

PHYSIOLOGICAL CORRELATES OF WATER CHEMISTRY
REQUIREMENTS IN FAIRY SHRIMPS (ANOSTRACA) FROM
SOUTHERN CALIFORNIA

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A B S T R A C T

In an effort to explain distributional differences among 4 species of fairy shrimps (Anostraca) in southern California, we correlated their ionoregulatory abilities with ion levels in their native pools. *Branchinecta sandiegonensis* Fugate and *Streptocephalus woottoni* Eng, restricted to dilute coastal vernal pools in San Diego county, were strong hyperregulators that maintained constant hemolymph $[Na^+]$ (average = 82 mmol l^{-1} , and 69 mmol l^{-1} , respectively) when external Na^+ levels were below 60 mmol l^{-1} . In higher concentrations, however, hemolymph levels rose significantly and mortality increased; no *S. woottoni* survived in 100 mmol l^{-1} Na^+ . Similarly, both species maintained constant hemolymph levels in alkalinities up to 800–1,000 mg l^{-1} , but did not survive higher concentrations. In contrast, *Branchinecta mackini* Dexter, which inhabits saline and alkaline pools, regulated hemolymph ion levels more loosely, but were tolerant of higher ion concentrations. Hemolymph Na^+ concentrations rose gradually from 50.0 ± 3.0 mmol l^{-1} in 1 mmol l^{-1} water to 105.1 ± 1.8 mmol l^{-1} in 100 mmol l^{-1} water, but there was no mortality. They could tolerate alkalinities up to 2,000 mg l^{-1} (double that of the other 2 species), although hemolymph $[Na^+]$ fell from 88.8 ± 1.5 mmol l^{-1} to 78.0 ± 3.8 mmol l^{-1} . The fourth species, *Branchinecta lindahli* Packard, which is found in both types of pools, exhibited intermediate ionoregulatory abilities. In high external Na^+ concentrations they allowed internal levels to rise, but in low external concentrations (<20 mmol l^{-1}) they were able to maintain elevated hemolymph levels. These results indicate that the distributions in southern California of the 4 species of fairy shrimps examined here were greatly influenced by the water chemistry of the pools due to different ionoregulatory capabilities.

Fairy shrimps (Anostraca) inhabit world-wide ephemeral pools that come in a wide variety of types ranging from small vernal pools to desert playa lakes. Many studies have documented that different species inhabit different types of pools, and several explanations have been offered (Belk, 1977; Brown and Carpelan, 1971; Eng *et al.*, 1990; Horne, 1967; McCarraher, 1970). One of the most common explanations is that distributional differences are based on the ion concentrations of the pools (Belk, 1977; Broch, 1969, 1988; Geddes, 1973; Horne, 1967, 1971). Some species appear to be restricted to waters with relatively low concentrations because they regulate hemolymph ion levels above the environment (hyperregulation), but they cannot tolerate water salinities higher than internal levels (Broch, 1969; Geddes, 1973). Others are able to tolerate higher ion concentrations by allowing body concentrations to rise with water levels (osmoconforming) or by hyporegulating.

In southern California, *Branchinecta*

sandiegonensis Fugate and *Streptocephalus woottoni* Eng are restricted to small coastal vernal pools in a few counties, while *Branchinecta mackini* Dexter is much more widely distributed (Eng *et al.* 1990; Fugate 1993). Eng *et al.* (1990) measured the water chemistry of pools inhabited by *S. woottoni* and *B. mackini* in California and found that *S. woottoni* was typically found in low ion waters, and *B. mackini* inhabited more concentrated waters. Additionally, Eng *et al.* (1990) found that pools inhabited by *B. mackini* had a significantly higher pH and alkalinity than those holding *S. woottoni*. No information about water chemistry of pools inhabited by *B. sandiegonensis* is available, but the fact that they are found in the same general localities as *S. woottoni* suggests that they too live in low ion pools. These observations suggest that water ion concentration and possibly pH and alkalinity are playing a key role in determining where these species live.

