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Cover Photo: Dr. Lynn Margulis, Symposium Keynote Speaker, describes the structure and ecology of living stromatolites. Some, visible as grayish mounds near her feet, line the shore of Storrs Lake whereas others occur farther out in deep water. (See paper by D. C. Edwards, this volume).

Back Cover Photo: Group photo of the 6th Symposium participants and speakers.

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SALINITY TOLERANCE OF *ERYTHEMIS SIMPLICICOLLIS* SAY (ODONATA: ANISOPTERA, LIBELLULIDAE)

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ABSTRACT

Dragonflies (Odonata:Anisoptera) are generally considered to be freshwater invertebrates, although a few clearly marine species have been described. Consequently, salt tolerances of the freshwater dragonflies are not well known. The purpose of this study was to examine the effects of salinity on survival and development, using nymphs of *Erythemis simplicicollis* Say, a species which occurs on San Salvador.

Commercially obtained nymphs were caged individually in dilutions of seawater ranging from 0-80%, and survival and molting patterns were recorded until death or adult eclosion. As expected, survival was higher in the lower seawater concentrations, and development to the adult stage appeared to parallel that of the control nymphs in terms of molting frequency and morphological changes. However, these nymphs exhibited much higher salinity tolerances than expected, and *Erythemis simplicicollis* is clearly able to survive in brackish water. If other San Salvadoran dragonflies have comparable salt tolerances, they may be able to utilize habitats ordinarily considered unsuitable, such as some of the tidal ponds or portions of the inland lakes, or to survive increases in salinity during evaporation of temporary pools.

INTRODUCTION

Dragonflies (Odonata:Anisoptera) have been recorded as occurring throughout the Bahamas, including the island of San Salvador. On San Salvador, adults of a total of thirteen species have been reported in the past, and nymphs of seven species have been collected (Smith and Smith, 1994), confirming that these species are breeding on this "dry" island. Possible breeding sites on San Salvador include temporary freshwater pools, more permanent freshwater marshes, and permanent saline and hypersaline inland lakes, some of which have underground connections to the sea. Resident dragonflies, therefore, must either be exploiting the limited permanent freshwater habitats, or they must be able to tolerate more saline conditions of the inland lakes or evaporating temporary pools.

Three species found on San Salvador have been reported to develop in brackish as well as fresh water (Dunkle, 1989): *Erythemis simplicicollis* Say, *Orthemis ferruginea* Fabricius, and *Pantala flavescens* Fabricius. However, little is known about the precise salinity tolerances of these species, or the effects of salinity on their development. Osburn (1903) listed *E. simplicicollis* as being able to live in "slightly salt water." Similarly, Wright (1943) reported finding *E. simplicicollis* in Mississippi River delta and central Gulf Coast salt marshes and brackish areas of varying salinities, but not in "strongly brackish or saline areas." He defined brackish areas as 14-57% seawater and saline areas as 57-143% seawater; the salinity of "strongly brackish" remains unclear.

A fourth species, *Erythrodiplax berenice* Drury, is considered a true marine dragonfly (Dunkle, 1989), and is apparently unable to develop in fresh water (Wright, 1943). Dunson (1980) found nymphs to be common in the Florida Keys in salinities of 36-48 ppt, and in the laboratory they lived in fresh water (tap water) and seawater for one month, with "no apparent ill effects" although no development data were given. In another study he held nymphs at salinities ranging from fresh water up to 260% seawater for 3 days with no mortality. Clearly, these brackish and marine dragonfly species have some physiological plasticity with regard to osmotic balance which allows them to develop in

habitats other than "typical" freshwater habitats.

Our purpose in this ongoing study is to examine more precisely the effects of salinity on nymphal survival and development for those species which may be breeding in saline sites on San Salvador. It is possible that as the salinity of a given habitat approaches the physiological limit of salinity tolerance for the dragonfly nymphs present, their development may be slowed as more energy is required for osmoregulation or maintaining fluid volume. We report here results of a preliminary laboratory study of the effects of six salinities on nymphal survival and development of *Erythemis simplicicollis* Say.

