# PROCEEDINGS OF THE NINTH SYMPOSIUM ON THE NATURAL HISTORY OF THE BAHAMAS

**Edited by:**

David L. Smith
Sherilyn Smith

**Conference Organizer**

Kenneth C. Buchan

**Production Editors**

David L. Smith
Sherilyn Smith
Vincent J. Voegeli

Gerace Research Center
San Salvador, Bahamas
2003

Cover photograph courtesy of David and Sherilyn Smith



Printed in the USA

ISBN 0-935909-73-7

# IONOCYTES IN THE DRAGONFLY NYMPH *ERYTHEMIS SIMPLICICOLLIS* (SAY)

Sherilyn G.F. Smith
Lynette Mendoza

Department of Biology, Le Moyne College, Syracuse, NY 13214

## ABSTRACT

Dragonfly nymphs (Odonata:Anisoptera) are commonly found in fresh water environments throughout the United States and in the Bahamas. Nymphs of the Green Jacket dragonfly, *Erythemis simplicicollis* Say, are known to inhabit brackish water environments as well. Presence and location of ionocytes, or ion transport cells, were compared in nymphs of two different species, *Anax junius* Drury (a freshwater species) and *E. simplicicollis*. The nymphs were held in tanks of three different salinities for a total of thirteen days, and then subjected to a silver nitrate staining technique to allow us to identify ionocytes on the gill tissues. Patches of ionocytes were found at the base of the rectal gill leaflets in *E. simplicicollis*. No similar patches were observed in *A. junius* nymphs, regardless of salinity. Patch density of ionocytes in *E. simplicicollis* increased as the salinity increased, which suggests that this species is able to respond to changes in salinity.

## INTRODUCTION

Osmoregulation allows organisms to adapt to a particular environment by regulating the osmotic pressure of body fluids and the intake or export of salt and/or water. Dragonflies (Odonata:Anisoptera) are aquatic insects commonly found in freshwater environments including marshes, ponds, lakes and streams. A few species have been found to inhabit both fresh water and brackish water environments. They seem to have the ability to tolerate and develop in water that varies in salinity, as has been demonstrated by previous research (Smith and Smith 1994, 1996). Unlike many other aquatic insects, the dragonfly nymph has no external gills; rather it possesses a rectal respiratory chamber where gas exchange can occur (Ross *et al.*, 1982). Normally there are six main gill folds in the anterior part of the rectum where water is drawn into the rectum by expansion of the body (Nation, 1985). It has been demonstrated that osmoregulatory ion transport occurs primarily in the gills' epithelial tissue. These epithelial cells are commonly referred to as ionocytes, which are epithelial cells specialized for ion transport (Holliday, 1985).

Two species commonly found in the United States, *Erythemis simplicicollis* Say and *Anax junius* Drury, have been found to inhabit freshwater habitats. However, *E. simplicicollis* is also common in the Bahamas, including the island of San Salvador, where it was observed to inhabit brackish waters (Smith and Smith, 1994). This species has been known to survive and develop to adulthood in salinities of up to 50% seawater (Smith and Smith, 1996). Clearly, this species is unique because of its broad range of salinity tolerance.

The purpose of this experiment was to identify more precisely the location of the ionocytes on the gill tissues where ion transport occurs. This study focused on examining the density of the ionocytes found and how this density was influenced by salinity in two species, one a salinity-tolerant species and the other not salinity-tolerant.

## MATERIALS AND METHODS

Twelve nymphs of *Anax junius* and thirty nymphs of *Erythemis simplicicollis* were obtained from a biological supply company, caged individually, and assigned to one of two treatment groups or the control group. Nymphs ranged in developmental stage from third to sixth instar; test groups were set up such that they were roughly equivalent in terms of the instars represented. Three aquaria were set up,

each containing three gallons of either 20% seawater solution, 40% seawater solution, or artificial pond water (APW). The seawater solutions were made by mixing artificial sea salt with conditioned tap water to make up artificial seawater at a concentration of 35 ppt (specific gravity of 1.022). The artificial seawater was then diluted with distilled water to the desired salinities (Table 1).