Our goal was to explain the differences in distribution of these species by correlat-

ing the water chemistry of pools inhabited by them with their ionoregulatory ability in relation to water Na^+ concentration, pH, and alkalinity. We first assayed pools in coastal San Diego county inhabited by *B. sandiegonensis* and *S. woottoni*, and pools in part of the Mojave desert inhabited by *B. mackini* for these variables. In addition, we assayed pools in both areas that were inhabited by a fourth species, *Branchinecta lindahli* Packard. Eng *et al.* (1990) found *B. lindahli* to be the most widely distributed species of fairy shrimp in California, and that it could live in a wide range of chemical conditions. For this reason, we thought it would be interesting to include it in our study.

Water chemistry measurements were followed by an examination of the effects of water Na^+ , pH, and alkalinity on the ability of each species to regulate hemolymph ion levels (particularly Na^+ and Cl^-). All three parameters are known to affect ionoregulatory ability of aquatic animals (Mantel and Farmer, 1983; Wilkie and Wood, 1991). These kinds of physiological measurements yield two useful pieces of information. First, they are a more sensitive indicator of overall condition than simple mortality. They indicate when treatments are stressful, but not outright lethal. This sort of sublethal stress can have enormous effects on the fairy shrimp population by reducing life span, growth, and fecundity (Dunson and Travis, 1991). By measuring these physiological indicators of stress, we produced a more realistic estimate of the concentration ranges within which populations can survive. Second, measurement of hemolymph ion levels provide useful information about the underlying mechanisms of ion regulation in these species that result in differences in tolerance ranges.

MATERIAL AND METHODS

Water Chemistry Analysis.—The water chemistry of pools on Edwards Air Force Base (EAFB) in the Mojave desert was examined several times during the winter and spring of 1993, and pools on Miramar Naval Air Station (MNAS) in San Diego county were examined during the same seasons of 1994. For each pond known to be inhabited by shrimp, the pH was measured with an Orion model 720 pH meter, and alkalinity was determined with a Hach titration kit. Water samples (approximately 125 ml) were filtered through 0.45- μm mesh, acidified with 100 μl concentrated HNO_3 , and returned to the laboratory for deter-

mination of Na^+ concentration with a Perkin-Elmer model 3100 atomic absorption spectrophotometer (AAS).

Experimental Animals.—We collected *B. sandiegonensis* from pools at MNAS, and in the laboratory placed them in aquaria containing water with the following salt levels: 1 mmol l^{-1} Na^+ , 0.5 mmol l^{-1} K^+ , 0.2 mmol l^{-1} Ca^{2+} and Mg^{2+} , 1.9 mmol l^{-1} Cl^- (pH = 8.0–9.0). Since *S. woottoni* were not available from San Diego county when experiments were started, we collected them from a pool in Riverside county. They were held in water with the same ion concentrations as for *B. sandiegonensis*. We collected *B. mackini* from Rosamond Lake on EAFB and placed them in aquaria containing 10 mmol l^{-1} Na^+ , 0.5 mmol l^{-1} K^+ , 0.2 mmol l^{-1} Ca^{2+} , 10.9 mmol l^{-1} Cl^- (pH = 8.0–9.0). All salt concentrations were chosen to simulate the levels in the native pools of each species. The pH of the water in which *B. sandiegonensis* and *S. woottoni* were held was slightly higher than that of their native ponds to facilitate the alkalinity exposures; they exhibited no obvious stress as a result of this exposure. Shrimps were held in the laboratory for at most a few days before being used in experiments, and were fed a yeast suspension several times daily during that time; they were not fed during tests.

We did not collect *B. lindahli* from the field, since they were not available when we started experiments. Instead, they were cultured from eggs collected from soil samples of pools from the Mojave Desert taken earlier in the year. The water chemistry in which we held *B. lindahli* was the same as that used for *B. mackini*.

