

ACUTE RESPONSE OF THE ESTUARINE CRAB *EURYPANOPEUS DEPRESSUS* TO SALINITY AND DESICCATION STRESS

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ABSTRACT

Hemolymph osmolality changes following exposure to abrupt salinity change in the range of 5–40 ppt ($T = 26^{\circ}\text{C}$, full air saturation) and upon exposure to air ($T = 23^{\circ}\text{C}$, r.h. = 30%) were investigated in the flatback mud crab *Eurypanopeus depressus* (Smith, 1869), a dominant species on oyster reefs in Southwest Florida. During salinity trials, hemolymph osmolality of *E. depressus* ranged from $751 \pm 123 \text{ mOsmol kg}^{-1}$ at 5 ppt ($214 \pm 32 \text{ mOsmol kg}^{-1}$) to $1188 \pm 81 \text{ mOsmol kg}^{-1}$ at 40 ppt ($1188 \pm 29 \text{ mOsmol kg}^{-1}$). In the salinity range of 5–15 ppt *E. depressus* exhibited a hyperosmotic pattern of osmoregulation while at 30 and 40 ppt it conformed. In all cases stable hemolymph osmotic concentration was reached in less than 24 h. During desiccation trials, hemolymph osmolality of *E. depressus* ranged from $971 \pm 121 \text{ mOsmol kg}^{-1}$ for unexposed crabs to $1132 \pm 169 \text{ mOsmol kg}^{-1}$ after 90 min of exposure. The information obtained from this study adds to knowledge of crustacean stress physiology and may give a clearer picture of the important factors involved in population distribution and the consequences of multiple stressors that may affect the crabs or their oyster-reef habitat.

KEY WORDS: desiccation, *Eurypanopeus depressus*, osmoregulation, oyster reef, salinity

DOI: 10.1651/08-3123.1

INTRODUCTION

Organisms can use behavioral or physiological responses to survive in challenging, changing environments. For example, estuarine organisms may battle drastic and rapid changes in salinity and tackle the difficulties of keeping fluids and ions in balance daily (Mangum and Towle, 1977; Kirschner, 1979). Desiccation is another important threat to intertidal organisms, as a high percentage of tissue water content is essential to the majority of life's functions (Hochachka and Somero, 2002).

The behavioral ability to find and take advantage of hospitable microhabitats decreases metabolic strain in motile organisms and makes up for physiological shortcomings in regards to environmental tolerance (Grant and McDonald, 1979). Crabs may avoid extreme salinities, limit activity to certain times of day, make use of burrows or other shelter, or visit water regularly to combat salinity, thermal or desiccation stress (Warner, 1977). In addition to behavioral responses, organisms may also have an array of cellular mechanisms to deal with stress. Estuarine organisms especially must maintain control over the movement of water into and out of their cells under fluctuating salinities to remain competitive in this challenging environment (Mangum and Towle, 1977; Kirschner, 1979).

The flatback mud crab *Eurypanopeus depressus* (Smith, 1869) is a dominant inhabitant of intertidal oyster reefs in Southwest Florida, depending upon them for food and refuge (Grant and McDonald, 1979; Tolley et al., 2006). They are a euryhaline and ubiquitous species, found in salinities above 4 ppt in estuarine oyster

beds from Massachusetts to Texas (Shirley and McKenney, 1987). A few researchers have studied the environmental tolerances and distribution of *E. depressus*. Garcés (1987) investigated desiccation tolerance of *E. depressus* and found it significantly different from the closely related *E. dissimilis* (Benedict and Rathbun, 1891). Grant and McDonald (1979) examined two populations of *E. depressus*, one intertidal and one subtidal, and compared their desiccation tolerances in the laboratory. With no significant difference in survival observed, they concluded that hospitable conditions provided by oyster-reef refugia were more important to crab survival in the intertidal zone than any inherent physiological differences between the two populations. Two studies (Meyer, 1994; Brown et al., 2005) found that *E. depressus* was much more common in subtidal habitats with available shelter.