Four to six nymphs were placed into each treatment group or control group. The nymphs were held in the tanks for a total of thirteen days. To make the transition from freshwater to the 40% seawater treatment, nymphs assigned to that treatment group were first kept in 20% seawater for four days and were then transferred to the 40% seawater tank. After nymphs had spent at least four days in their respective solutions, silver nitrate staining was used to identify ionocytes. Two separate trials were conducted with *E. simplicicollis* and only one trial was done with *A. junius*.

**Table 1.** Control and artificial seawater treatments, with their corresponding measurements of specific gravities and calculated salinities.

| Seawater Solution | Specific gravity | Salinity |
|---|---|---|
| APW(0%) | 1.000 | <0.5 ppt |
| 20% | 1.008 | ~7 ppt |
| 40% | 1.010 | ~15 ppt |

### Silver Nitrate Staining Technique

Prior to staining, the nymphs were rinsed and forced to swim around in deionized water for three 45-second periods each to remove any excess chloride ions from the surface and rectal chamber. They were then transferred to a 0.5% $AgNO_3$ solution for a 45-second exposure and again forced to swim around so that the solution would be taken into the rectal chamber. The nymphs were rinsed again as described for 45 seconds and then transferred to a Dektol solution for another 45 seconds for "development." The nymphs underwent a final 45-second rinse and were then placed in vials containing 70% ethanol.

### Dissection

The nymphs were transversely bisected where the abdomen meets the thorax. The rectal chamber was dissected out of the abdomen and rows of gills were dissected apart to expose the gills. The gills were then observed and photographed using a dissecting microscope (Olympus Model SZH10) and camera. Evaluation of ionocyte density was done by determining whether each gill's basal pad was black over more than 50% of its area, indicating precipitation of AgCl in ion-transporting cells. The number of gills which were more than 50% stained and the total number of gills in randomly chosen rows of gills were counted.

## RESULTS AND DISCUSSION

The arrangement of gills in all the dragonfly nymphs dissected was basically the same for both species. The gill chamber contained six pairs of longitudinally-oriented rows of gill lamellae (Figure 1A, B), each pair of rows about twice as far apart as the distance between the rows of the pair. Gill lamellae were largest at the posterior end of each row and gradually diminished in size toward the anterior end (Figure 1C). The lamellae all contained granules of melanin, and ventral lamellae were generally darker than dorsal lamellae in both *Anax* and *Erythemis*.

Gill structure differed between the two genera (Figures 1A, B). The gill lamellae in *Erythemis* were roughly subtriangular and were attached to the chamber wall at one angle, near which a triangular-to-oval thickened area, opaque and yellowish to golden brown in color, was observed. These pads occurred at the base of most gills as well as in the membranous posterior wall of the gill chamber in *Erythemis*. The number of lamellae varied from 23 to 36 per row, with an average of 29.1.

In *Anax,* rows of gill lamellae in each pair were very close to each other, almost appearing to have a common base. The gill lamellae were more leaf-shaped and were attached to the chamber wall at the base of the leaflet, as well as to each other by a membrane

which connected successive lamellae in alternate rows, so that both rows of lamellae in a pair were actually interconnected. The number of lamellae per row ranged from 14 to 21, with an average of 18.8. Thickened pads, generally translucent yellowish-brown and oval in shape were present but appeared to be considerably thinner than those in *Erythemis*; no patches of ionocytes were visible. As in *Erythemis,* all the gill lamellae contained granules of melanin which could be seen between the tracheoles. Scattered irregular-to-spherical black spots are visible in some gills, but these seemed to be unusual growths in or on the membranes; their cause is not known.

No patches of ionocytes were found on gills of *Anax junius*, regardless of salinity treatment (Table 2). It appears unlikely that this dragonfly nymph has the ability to adapt to different salinities based on ion transport in the gills.

In *Erythemis simplicicollis*, ionocytes, as indicated by the darkening caused by silver nitrate, did appear to become more numerous or active with an increase in salinity (Figures 2, 3, Table 2). The proportion of gill lamellae having more than half of the pad area black increased from 1.2% in the control group to 100% in the 40% seawater group. While most lamellae in the control group showed some staining, that staining was quite limited in extent; lamellae in *Anax junius* showed no staining whatsoever (Table 2).