Experimental Protocol.—In order to examine the effects of water chemistry on ion regulation in fairy shrimps we exposed individuals of each species to a range of water pH, Na^+ concentration, and alkalinity, and then analyzed their hemolymph ion levels. For test exposures, shrimps were placed in a series of 500-ml containers (up to 20 shrimp per container) filled with water at room temperature (21–23 °C). For the Na^+ tests, Na^+ concentrations ranged from 0.5–100 mmol l^{-1} (other salt concentrations were maintained at the holding concentrations as appropriate for specific species). For alkalinity tests, a preliminary test was performed for each species by exposing them to alkalinities (KHCO_3) ranging from 0–3,000 mg l^{-1} (10 shrimps per concentration) for 24 h. Based upon mortalities from the preliminary tests, *B. sandiegonensis*, *S. woottoni*, and *B. lindahli* were then exposed to alkalinities from 0–1,000 mg l^{-1} (they did not survive higher concentrations) for 24 h. For *B. mackini*, alkalinities ranged from 0–2,000 mg l^{-1} . In the pH test, the pH ranged from 8–10.9, and was adjusted with concentrated KOH. Shrimps were exposed to the pH test for only 8 h, because atmospheric CO_2 constantly dissolved in our baths, lowering the pH. Consequently, the baths were monitored and adjusted regularly.

At the end of all the exposure periods, we removed the shrimps and collected their hemolymph with a microcapillary tube. An aliquot of hemolymph was used undiluted to measure chloride concentration with a Radiometer model CMT 10 chloridometer or to measure osmotic pressure with a Wescor model 5500 vapor pressure osmometer, and a portion was diluted 200–1,000 times for analysis of Na^+ with AAS.

Table 1. Water chemistry of pools in San Diego county containing *Branchinecta sandiegonensis* during two sampling periods, and *Streptocephalus woottoni* or *Branchinecta lindahli* (all dates combined for both). Values are means \pm SE.

Species	N	pH	Na ⁺ (mmol l ⁻¹)	Alkalinity (as mg l ⁻¹ HCO ₃ ⁻)
<i>B. sandiegonensis</i>				
15 Feb. 1994	21	7.1 \pm 0.1	1.98 \pm 0.73	55 \pm 4
15 Mar. 1994	9	7.2 \pm 0.2	0.70 \pm 0.08	40 \pm 5
<i>S. woottoni</i>	4	7.0 \pm 0.3	0.73 \pm 0.19	41 \pm 1
<i>B. lindahli</i>	3	7.8 \pm 0.3	1.52 \pm 0.04	64 \pm 11

Statistical Analyses.—Significant differences among means were determined using analysis of variance ($P < 0.05$). If significant, multiple comparisons were made between individual means using Sheffe F -test.

RESULTS

Water Chemistry Analysis

Table 1 summarizes water chemistry measurements for pools at MNAS. For *S. woottoni* we have included the chemistry of the pool in Riverside county, where they were collected for laboratory use. That gave us a total of two pools. Regardless of which species was found in the pools, their water chemistries were very similar. In general, they were neutral in pH (although the pH of pools containing *B. lindahli* were a bit higher), and low in ions and alkalinity. The Na⁺ concentrations of all the pools (with three exceptions) were all below 2 mmol l⁻¹. The three exceptions were responsible for the higher Na⁺ concentration of pools containing *B. sandiegonensis* during the first sampling period; they dried before the second sampling period.

In sharp contrast to pools in San Diego county, those in the Mojave Desert had much higher pH, Na⁺ concentration, and alkalinity when they filled (Table 2), and, as the pools dried, Na⁺ concentration and alkalinity rose significantly. This latter point is

highlighted by tracking the Na⁺ concentration of Rosamond Lake, which contained water well into the summer (Fig. 1). When the lake initially filled, its water had a Na⁺ concentration of about 9 mmol l⁻¹. Over the spring, as the lake slowly dried, the concentration rose gradually, until by mid-May it was around 42 mmol l⁻¹. Drying accelerated in the summer and by August the Na⁺ concentration was approaching 400 mmol l⁻¹, a more than 40-fold increase in Na⁺ concentration over the life of the lake. Similarly, alkalinities of Mojave Desert pools at filling were about 10 times higher than those in San Diego county; they rose as the pools dried. Unfortunately, we could not get accurate alkalinity measurements later in the season, because the water contained large quantities of suspended clay particles, making colorimetric titration impossible. Pools on EAFB that were inhabited by *B. lindahli* had slightly lower Na⁺ concentrations and significantly lower alkalinities than those inhabited by *B. mackini*, but they were still much higher than pools containing *B. lindahli* on MNAS. These pools were shorter-lived, and had generally dried before the March sampling period.

