, W.S.** and S.D. Klyce. Numerical procedures for ion-selective electrodes. J. Electrophysiol. Tech. 9:113-121 (1982).
15. **Marshall, W.S.** and S.D. Klyce. Membrane and shunt resistances in the rabbit corneal epithelium. J. Memb. Biol. 73:275-283 (1983).
16. Lessman, C.A. and **W.S. Marshall.** Electrophysiology of insulin- and progesterone-induced meiosis in *Rana pipiens* oocytes. J. Exp. Zool. 231:257-266, (1984).
17. **Marshall, W.S.** and S.D. Klyce. Cellular mode of serotonin action in the rabbit corneal epithelium. Biochim. Biophys. Acta., 778:139-143 (1984).
18. **Marshall, W.S.** Paracellular ion transport in trout opercular epithelium models osmoregulatory effects of acid precipitation. Can. J. Zool. 63:1816-1822 (1985).
19. **Marshall, W.S.**, H.R. Habibi and C.A. Lessman. Electrophysiology of oocytes during meiotic maturation and after ovulation in brook trout (*Salvelinus fontinalis*). Can. J. Zool. 63:1904-1908 (1985).
20. **Marshall, W.S.** Independent Na^+ and Cl^- active transport by urinary bladder epithelium of brook trout. Am. J. Physiol. 250:R227-R234 (1986).
21. Lessman, C.A., **W.S. Marshall** and H.R. Habibi. Nuclear movement and dissolution during *Rana* oocyte meiosis: Effect of demecolcine. Gamete Res. 14:11-23 (1986)
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24. **Marshall, W.S.** and S.E. Bryson. Evidence for Cl^- dependent K^+ secretion by the blood-testis barrier of brook trout. Can. J. Zool. 66:1603-1609 (1988).
25. **Marshall, W.S.**, S.E. Bryson, and D.R. Idler. Gonadotropin stimulation of K^+ secretion and Na^+ absorption by brook trout (*Salvelinus fontinalis*) sperm duct epithelium. Gen.

Comp. Endocrinol. 75: 118-128 (1989).

26. **Marshall, W.S.**, S.E. Bryson and M.M. *Sapp. Volume regulation in glutathione-treated brook trout (*Salvelinus fontinalis*) erythrocytes. Fish Physiol. Biochem. 8:19-28 (1990).
27. **Marshall, W.S.** and J.W. Hanrahan. Anion channels in the apical membrane of mammalian corneal epithelium primary cultures. Invest. Ophthalmol. Vis. Sci. 32:1562-1568 (1991).
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29. **Marshall, W.S.**, S.E. Bryson and C.M. Wood. Calcium transport by isolated skin of rainbow trout. J. exp. Biol. 166:297-316 (1992).
30. **Marshall, W.S.**, S.E. Bryson and D. *Garg. _2-adrenergic inhibition of chloride transport by opercular epithelium is mediated by intracellular Ca^{2+}. Proc. Nat. Acad. Sci. U.S.A. 90:5504-5508 (1993).
31. **Marshall, W.S.**, S.E. Bryson and D.R. Idler Gonadotropin action on sperm duct epithelium: Ion transport stimulation mediated by cAMP and Ca^{2+}. Gen. Comp. Endocrinol. 90:232-242 (1993).
32. Wood, C.M. and **W.S. Marshall**. Ion balance, acid-base regulation, and chloride cell function in the common killifish *Fundulus heteroclitus* - a freely euryhaline estuarine teleost. Estuaries 17:34-52 (1994).
33. Marshall, W.S., S.E. Bryson, A. Midelfart and W.F. Hamilton. Low conductance anion channel activated by cAMP in teleost Cl^- secreting cells. Am. J. Physiol. 268:R963-R969 (1995).
34. **Marshall, W.S.**, S.E. Bryson, J.S. *Burghardt and P.M. Verbost. Ca^{2+} transport by opercular epithelium of the freshwater adapted euryhaline teleost *Fundulus heteroclitus*. J. Comp. Physiol. B. 165:268-277 (1995).
35. Patrick, M.L., Part, P., **Marshall, W.S**. and C.M. Wood. Characterization of ion and acid-base transport in fresh water adapted mummichog (*Fundulus heteroclitus*). J. Exp. Zool. 279:208-219 (1997).
36. **Marshall, W.S.**, S.E. Bryson, P. *Darling, C. *Whitten, M. Wilkie, C.M. Wood and J. Buckland-Nicks. NaCl transport and ultrastructure of opercular epithelium from a freshwater adapted euryhaline teleost, *Fundulus heteroclitus* J. Exp. Zool. **277**:23-37 (1997).
37. Verbost, P.M., S.E. Bryson, S.E. Wendelaar Bonga and **W.S. Marshall.** Na^+-dependent Ca^{2+} uptake in isolated opercular epithelium of *Fundulus heteroclitus*. J. Comp. Physiol. B. **167**:205-212 (1997).
38. Patrick, M.L., C.M. Wood and **W.S. Marshall**. Calcium regulation in the freshwater adapted mummichog (*Fundulus heteroclitus*). J. Fish Biol. **51**:135-145, (1997).
39. Patrick, M.L., Part. P., **Marshall, W.S**. and C.M. Wood. Ionoregulation and acid-base balance in the freshwater adapted mummichog (*Fundulus heteroclitus*). J. Exp. Zool. **279**:208-219 (1997).
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42. **Marshall, W.S.**, *DuQuesnay, R.M., *Gillis, J.M. Bryson, S.E. and C.M. Liedtke. Neural modulation of salt secretion in teleost opercular epithelium by α_2-adrenergic receptors and inositol 1,4,5-trisphosphate. J. exp. Biol. **201**:1959-1965 (1998)
43. **Marshall, W.S.**, T.R. Emberley*, S.E. Bryson and S.D. McCormick. Time course of salinity adaptation in a strongly euryhaline estuarien teleost *Fundulus heteroclitus*: A multivariable approach. J. exp. Biol. 202:1535-1544 (1999).
44. **Marshall, W.S**., S.E. Bryson and *T. Luby. Control of epithelial Cl- secretion by basolateral osmolality in the euryhaline teleost, *Fundulus heteroclitus*. J. exp. Biol. **203**:1897-1905. (2000).
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46. **Marshall, W.S.,** J.A. Howard*, R.R.F. Cozzi, and E.M. Lynch* NaCl and fluid secretion by the intestine of the teleost *Fundulus heteroclitus*: Involvement of CFTR. J. exp. Biol. **205**:745-758 (2002)
47. **Marshall, W.S.,** E.M. Lynch* and R.F. Cozzi. Redistribution of immunofluorescence of CFTR anion channel and NKCC cotransporter in chloride cells during adaptation of the killifish Fundulus heteroclitus to sea water. J. exp. Biol. **205**: 1265-1273 (2002).
48. **W.S. Marshall**, R.R.F. Cozzi, R.M. Pelis and S.D. McCormick. 2004. Cortisol receptor blockade and seawater adaptation in the euryhaline teleosts *Fundulus heteroclitus*. J. Exp. Zool. xxx:xxx, in press
49. **Marshall, W.S.,** C.G. Ossum and E.K. Hoffmann. Hypotonic shock mediation by p38 MAPK, JNK, PKC, FAK, OSR1 and SPAK in osmosensing chloride secreting cells of killifish opercular epithelium. J. exp. Biol. 2005 *in press*.