Stress conditions produce physiological responses. Salinity stress has been shown to affect hemolymph osmolality (Chen and Chia, 1997; Walls, 2006) and desiccation stress has been demonstrated to impact both hemolymph osmolality and water loss (Grant and McDonald, 1979; Jones and Greenwood, 1982; Warburg and Goldenberg, 1984; Garcés, 1987; Luquet and Ansaldo, 1997). This study tracked changes in the hemolymph osmotic concentration over a 24-h period after sudden transfer to both dilute and concentrated seawater to determine how well *E. depressus* is able to adapt to salinity shock. Desiccation tolerance of *E. depressus* was determined in the laboratory by measuring water-weight loss and changes in hemolymph osmolality in response to emersion.

MATERIALS AND METHODS

Animal Collection

Juvenile and adult male and female *E. depressus* were collected by hand from the intertidal zone of oyster reefs in the Caloosahatchee and Estero Bay estuaries in Southwest Florida, USA and transported to the laboratory. Sizes ranged from 8.7–18.6 mm carapace width and wet masses from 0.15–2.42 g. Salinity and temperature at time of capture averaged 35.20 ± 2.15 ppt and $26.02 \pm 2.35^\circ\text{C}$, respectively. Crabs were held in aquaria at full air saturation, 26°C , and 30 ppt for a period of one week prior to the start of the experiment. Target salinities were achieved using commercially available sea salt (Instant Ocean[®], Aquarium Systems) and dechlorinated tap water. Crabs were fed with commercially available sinking wafer fish food.

Salinity Shock and Hemolymph Osmolality

Hemolymph osmotic concentration was measured at the following salinities: 5, 15, 30 (control) and 40 ppt, with other parameters constant ($T = 26^\circ\text{C}$ and full air saturation). Intermolt, non-ovigerous crabs of various sizes without concern for sex were randomly selected and placed into 40-L experimental aquaria. For each of the four salinity treatments (3 replicates each), five crabs were sampled six times over the course of 24 h for a total of 360 individuals. Sampling was without replacement. Hemolymph was withdrawn after 0, 1, 4, 8, 12 and 24 h by puncturing the ventral medial carapace and collecting exuded fluid via capillary action with 5- μL Drummond[®] microcapillary glass pipettes (Shirley and McKenney, 1987). The first sample represented osmolality at the control salinity. Osmolality was immediately measured on a Wescor Vapro 5520[®] vapor pressure osmometer using the small sample size protocol provided by the manufacturer.

Desiccation and Hemolymph Osmolality

Hemolymph of crabs undergoing emersion in plastic desiccators (FisherBrand[®]) was measured after 45 and 90 min. Each crab was placed in an individually numbered paper cup at a density of twelve per chamber on a screen suspended above 100 g of color change desiccant (Drierite[®], Mesh size 8). Relative humidity (r.h.) and temperature were monitored with a pen-type ThermoHygrometer[®] inside one of the chambers and remained stable at 30–35% and 23°C , respectively. Five each of small and large crabs were sampled at three time intervals in triplicate trials for a total of 90 crabs used. Hemolymph of crabs not exposed to desiccation (emersion) was extracted and recorded as time zero. Hemolymph sampling protocol was identical to that described for salinity shock trials.

Desiccation Tolerance

Water-weight loss in plastic desiccators (FisherBrand[®]) was measured at 30-min intervals until movement ceased, limbs were pulled inward and touch did not result in a response. At this point, the crabs were considered stressed (Garcés, 1987). Fifty crabs were challenged and sampling was again without replacement.

Statistical Analysis

Differences in hemolymph osmolality among salinity treatments and exposure times were tested using 2-way analysis of variance (ANOVA). Desiccation tolerance differences in hemolymph osmolality with respect to sex, size class and exposure time were tested using analysis of variance (ANOVA). The Levine statistic was used to test for homogeneity of variance. Where significant differences ($P \leq 0.05$) were detected by ANOVA and variances were equal, Hochberg's GT2 multiple-comparison test was used to distinguish among factors; otherwise, the Games-Howell post-hoc test was used. Analyses were performed using SPSS (SPSS, Inc., USA). Slopes of the regressions relating cumulative weight loss over time during the desiccation trial were compared using analysis of covariance to identify differences between small and large crabs. Values are presented as mean \pm standard deviation unless otherwise stipulated.