**Table 2.** Percentage of gill lamellae exhibiting >50% black pigmentation in *Erythemis simplicicollis* and *Anax junius*. Six nymphs of *E. simplicicollis* and four nymphs of *A. junius* in each treatment were dissected and gill pigmentation estimated.

| Treatment | *E. simplicicollis* | *A. junius* |
|---|---|---|
| Control (0%) | 1.2% | 0% |
| 20% seawater | 11.1% | 0% |
| 40% seawater | 100% | 0% |

## CONCLUSION

Our results demonstrated that there is a difference in the number of ionocytes between the two species as well as with varying salinities. Clearly there are structural and physiological differences between the two species that affect the ability of each either to adapt or not to adapt to a particular aquatic environment. Overall, these results suggest that in *Erythemis simplicicollis*, but not in *Anax junius*, ionocytes associated with the rectal gills do respond to the changes in salinity.

## ACKNOWLEDGEMENTS

We thank the Biology Department faculty for their suggestions and for providing supplies for this project. Dr. Beth Mitchell printed the title for the poster. We also thank the Student Research Committee and the Office of the Academic Vice-President of Le Moyne College for providing the funds to attend the Symposium. Finally, we would like to thank the Bahamian Field Station (now Gerace Research Center) for providing the opportunity to present this research.

## LITERATURE CITED

Holliday, C.W. 1985. Salinity-induced changes in gill Na,K-ATPase activity in the mud fiddler crab, *Uca pugnax.* J. Exp. Zool. 233: 199-208.

Nation, J.L. (1985). Respiratory systems. In M.S. Blum (Ed.), *Fundamentals of Insect Physiology* (pp 185-225). New York: John Wiley & Sons.

Ross, H.H., Ross, C.A. and Ross, J.A. A *Textbook of Entomology.* 4th ed. New York: John Wiley & Sons; 1982. p. 293-294.

Smith S.G.F. and Smith, D. 1996. Salinity tolerance of *Erythemis simplicicollis* Say (Odonata: Anisoptera, Libellulidae). Symposium on the Natural History of the Bahamas. Bahamian Field Station, San Salvador, Bahamas, p. 139-143.