BOOK CHAPTERS AND REVIEWS CONTAINING NEW DATA

50. Pequeux, A., R. Gilles and **W.S. Marshall**. NaCl transport in gills and related structures: In: Advances in Comparative and Environmental Physiology I. NaCl Transport in Epithelia. R. Greger, ed, Springer-Verlag, Berlin. Pp 1-73 (1988) Invited book chapter.
51. **Marshall, W.S**. Transport processes in isolated teleost epithelia: Opercular epithlium and urinary bladder. Book Chapter in Fish Physiology Vol 14, T. Shuttleworth & C.M. Wood, eds., Academic Press NY pp 1-23 (1995) Invited book chapter
52. **Marshall, W.S**. Na^+, Cl^-, Ca^{2+} and Zn^{2+} transport by fish gills: retrospective review and prospective synthesis. J. Exp. Zoology 293:264-283 (2002) Invited review
53. **Marshall, W.S**. and T.D. Singer. Cystic Fibrosis Transmembrane Conductance Regulator in teleost fish. Biochim. Biophys Acta, Biomembranes 1566: 16-27 (2002).
54. **Marshall, W.S**. Rapid regulation of NaCl secretion by estuarine teleost fish: Coping strategies for short duration fresh water exposures. Biochim. Biophys. Acta. Biomembranes 1618:95-105 (2003)

55. **Marshall, W.S**. and M. Grosell. Osmoregulation and acid-base balance. In: The Physiology of Fishes. D.H. Evans, Ed. CRC Press, Boca Raton FL., 3rd Ed. 2005 (in press)

INVITED CONFERENCE PRESENTATIONS

FULL PAPERS

56. **Marshall, W.S.** and Bern, H.A. Ion transport across the isolated skin of the teleost, *Gillichthys mirabilis*. *In*: Epithelial transport in the lower vertebrates. B. Lahlou, ed., Cambridge Univ. Press, pp. 337-350 (1980).
57. **Marshall, W.S.** and S.D. Klyce. Control mechanisms of Cl^- secretion in some vertebrate epithelia. *In* Current Trends in Comparative Endocrinology B. Lofts and W.N. Holmes, ed., Hong Kong Univ. Press, pp 971-976 (1985).
58. **Marshall, W.S.** Control of K^+ secretion in the blood-testis barrier of brook trout. **In:** Proceedings of the 3rd International Symposium on Reproductive Physiology of Fish. D.R. Idler, L.W. Crim and J.M. Walsh, ed., Memorial Univ. Newfoundland, 264 (1987). (refereed conference paper)
59. **Marshall, W.S.**, S.E. Bryson and D.R. Idler. Control of ion transport by the blood-testis barrier in brook trout. 1st Intl. Sympos. Fish Endocrinol. Fish Physiol Biochem. 7:331-336 (1989)
60. **Marshall, W.S.** and S.E. Bryson. Transport mechanisms of seawater chloride cells: An inclusive model of a multifunctional cell. Comp. Biochem. Physiol. 119A:97-106 (1998).

PAPERS SUBMITTED AND IN PREPARATION

Marshall, W.S., S.D. McCormick and T. Doyle. Time course of adaptation of the euryhaline teleost *Fundulus heteroclitus* to fresh water. In preparation

Marshall, W.S. Short term regulation of chloride cells in gills of euryhaline teleosts. Book Chapter in preparation M. Mancera, ed. 2005

Marshall, W.S. and A. Epega. CFTR expression in fish brain; ancient association with hypothalamic releasing factors. In preparation