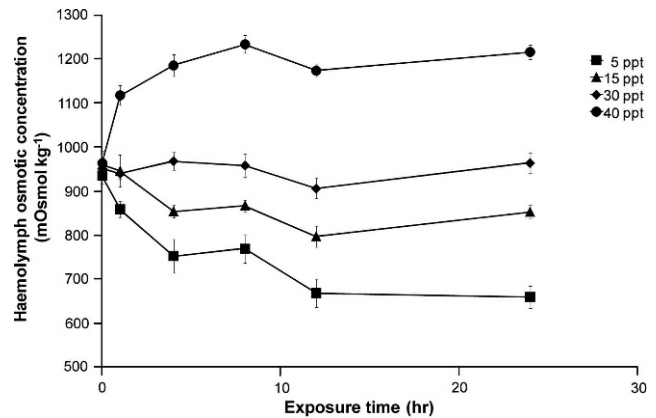


Fig. 1. Mean hemolymph osmotic concentration of *Eurypanopeus depressus* at different salinities and exposure times. Values are mean \pm standard error.

RESULTS

Salinity Shock and Hemolymph Osmolality

Osmotic values ranged from 659 ± 87 mOsmol kg^{-1} after 24 h at 5 ppt (214 ± 32 mOsmol kg^{-1}) to 1234 ± 76 mOsmol kg^{-1} after 8 h at 40 ppt (1188 ± 29 mOsmol kg^{-1}) (Fig. 1). There were significant differences in hemolymph osmolality with respect to salinity and exposure time (ANOVA, $P \leq 0.05$). Mean hemolymph osmolality differed significantly among all treatments ($F [3, 299] = 276.3, P < 0.001$) and, in general, increased with salinity. At 5 ppt (214 ± 32 mOsmol kg^{-1}), hemolymph osmolality averaged 751 ± 123 mOsmol kg^{-1} compared with 862 ± 92 mOsmol kg^{-1} at 15 ppt (501 ± 26 mOsmol kg^{-1}), 1188 ± 81 at 40 ppt (1188 ± 29 mOsmol kg^{-1}) and 956 ± 77 mOsmol kg^{-1} for the control treatment at 30 ppt (921 ± 48 mOsmol kg^{-1}). Osmoregulation showed a similar pattern of change for all salinity dilution treatments, but transfer to low (5 ppt) and high (40 ppt) salinities were characterized by a rapid change that appears to be related to the degree of deviation from the control salinity (Fig. 1).

Hemolymph osmolality was hyperosmotic at 5 and 15 ppt and isosmotic at 30 and 40 ppt (Fig. 2). Exposure to 5 ppt resulted in significantly reduced hemolymph osmolality from the control for all exposure times after one hour (Hochberg's GT2, $P \leq 0.001$). At 15 ppt, osmolality after twelve hours of exposure was significantly lower than that at both the initial sampling and after one hour (Hochberg's GT2, $P \leq 0.033$). No differences in osmolality were detected among exposure times for the control (30 ppt) treatment. At 40 ppt, osmolality at all exposure times differed significantly from the initial hemolymph osmotic concentration (Hochberg's GT2, $P \leq 0.034$). For the 15- and 30-ppt treatments, final hemolymph osmotic concentration did not significantly differ from the initial measurement at time zero. For the 40-ppt treatment, osmolality measurements did not differ significantly after one hour.

Desiccation and Hemolymph Osmolality

Crabs were divided into two size classes based on a median wet mass of 0.61 g. Statistical tests detected only a

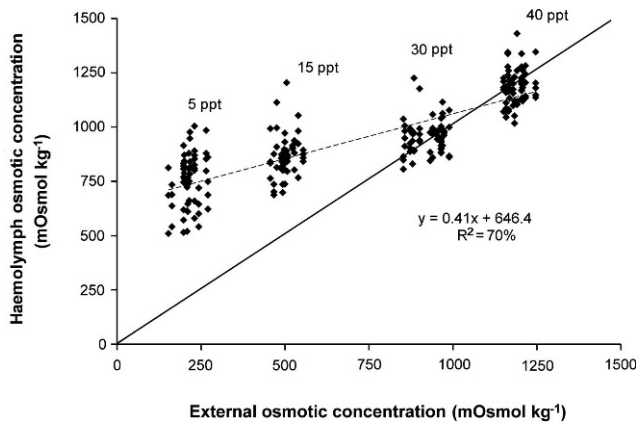


Fig. 2. Linear regression relating *Eurypanopeus depressus* hemolymph osmotic concentration and external osmotic concentration. The solid line represents the isosmotic line.