METHODS

Sixty commercially obtained nymphs of *Erythemis simplicicollis* Say were arbitrarily categorized by relative wing pad length and body size into approximate instars. Three distinct size ranges were evident, 7-9 mm ("3rd instars"), 10.5-14.0 mm ("4th instars"), and 14.5-16mm ("5th instars"). This was necessary since dragonflies go through a variable number of instars, so the exact stages of the nymphs obtained could not be precisely known. All nymphs were, however, at least midway through their development.

Six salinities were made using artificial sea salt in aged tap water. This artificial seawater (sp. gr. 1.022; 35 ppt) was then diluted with aged tap water to the salinities shown in Table 1.

Table 1. Artificial seawater treatments expressed as percent seawater, with their corresponding measured specific gravities and calculated salinities.

Seawater dilution	Specific gravity	Salinity
0% seawater	1.0000	<0.5 ppt
10% seawater	1.0035	3.5 ppt
20% seawater	1.0080	7.0 ppt
50% seawater	1.0120	18.0 ppt
60% seawater	1.0180	22.0 ppt
80% seawater	1.0195	29.0 ppt

Ten nymphs were randomly assigned to each of the salinities. Nymphs were caged individually in one liter containers with 500 ml of water and maintained at 22 C on a day-night cycle of 16 hrs l: 8 hrs d. They were fed tubifex worms and brine shrimp *ad libitum* every two days, and water was changed weekly. A piece of plastic mesh 8 cm x 14 cm was placed in each container to provide both a perching site in the water and a substrate above the water upon which adult eclosion could occur.

Effects of salinity on development were compared using a mean instar index, which is calculated as $\Sigma [(n_i)(i)] / \Sigma n_i$, where i = instar and n = numbers of nymphs. The Mann-Whitney U test was used to compare survival times for nymphs in the six salinities.

RESULTS AND DISCUSSION

E. simplicicollis nymphs survived for at least 24 hours in all salinities tested, although nymphs in salinities above 20% exhibited significantly decreased survival times (Mann-Whitney U test, α=0.05) (Figure 1). Mean survival time was not significantly different in 0% and 10% seawater.

There was never 100% eclosion in any treatment, but at least half the nymphs did eclose (emerge as adults) in treatments of up to 20% seawater (Figure 1). One nymph eclosed after 11 days in 50% seawater. These results suggest that water of up to 3.5 ppt salinity may have little effect on the survival of *E. simplicicollis* nymphs, and further, some individuals of this species are capable of completing development at salinities of up to about 7 ppt.

Mean instar data suggest that compared to the freshwater control, development was delayed in 10% and 20% seawater (Figure 2). While nymphs did molt in the 50% seawater, only one completed development, and most molted only once. Little development occurred in the 60% and 80% seawater treatments, no doubt reflecting the much shorter survival times for nymphs in these salinities. Delayed development under adverse environmental conditions is common in many terrestrial insects such as grasshoppers (e.g. Smith and Grodowitz, 1983), and the delayed