Physiological Tests

Measurement of hemolymph Na⁺ concentrations in relation to external Na⁺ levels in-

Table 2. Water chemistry of pools in the Mojave desert containing *Branchinecta mackini* during three sampling periods, and *Branchinecta lindahli* during one sampling period. Values are means \pm SE.

Species	N	pH	Na ⁺ (mmol l ⁻¹)	Alkalinity (as mg l ⁻¹ HCO ₃ ⁻)
<i>B. mackini</i>				
24 Jan. 1993	17	8.8 \pm 0.1	6.4 \pm 1.3	511 \pm 107
20 Mar. 1993	13	8.8 \pm 0.1	11.1 \pm 1.9	737 \pm 197
12 May 1993	4	9.2 \pm 0.1	40.7 \pm 7.6	—
<i>B. lindahli</i>				
24 Jan. 1993	6	8.8 \pm 0.3	5.2 \pm 2.3	179 \pm 26.8

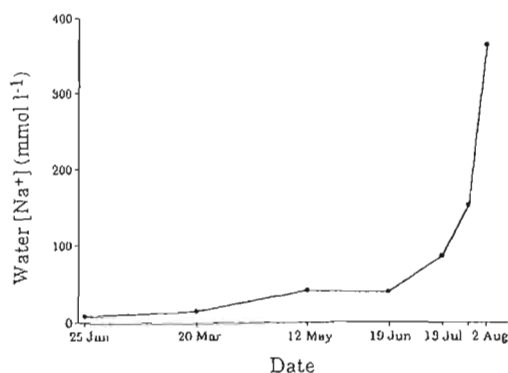


Fig. 1. Water Na⁺ concentration of Rosamond Lake, which is inhabited by *Branchinecta mackini*, in the Mojave Desert during the winter, spring, and summer of 1993. Shrimp were present until late July.

indicated that both *B. sandiegonensis* and *S. woottoni* could maintain internal levels fairly constant over a wide range of water Na⁺ concentrations, but were sensitive at extreme concentrations (Fig. 2). Between 0.5 and 60 mmol l⁻¹ Na⁺ they tightly regulated their hemolymph Na⁺ concentrations at about 82 and 69 mmol l⁻¹, respectively. Above that range, however, hemolymph Na⁺ rose steadily, remaining about 20 mmol l⁻¹ above water levels. At the greatest water Na⁺ concentration (100 mmol l⁻¹) mortality was elevated. Less than half of *B. sandiegonensis* and no *S. woottoni* survived.

In contrast, *B. mackini* and *B. lindahli* did not regulate hemolymph Na⁺ concentration as tightly as the other two species (Fig. 3), but were more tolerant of high external Na⁺ concentrations; there was no mortality observed at the highest salt levels. Across the whole range of water Na⁺ concentrations, hemolymph levels rose gradually and approached the isotonic line. In the highest Na⁺ concentration (100 mmol l⁻¹) they were virtually isotonic with their environment. At the lower end of the range (< 20 mmol l⁻¹), hemolymph Na⁺ concentrations of *B. mackini* fell particularly steeply, and, in 1 mmol l⁻¹ water, their hemolymph was only one-half as concentrated as in individuals in 100 mmol l⁻¹ water. Over this same lower range, *B. lindahli* exhibited a strong ability to regulate internal Na⁺ levels. In fact, at external Na⁺ concentrations of 1 and 5 mmol l⁻¹, hemolymph levels rose significantly above those at 10 mmol l⁻¹.