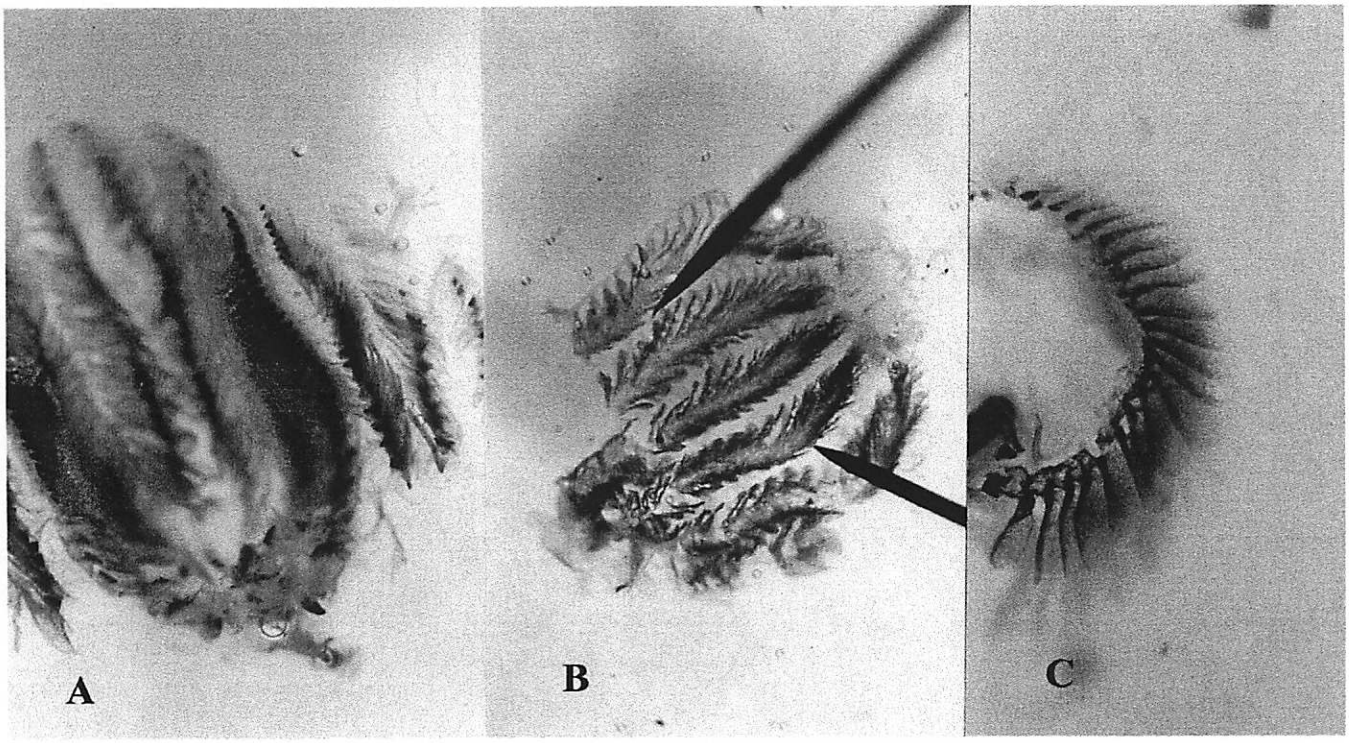


**Figure 1.** Rectal gill chambers of A. *Erythemis simplicicollis*, and B. *Anax junius*. Note general darkness of gills, a result of melanin pigment granules, and the difference in arrangement of the gill lamellae in these species. C. Single row of lamellae showing decrease in size from posterior to anterior end.