marginal difference between size classes, with larger crabs having a mean hemolymph osmolality ($1025.6 \pm 107 \text{ mOsmol kg}^{-1}$) that was lower than that of small crabs ($1059.2 \pm 179.6 \text{ mOsmol kg}^{-1}$) ($F [1, 106] = 3.842, P = 0.0530$). Because the difference was not significant, data for small and large crabs were combined for further consideration. Hemolymph osmolality increased with exposure time, with values averaging $971.5 \pm 121.1 \text{ mOsmol kg}^{-1}$ for the control group that was not exposed, $1032.9 \pm 116.0 \text{ mOsmol kg}^{-1}$ for the 45-min exposure, and $1132.0 \pm 169.1 \text{ mOsmol kg}^{-1}$ for the 90-min exposure (Fig. 3). Osmolality of the aquarium water averaged $876.2 \pm 14 \text{ mOsmol kg}^{-1}$.

Desiccation Tolerance

Crabs were divided into two size classes based on a median wet mass of 1 g (Table 1). Total wet mass lost ranged from 0.07 g to 0.23 g and averaged $0.12 \pm 0.04 \text{ g}$. Time to stress response ranged from 230 to 400 min. The average time to the beginning of stress response was $309.2 \pm 50.9 \text{ min}$ and did not differ significantly between size classes or sex.

Although there was no difference in total wet-mass loss between sexes, female crabs lost a significantly higher (12.16 ± 3.95) percentage of their starting wet mass than males (8.89 ± 2.00), ($F [1, 46] = 12.983, P = 0.001$). Sex effects could be attributed to size, as males ($1.42 \pm 0.47 \text{ g}$) were significantly larger than females ($0.97 \pm 0.29 \text{ g}$) ($F [1, 46] = 15.9, P < 0.001$). The average percent wet-mass loss at time of stress response was 10.49 ± 3.49 . No significant effect of sex was detected on time to stress response. Regression analysis found no significant relationship between starting wet mass and time to the beginning of stress response. Towards the end of the

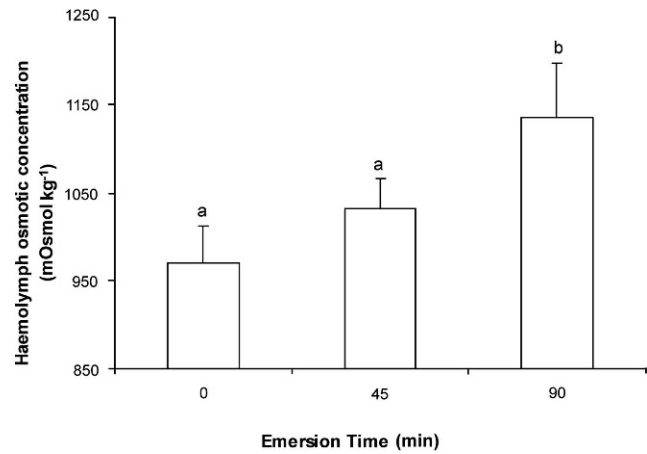


Fig. 3. Hemolymph osmotic concentration of *Eurypanopeus depressus* at different emersion times. Bars represent average hemolymph osmotic concentration at each sampling time with lines representing standard deviation. Different letters are significant differences in means ($P < 0.05$).

desiccation trials, mean wet-mass loss of large crabs and male crabs surpassed that of small crabs and female crabs.

Both wet-mass loss and cumulative percent wet-mass loss increased with exposure time. Linear regression of cumulative percent wet-mass loss over time explained 95 and 98 percent of variation between small and large crabs, respectively (Fig. 4). An analysis of covariance comparing the slopes of the two regressions found that they were significantly different ($F_{22} = 18.77, P < 0.001$), with the slope being greater for small crabs, indicating a higher rate of cumulative percent loss. Larger crabs lost significantly more mass per unit time than small crabs ($F [1, 46] = 15.855, P < 0.001$) and small crabs lost a higher percentage of their total body mass ($F [1, 46] = 9.862, P = 0.003$).

DISCUSSION

The changing tide in the shallow environment of the oyster reef means inhabitants risk exposure and desiccation, yet the reefs support rich communities. *Eurypanopeus depressus* is one of the most abundant decapods found on Southwest Florida oyster reefs (Tolley et al., 2006) despite the harsh conditions found there, suggesting that this species has the ability to cope with both abrupt salinity changes and desiccation.