Although *B. sandiegonensis* and *S. woot-*

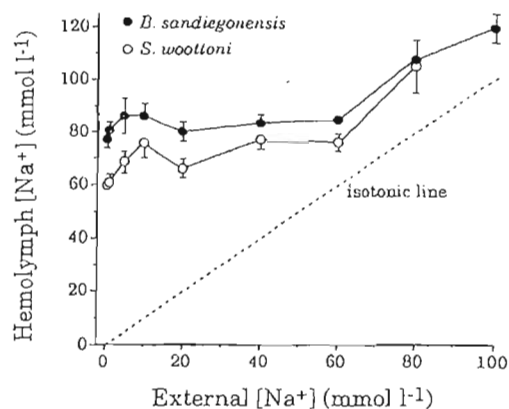


Fig. 2. Hemolymph Na⁺ concentrations of *Branchinecta sandiegonensis* ($N = 142$) and *Streptocephalus woottoni* ($N = 130$) in relation to water Na⁺ concentration. Dotted line represents hemolymph concentration isotonic with water levels. Values are means \pm SE. Values that seem to be without error bars mean that the error estimates are contained within the symbol.

toni did not survive exposure to alkalinities greater than 800–1,000 mg l⁻¹, alkalinity below this range had no effect on hemolymph Na⁺ concentration of the two species (Fig. 4), and hemolymph Cl⁻ levels of *B. sandiegonensis* (Fig. 4a). We did, however, see a significant drop (30%) in Cl⁻ levels of *S. woottoni* (Fig. 4b). In comparison, *B. mackini* could tolerate alkalinities up to 2,000 mg l⁻¹ (2–3 times greater than *B. san-*

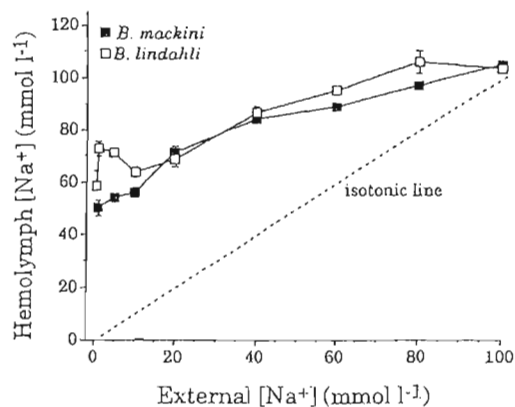


Fig. 3. Hemolymph Na⁺ concentration of *Branchinecta mackini* ($N = 79$) and *B. lindahli* ($N = 70$) in relation to water Na⁺ concentration. Dotted line represents hemolymph concentration isotonic with water levels. Values are means \pm SE. Values that seem to be without error bars mean that the error estimates are contained within the symbol.

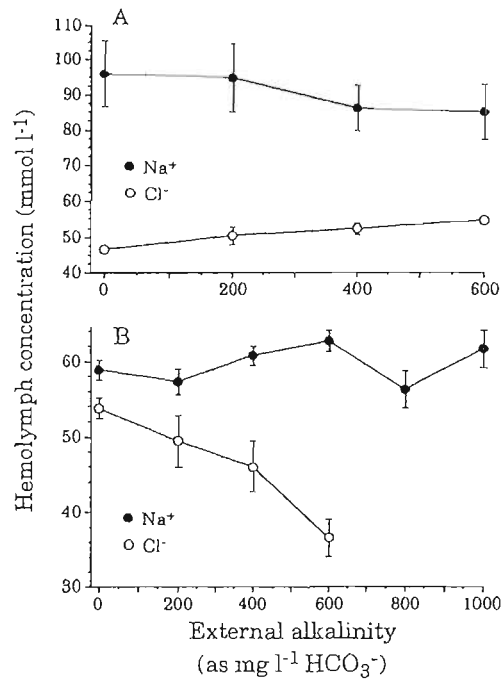


Fig. 4. Hemolymph Na⁺ and Cl⁻ concentration of (A) *Branchinecta sandiegonensis* ($N = 28$ for Na⁺ and 44 for Cl⁻) and (B) *Streptocephalus woottoni* ($N = 50$ for Na⁺ and 49 for Cl⁻) in relation to water alkalinity. Values are means \pm SE.

diegonensis or *S. woottoni*) even though alkalinity had a more pronounced effect on their ability to regulate ions. Hemolymph Na⁺ concentration dropped about 12% over the range tested (Fig. 5a). While we do not have hemolymph Cl⁻ values, the drop in hemolymph osmotic pressure, which is made up primarily of Na⁺ and Cl⁻, indicates that Cl⁻ levels fell by the same amount as Na⁺.