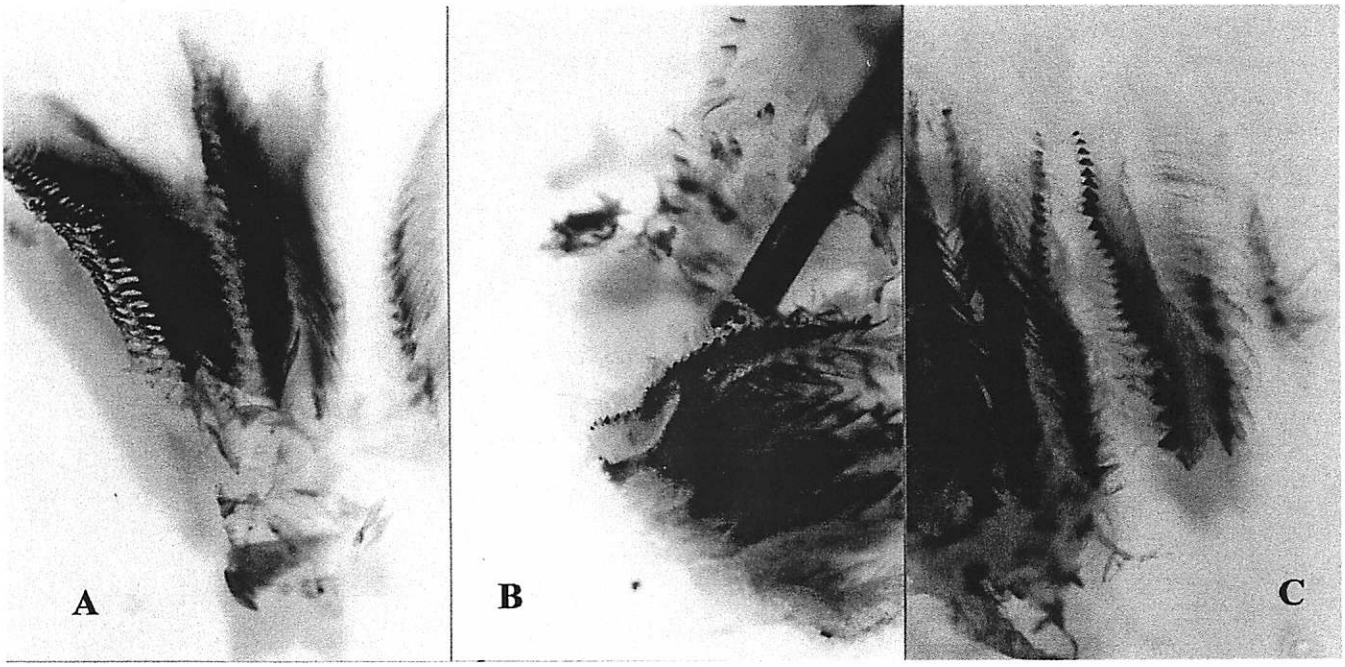


**Figure 2.** Rows of gills and pads in the posterior gill chamber wall in *Erythemis simplicicollis* showing effects of salinity on black pigmentation of the thickened pads. More pigmentation indicates greater ionocyte activity. A. Control, freshwater; B. 20% seawater; C. 40% seawater.

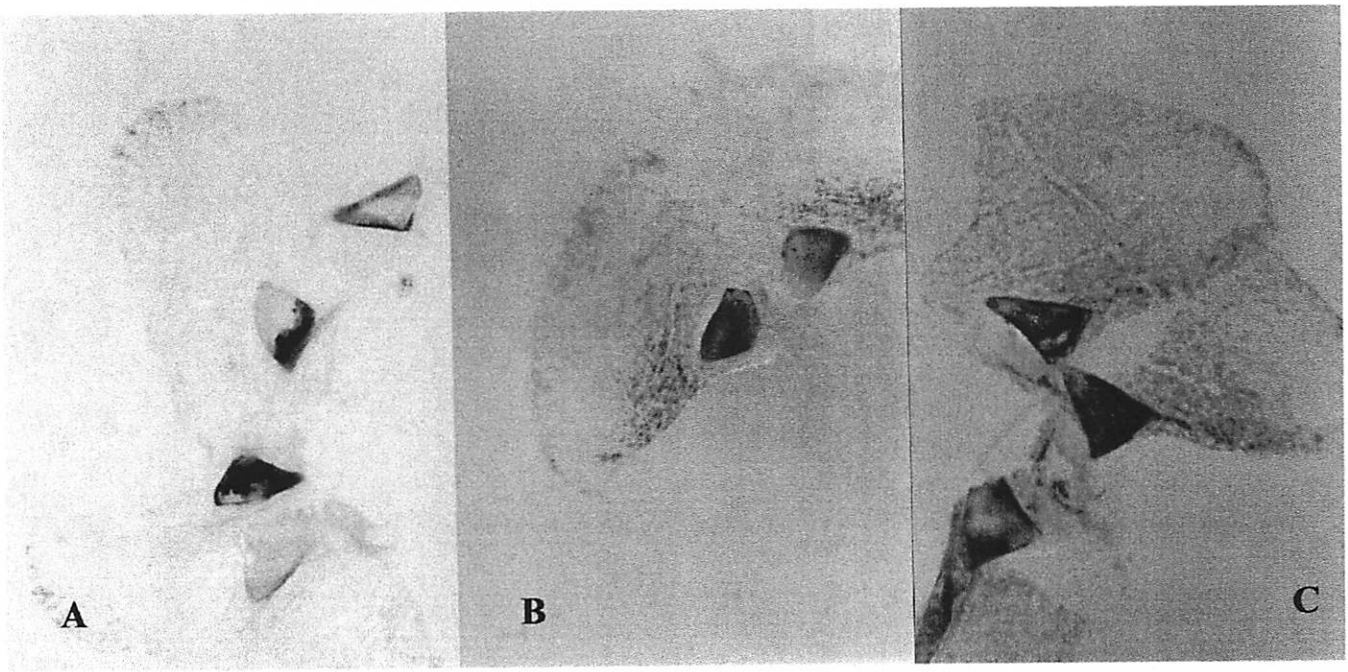


**Figure 3.** Comparison of silver chloride staining of gill lamellae for *Erythemis simplicicollis* in varying salinities. Control = A; 20% salinity = B; 40% salinity = C.