In Southwest Florida, seasonal rains bring about localized drastic drops in salinity during the wet season, and hypersaline conditions are common downstream during the dry season. In response to rapid changes in salinity, crustaceans typically endure some dilution of hemolymph at low salinities and conform to the osmolality of full

Table 1. Summary of data for desiccation tolerance trials of *Eurypanopeus depressus*. Sample size in parentheses.

Size class	Sex	Starting WM (g)	WM lost (g)	WM lost (%)	Time to stress response (min)
≤ 1.00 g	F	0.85 ± 0.87 (18)	0.10 ± 0.03 (18)	12.60 ± 4.20 (18)	315.00 ± 52.38 (18)
	M	0.90 ± 0.13 (7)	0.09 ± 0.03 (7)	9.96 ± 1.81 (7)	274.29 ± 49.95 (7)
> 1.00 g	F	1.41 ± 0.21 (5)	0.15 ± 0.05 (5)	10.56 ± 2.60 (5)	324.00 ± 54.1 (5)
	M	1.64 ± 0.37 (17)	0.13 ± 0.03 (17)	8.45 ± 2.00 (17)	312.94 ± 46.87 (17)

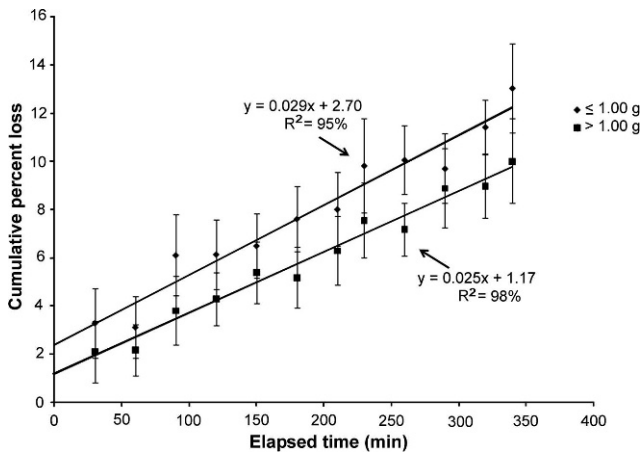


Fig. 4. Linear regression relating cumulative percent water loss over time of small and large *Eurypanopeus depressus* in 30% relative humidity and 23°C. Slopes are significantly different ($F_{22} = 18.77$, $P < 0.001$).

strength seawater (Mantel and Farmer, 1983; Henry et al., 2003). Most crabs are able to stabilize their hemolymph osmotic concentration within 24 h after sudden salinity change (Lovett et al., 2001). In the current study, *E. depressus* exhibited the expected pattern of osmoconformation at higher salinities and hyperregulation at dilute salinities but was able to stabilize its hemolymph osmotic concentration in four hours or less after dilute salinity shock.

This acclimation to sudden salinity dilution was accomplished much more quickly than has been observed in most other crustaceans. Species such as the blue crab *Callinectes sapidus* (Rathbun, 1896) (Towle, 1997), sharp-toothed swimming crab *C. rathbunae* (Contreras, 1930) (Alvarez et al., 2002), Chinese mitten crab *Eriocheir sinensis* (Milne Edwards, 1853) (Roast et al., 2002) and the isopod *Idotea balthica* (Pallas, 1772) (Bulnheim, 1974) require at least 24 h to stabilize osmolality. In contrast, rapid acclimation such as that demonstrated by *E. depressus* has been observed only rarely. As an example, the green shore crab *Carcinus maenus* (Linnaeus, 1758) was able to adjust its hemolymph osmotic concentrations following salinity reduction in six hours (Towle, 1997). The small size of *E. depressus* compared to most of the other crustaceans studied may account for the speed of acclimation because of their larger surface area to volume ratio. Charmantier (1998) reported that adaptation time to salinity change is much shorter in larvae versus adult crustaceans for the same reason.

Crustaceans generally reach a stable hemolymph osmotic concentration more quickly after transfer from dilute to full strength or higher seawater than after exposure to dilute salinities. This is probably because of the time delay involved in ramping up enzymatic mechanisms for active ion transfer (Bulnheim, 1974; Towle, 1997; Alvarez et al., 2002). In the current study, conformation to 40 ppt occurred after only one hour in contrast to acclimation to 5 ppt, which required four hours.