Branchinecta lindahli was as sensitive to elevated alkalinity as *S. woottoni* and *B. sandiegonensis*; they could not survive alkalinity above 1,000 mg l⁻¹. In addition, as in the two species from San Diego county, Na⁺ regulation of *B. lindahli* was not disturbed within the range tested (Fig. 5b); there were no significant differences among the concentrations.

Water pH appeared to have very little effect on Na⁺ regulation of hemolymph ions in any of the species tested (Fig. 6). All shrimps survived the 8-h pH exposures and there was no significant change in hemolymph Na⁺ concentration during that time.

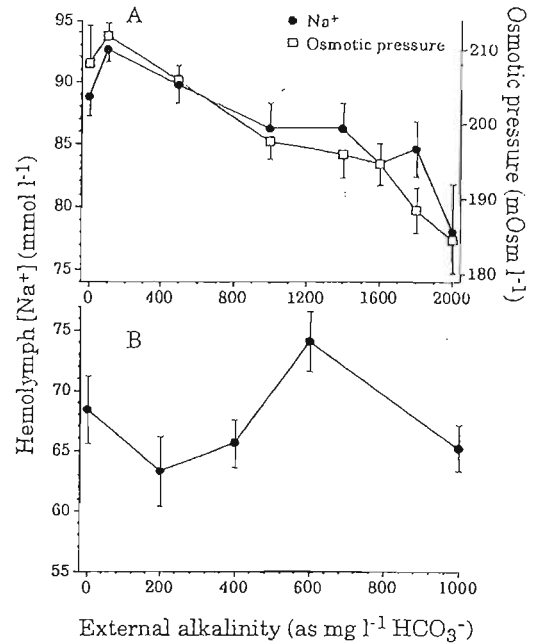


Fig. 5. Effect of water alkalinity on (A) hemolymph Na⁺ ($N = 63$) and osmotic pressure ($N = 73$) of *Branchinecta mackini* and (B) hemolymph Na⁺ ($N = 35$) of *B. lindahli*. Values are means \pm SE.

DISCUSSION

Our results indicate that the distributions in southern California of the four species of fairy shrimps examined here were greatly influenced by the water chemistry of the pools due to different ionoregulatory capa-

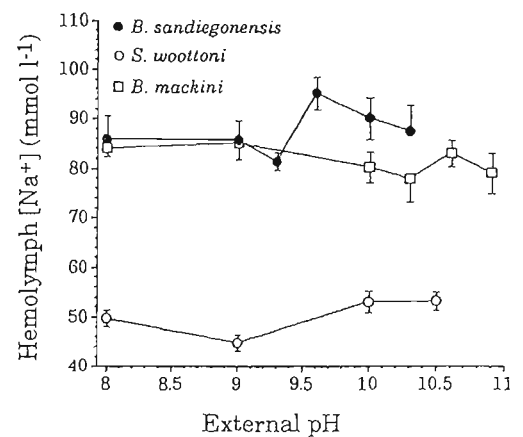


Fig. 6. Effect of water pH on hemolymph Na⁺ concentration of three species of fairy shrimps. Values are means \pm SE. $N = 55$ for *Branchinecta sandiegonensis*, $N = 34$ for *Streptocephalus woottoni*, and $N = 49$ for *B. mackini*.

bilities. Two species, *B. sandiegonensis* and *S. woottoni*, were unable to maintain ion balance in Na^+ concentrations greater than 60 mmol l^{-1} , and were unable to survive alkalinities higher than $800\text{--}1,000 \text{ mg l}^{-1}$. Consequently, they are unable to inhabit pools with concentrations higher than these values. A third species, *B. mackini*, is not excluded from concentrated pools, because it could tolerate much higher salinities and alkalinities. However, this species appeared to have difficulty at low ion concentrations, which may explain why it is not found in such dilute pools. The fourth species, *B. lindahli*, which is found in both types of habitats, appeared to be intermediate. It could tolerate high ion concentrations, but it could also maintain hemolymph levels even in the most dilute waters.