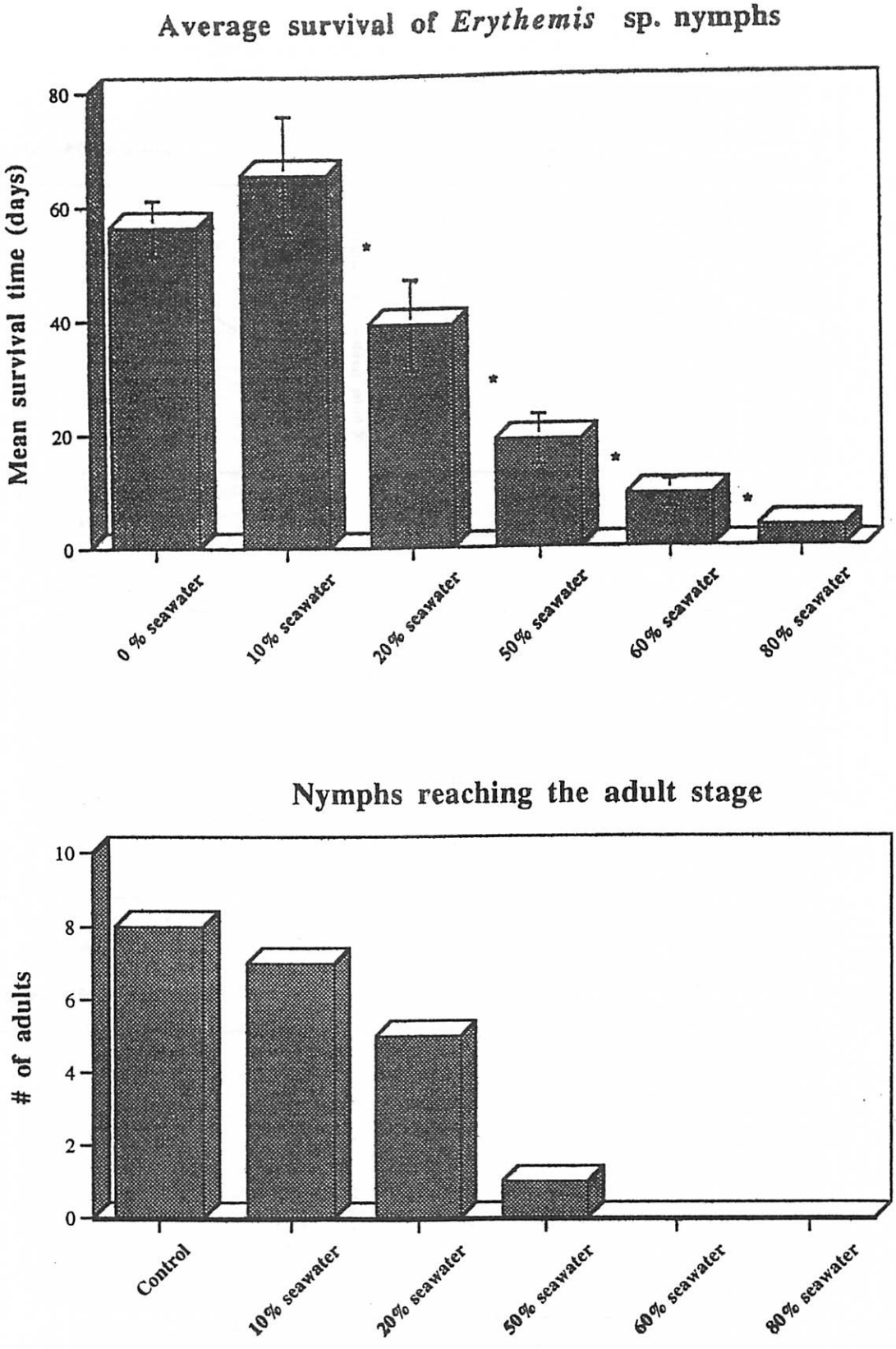

Figure 1. Mean survival in days and development to adulthood in *Erythemis simplicicollis* at varying salinities.