The current study confirms that *E. depressus* is a euryhaline organism able to hyperosmoregulate in the face of rapid and extreme drops in salinity as well as conform to

concentrated seawater over the short term. Other studies lend evidence in support of this crab's ability to survive over a longer period of time in various salinities (Shirley and McKenney, 1987; Walls, 2006; Hulathduwa et al., 2007).

When forced to combat desiccation without shelter, *E. depressus* is not as resilient as other intertidal crustaceans. Grant and McDonald (1979) and Garcés (1987) estimated total percent water lost at death for *E. depressus* to be 30% on average, less than that measured for an intertidal hermit crab with 50% but greater than that measured for the porcelain crab *Petrolisthes elongatus* (Milne Edwards, 1837) with 20% (Jones and Greenwood, 1982). Assuming a body water content of sixty-four percent (Garcés, 1987), *E. depressus* in the present study began the stress response at an average water loss of 16%, which is in close agreement with Garcés measurement of 17% (1987). Tolerance of water loss cannot be considered an absolute measure of desiccation resistance though, because the lethal water-weight loss of land crabs and marine crabs is not much different (Warner, 1977).

Sex differences in desiccation resistance or other aspects of physiology have been observed by other researchers and attributed to factors related to size (Cházaro-Olvera and Peterson, 2004; Novo et al., 2005; Yoder et al., 2007). Smaller animals have a higher surface area to volume ratio and are as a result more vulnerable to evaporative loss, both through the integument and gills. An investigation of equal sized male and female giant river prawns *Macrobrachium rosenbergii* (De Man, 1879) revealed no differences in hemolymph osmolality after acclimation to hyperosmotic conditions (Cheng et al., 2003).

The structure of the oyster reef traps water as the tides recede, providing moist refuges for *E. depressus*. Other studies have shown that crabs choose habitats or are more common where shelter is available (Day and Lawton, 1988; Meyer, 1994; Brown et al., 2005; Tolley and Volety, 2005). Also, researchers have found that species that tend to hide in sheltered crevices are less tolerant of desiccation than those inhabiting more exposed areas, even if the species coexist closely (Kensler, 1967). These findings fit with what is known about *E. depressus* behavior, because although individuals are common on oyster reefs, they are not seen venturing from their shelter unless the reef is disturbed; even then they emerge reluctantly (May, 1974).

The current study's finding that hemolymph osmotic concentration increases upon exposure to air is supported by the work of Taylor and Butler (1978) with the shore crab, *C. maenus*, Jones and Greenwood (1982) with the porcelain crab, *P. elongatus*, and Huang and Chen (2001) with the Japanese spiny lobster *Panulirus japonicus* (von Siebold, 1824). The range of measurements (900–1250 mOsmol kg⁻¹) was also similar to that of the current study. Jones and Greenwood (1982) concluded that the *P. elongatus* was able to osmoregulate in air because of an observed plateau-effect in hemolymph osmotic pressure over time for large individuals. Hemolymph osmolality in *E. depressus* increased significantly over a 90-min period; it can therefore be assumed that this species is unable to osmoregulate in air. Because hemolymph became increas-

ingly difficult to analyze over time, an examination of the effects of longer exposure periods was not possible.

Although *E. depressus* does not appear to be physiologically suited to withstand air exposure, the shelter provided by oyster reefs allows it to inhabit the intertidal environment successfully, while a strong osmoregulatory response allows survival of sudden salinity changes. The great abundance of this species suggests that *E. depressus* has quite successfully overcome, through a combination of physiology and behavior, obstacles presented by salinity gradients and desiccation on its oyster-reef home.

ACKNOWLEDGEMENTS

Many thanks are extended to Lesli Haynes, Amanda Booth and the Coastal Watershed Institute interns, who collected and helped maintain the hundreds of specimens used in this study. Our sincere thanks go to Vincent Encomio and Laura Walls for help with the osmometer. This work was funded by a grant from the South Florida Water Management District (C-13252) and by Congressional Grant X7-96403504-0 awarded through the U.S. Environmental Protection Agency.

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RECEIVED: 24 November 2008.

ACCEPTED: 15 January 2009.