Branchinecta sandiegonensis and *S. woottoni* were strong hyperregulators that tended to maintain fixed hemolymph Na^+ and Cl^- levels even in very low environmental Na^+ concentrations. This allowed them to inhabit low-ion pools in San Diego county. However, at higher water concentrations ($>60 \text{ mmol l}^{-1}$), they had difficulty maintaining internal levels; *S. woottoni* did not survive above 100 mmol l^{-1} . Similarly, Geddes (1973) and Broch (1969) found that *Branchinella australiensis* Richters and *B. campestris* Lynch, respectively, were strong hyperregulators in low-ion waters, but could not survive higher concentrations. This appears to be the typical pattern for fresh-water branchiopods (Potts and Durning, 1980).

Meanwhile, while *B. mackini* did not maintain their hemolymph ion concentrations as rigidly as the previous two species, they were able to tolerate much higher salt concentrations. In our tests, they survived the highest concentrations (100 mmol l^{-1}) without increased mortality. Furthermore, Broch (1988) demonstrated that *B. mackini* could survive considerably higher salinities than the range tested here (up to 3 times higher) by osmoconforming. It appears, however, that, while they can tolerate very high salt concentrations, they had a reduced ability to ion regulate in low salt levels. In the low Na^+ concentrations, *B. mackini* was a much weaker hyperregulator, and appeared to have difficulty maintaining hemolymph ion levels. For example, in water

with a $1 \text{ mmol l}^{-1} \text{Na}^+$ concentration their hemolymph Na^+ levels were more than 30 mmol l^{-1} below that of *B. sandiegonensis* or *S. woottoni*.

Branchinecta mackini survived the 24-h exposures to the low salt concentrations. They were found in pools with moderately low salt concentrations, at least early in the season, though not as low as those inhabited by *B. sandiegonensis* and *S. woottoni*. They appear to be able to survive these conditions. This raises the interesting possibility that the low salt concentrations are not toxic, but are indirectly causing the exclusion of *B. mackini* from otherwise suitable pools. There are a number of ways this could happen. For example, the low-ion concentrations could be unsuitable for larval survival or could cause the exclusion of some required food item. One interesting possibility is that the reduced hemolymph Na^+ levels experienced in these low salinities could represent a sublethal stress that adversely affects their competitive ability. Similar arguments have been made for reptiles and fish (Dunson and Mazzotti, 1989; Dunson and Travis, 1991; Gonzalez and Dunson, 1991).

Branchinecta lindahli, which is found at both areas, displayed intermediate ionoregulatory capabilities. They were able to survive at least $100 \text{ mmol l}^{-1} \text{Na}^+$, and, like *B. mackini*, their hemolymph Na^+ concentration rose with external levels. However, at lower water Na^+ levels, they strongly regulated hemolymph Na^+ much like *B. sandiegonensis* and *S. woottoni*. These results suggest that *B. lindahli* is a physiological generalist that can inhabit a wide variety of habitats. Not surprisingly, Eng *et al.* (1990) found *B. lindahli* to be the most widespread fairy shrimp in the state of California. Indeed, while they were collected in waters as low in alkalinity and ion concentration as any shrimp in the state, they were also taken from pools with very high levels for these parameters. Our results further suggest that *B. lindahli* is an opportunistic species that survives on the edges of habitats. For example, in the Mojave Desert, they were found in pools with significantly lower alkalinities than those containing *B. mackini*, and in San Diego county, they were found in pools slightly higher in pH and Na^+ concentration than those in-

habited by *B. sandiegonensis*. Furthermore, our records indicate that the typical pools containing *B. lindahli* in both areas tended to be disturbed sites, such as tire ruts and depressions in roads.

When the different shrimp species were exposed to elevated alkalinity, the ionoregulatory patterns continued to be observed. Again, *B. sandiegonensis* and *S. woottoni* were more sensitive than *B. mackini*, but they seemed to more tightly regulate ion levels over the range that they tolerated. *Branchinecta sandiegonensis* could not survive alkalinity greater than 800–1,000 mg l⁻¹, but below that point it did not affect hemolymph Na⁺ or Cl⁻ concentrations. *Streptocephalus woottoni* displayed similar tolerance and ion balance was only mildly affected; Cl⁻ levels fell 30%. In contrast, *B. mackini* tolerated alkalinities up to 2,000 mg l⁻¹, even though hemolymph Na⁺ and Cl⁻ (as indicated by measurement of osmotic pressure) levels fell steadily.