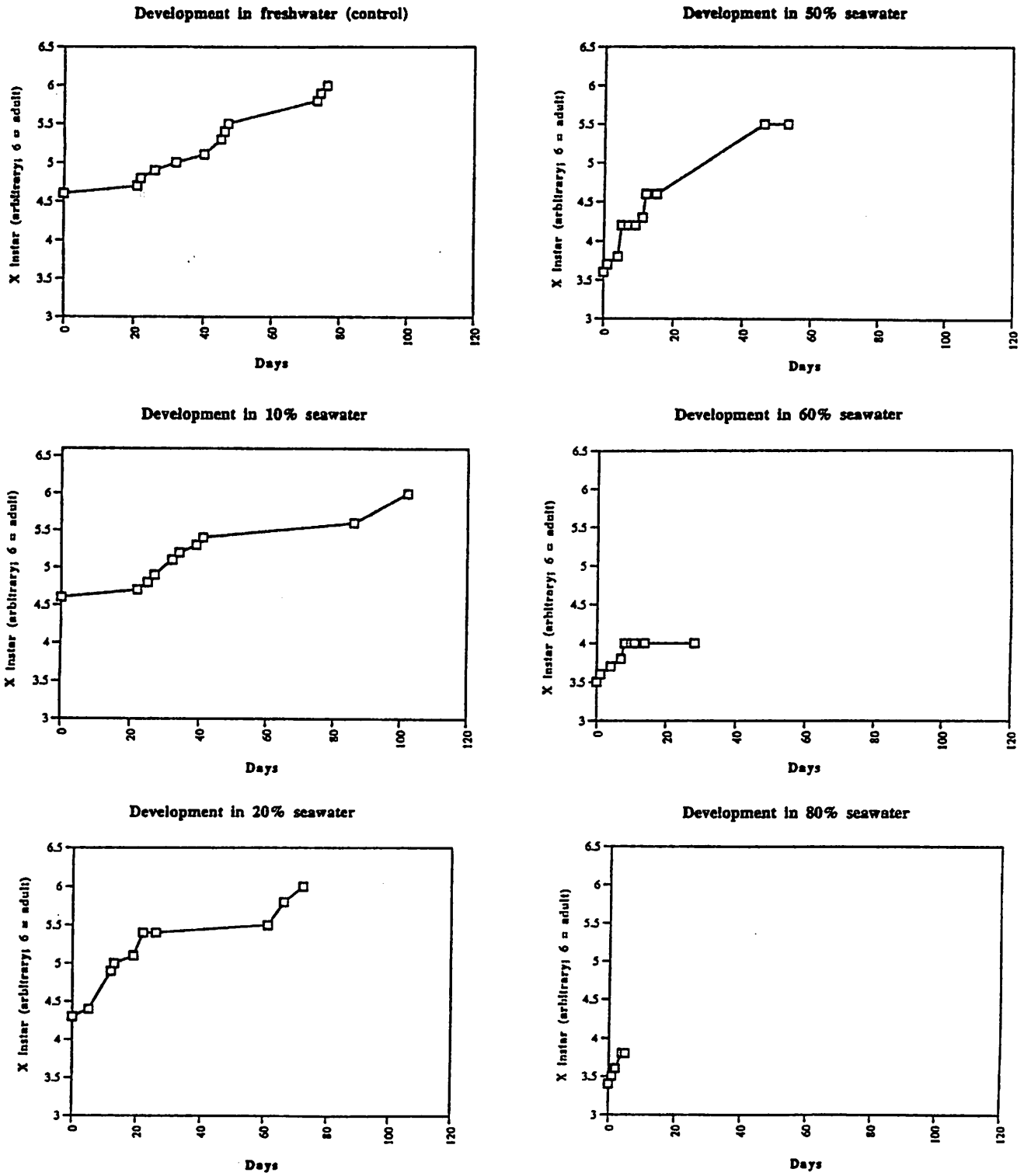

Figure 2. Development expressed as mean instar index for *Erythemis simplicicollis* at varying salinities.

development seen in this study suggests that increasing salt concentrations may physiologically stress *E. simplicicollis* nymphs even within their range of salt tolerance.

The results of this study support previous observations that this species can tolerate brackish conditions and show that salinities above 50% have a clear negative impact on survival and development; these salinities may well exceed the limits of salt tolerance for this species, which have not yet been established. Further study of the effects of salinities between 20% and 50% seawater (7-18 ppt) may reveal more precisely the limits of salt tolerance for *E. simplicicollis* and the consequences of physiological stress as these limits are approached.

While it is known that some species of dragonflies can develop under the constraints of a temporary water supply, which may increase in salinity as evaporation proceeds, it is also known that most do not (Dunkle, 1976; Corbet, 1980). Our survey of aquatic habitats on San Salvador is not yet sufficient to thoroughly document where dragonflies are developing on the island, but it does confirm that some species, including two of the three recognized as brackish water species, are reproducing here (Smith and Smith, 1994). The sinkholes, most transient of aquatic habitats, have yet to yield any nymphs. To date, the only species found in the saline inland lakes and blue holes is the marine dragonfly, *Erythrodiplax berenice* (Dunson, 1980; Dunkle, 1989) However, as *Erythemis simplicicollis* is also salt tolerant, further collection may reveal the presence of this and/or other species of nymphs in brackish water habitats.

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