Branchinecta lindahli appeared to be as sensitive to elevated water alkalinity as the two species from San Diego county. These results correlate well with water chemistry measurements of pools that they inhabit in the Mojave Desert. The alkalinity of these pools was significantly lower than those inhabited by *B. mackini*. It seems likely that *B. lindahli* is excluded from many pools in the Mojave with high alkalinity. McCarragher (1970) reported *B. lindahli* in pools in Nebraska with alkalinities much higher than those reported here. This suggests the presence of geographic variation in the tolerance to alkalinity.

The inconsistent effect of water alkalinity on ion regulation in these species raises some questions as to how elevated alkalinities are exerting their toxic effects. In the pH range used in our experiments, almost all the alkalinity would be in the form of bicarbonate ions (Goldman and Horne, 1983). In fresh-water organisms, Cl⁻ is actively taken up from the water in exchange for HCO₃⁻ (Péqueux *et al.*, 1988). It seems possible that elevated HCO₃⁻ levels could inhibit Cl⁻ uptake, which would act to lower hemolymph Cl⁻ levels. *Streptocephalus woottoni* and *B. mackini* experienced reduced hemolymph Cl⁻ levels with rising alkalinity. Similar consequences were observed in fresh-water fishes exposed to high

alkalinity (Wilkie and Wood, 1991; Wilkie *et al.*, 1993). However, Cl⁻ levels of *B. sandiegonensis* were unaffected, and *B. mackini* exhibited a drop in hemolymph Na⁺ as well as Cl⁻. It is thought that Na⁺ is actively taken up in exchange for H⁺ (Péqueux *et al.*, 1988), and there is no direct connection with HCO₃⁻. It has been suggested, however, that the H⁺ and HCO₃⁻ required for ion transport are supplied by hydration of CO₂ in the presence of carbonic anhydrase (Mantel and Farmer, 1983). This raises the possibility that in high alkalinity waters HCO₃⁻ is entering the gill epithelial cells and reacting with H⁺, thus limiting them for Na⁺ uptake. Further work is necessary to clarify these issues.

Measurement of hemolymph ion levels showed that none of the species of shrimps tested here were sensitive to high pH (up to pH 10.9). These results were somewhat surprising, since a number of studies have shown that high pH (>9.5) can fatally disrupt ion regulation in aquatic organisms (Wilkie and Wood, 1991). Eng *et al.* (1990) reported *B. mackini* in pools with a pH as high as 9.8. It should be noted that the shrimp was exposed to the high pH for only 8 h, because of problems maintaining the target pH. It is possible that the pH range used was toxic, but required a longer exposure to exert its effects.

It would be a gross oversimplification to argue that the distributions of the species of fairy shrimps examined here were solely the result of differences in water Na⁺ concentration and alkalinity. A variety of factors ultimately determine where a species can live. Usually, the appropriate conditions are a combination of biotic interactions, such as competition and predation, and abiotic factors. However, in particularly harsh environments, such as alkaline lakes, physical factors can be of overriding importance (Dunson and Travis, 1991; Gonzalez and Dunson, 1991). In these habitats, whether or not a species can survive will be determined by its physiological ability to deal with the challenges of the environment. Our examination of the ionoregulatory abilities of these species indicates that, in this instance, differences in the Na⁺ concentration and alkalinity of pools from the two areas plays a significant role in determining (di-

rectly or indirectly) where these species can live.

ACKNOWLEDGEMENTS

The authors thank M. Patrick and M. Boudrias for helpful comments on early drafts of this manuscript, and C. Esparza, D. Parsick, D. Mitchell, and C. Stein for technical assistance. We also thank R. W. Wilson and C. M. Wood for useful discussions.

The work was supported in part by grants from the Department of Defense and the University of San Diego. Some samples were collected at the Air Force Flight Test Center of Edwards Air Force Base, California, under contract to Tetratex Inc. and GRW Engineers Inc. (Contract number DCA 05-C-91-0130) for the United States Army Corps of Engineers, Sacramento District, and the Air Force Training Center Environmental Management Office.

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RECEIVED: 17 July 1995.

ACCEPTED: 11 September 1995.

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