Scaling of Gill Metabolic Potential as a Function of Salinity in the Euryhaline Crab, *Callinectes sapidus* Rathbun

Stephen T. Kinsey*
Elizabeth Buda
Jennifer Nordeen
Department of Biological Sciences, University of North Carolina at Wilmington, 601 South College Road, Wilmington, North Carolina 28403-5915

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ABSTRACT

The body-size scaling pattern of enzymes that are important in energy metabolism was examined in gills of the blue crab as a function of acclimation salinity. We hypothesized that the higher surface-area-to-volume ratio of small crabs would impose a greater metabolic cost for hyperosmoregulation, leading to an increase in the capacity for ATP production in gills. Postmetamorphic crabs spanning a 2,500-fold range in body mass were examined following a 7-d exposure to a salinity of 35, 17, or 5 ppt. The posterior gills, which are the principal site of osmoregulatory ion pumping, generally had higher activities than the anterior gills, which are primarily used for gas exchange, and this discrepancy was greatest in small crabs. A significant effect of salinity was found only for the enzyme citrate synthase, where the activity was highest at the lowest salinity. Although most enzymes scaled negatively with body mass, the activity was independent of size over a 250-fold size range that encompassed the body masses of juvenile crabs but decreased abruptly in the adult crabs. These data suggest that ion pumping associated with osmoregulation may represent a greater energetic challenge in smaller crabs, and this is reflected in the relatively higher metabolic potential of the posterior gills. However, acclimation to different salinity regimes does not lead to dramatic global changes in the capacity for energy metabolism.

Introduction

The blue crab, *Callinectes sapidus*, is highly euryhaline and can be found in salinities ranging from 0 to 35 ppt (Mangum and Amende 1972; Lynch et al. 1973; Cameron 1978). At salinities below 25 ppt, *C. sapidus* is a hyperosmoregulator, actively transporting ions from the water into its hemolymph, while at higher salinities blue crabs osmoconform (Ballard and Abbot 1969; Mangum et al. 1985; Robinson 1994). The principal sites of active ion transport in adult blue crabs are the posterior gills, while the anterior gills have a largely respiratory role (Mantel and Farmer 1983). This functional division is evident from the greater number of transport epithelial cells in posterior gills (Copeland and Fitzjarrell 1968; Towle and Kays 1986), which have a higher capacity for ATP production (Copeland and Fitzjarrell 1968; Piller et al. 1995) and ion transport (Neufeld et al. 1980; Lucu 1990; Péqueux 1995; Towle and Weihrauch 2001). In addition, fluctuations in salinity induce more dramatic effects on metabolism and gene expression/protein activation in posterior gills than in anterior gills. A reduction in salinity leads to responses in posterior gills that include increased rates of oxygen consumption (King 1965; Engel et al. 1975; Piller et al. 1995) and glucose/amino acid oxidation (Pressley and Graves 1983), a proliferation of transport epithelial cells (Aldridge and Cameron 1982), and an upregulation of transport-related enzymes (e.g., Towle et al. 1976; Neufeld et al. 1980; Savage and Robinson 1983; Towle 1990; Péqueux 1995; Henry 2001).

The capacity for hyperosmoregulation in euryhaline crustaceans changes with developmental stage, and in terms of maintenance of an osmotic gradient across the gills, adults are generally superior to larval and early juvenile stage animals (reviewed in Charmantier 1998). Although the osmoregulatory function of gills from juvenile blue crabs has received relatively little attention, small juveniles are capable of surviving for extended periods at low salinity (Cadman and Weinstein 1988; Guerin and Stickle 1992, 1997). In the Cape Fear River estuary in southeastern North Carolina, large numbers of *C. sapidus* juveniles (<12 mm carapace width) utilize upriver habitats with salinities that range from 4 to 12 ppt (M. H. Posey, unpublished results). Exploiting these environments may offer ecological benefits such as refuge from predators or favorable food resources. However, the postmetamorphic development of *C. sapidus* encompasses a range in body mass that is more than three orders of magnitude. This suggests that the extremely high surface area to volume ratio (SA : V) of small juvenile crabs imposes considerably higher metabolic costs associated with gill ion transport than would be expected for adult crabs. Previous work has shown that oxygen consumption in gills from immature blue crabs is elevated at low salinity in a manner

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* Corresponding author; e-mail: kinseys@uncw.edu.

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similar to that seen in adult crabs (King 1965; Engel and Eggert 1974). Guerin and Stickle (1992) found that the energy available for growth was lower in juvenile blue crabs exposed for short periods to low salinity than for those at high salinity. However, the salinity effects on scope for growth were not observed during experiments with longer acclimation times (Guerin and Stickle 1997).

These previous studies have not examined osmoregulation across the entire size range of postmetamorphic development in the blue crab. Based on SA : V constraints, the expectation is that low salinity will induce an upregulation of ATP production capacity that is proportionately greater in smaller crabs. In this study, we examined the effect of acclimation salinity on the scaling relationship between enzymes of energy metabolism and body mass in anterior and posterior gills of the blue crab. We hypothesized that mass-specific enzyme activity would scale negatively with body size and be inversely related to acclimation salinity in posterior gills but not anterior gills. At low acclimation salinity, we hypothesized, this pattern would result in an amplification of the negative allometric scaling relationship that has often been observed between mass-specific aerobic enzyme activity and body mass (Somero and Childress 1980; Emmett and Hochachaka 1981; Hochachka et al. 1988; Childress and Somero 1990; Goolish 1991). We measured enzymes of the citric acid cycle (citrate synthase), fatty acid oxidation (β-hydroxyacyl-CoA dehydrogenase), glucose oxidation (hexokinase), and amino acid oxidation (glutamate dehydrogenase). In addition, we examined the activity of arginine kinase, which is important in the temporal, and possibly spatial, buffering of cellular ATP concentrations (reviewed in Ellington 2001) and which has recently been shown to be induced by low salinity in posterior gills of adult blue crabs (Kotlyar et al. 2000).

Methods

Juvenile blue crabs were collected in the lower Cape Fear River estuary at a collection site where salinity ranged from 0 to 20 ppt. Adult crabs were purchased at a local seafood market. Juvenile crabs were housed in plastic containers that were partitioned to isolate each crab while adults were maintained in aquaria. All animals were maintained at 20°C for 7 d at a salinity of 5, 17, or 35 ppt. Crabs were fed daily, with adults receiving shrimp while juveniles were given brine shrimp pellets. After a 7-d period, weight and carapace width (CW) were measured, and crabs were categorized into one of three size classes: "small" juvenile crabs that were <30 mm CW, "medium" juvenile crabs that were 30–80 mm CW, and "large" adult crabs that were >125 mm CW.

Prior to dissection, the crabs were cooled on ice for 5–15 min (depending on animal size) to reduce movement. Anterior gills (gills 3 and 4) and posterior gills (gills 6 and 7) were removed, blotted, weighed, and placed in 9 volumes of ice-cold Tris buffer (0.05 M, pH 7.6). Due to the extremely small volume of metabolically active tissue in the gills of juvenile crabs, gills from several animals had to be pooled to generate a sufficient volume of extract for the enzyme assays (as well as a sufficient mass to be accurately weighed). Therefore, for the smallest size class, a single replicate consisted of gills that were pooled from as many as 22 animals. Gills from adult crabs and most of the medium-size crabs did not need to be pooled. The gills were then homogenized using a Powergen 125 sawtooth homogenizer followed by a 10-s burst with a Fisher Scientific 60 sonic dismembrator. The homogenate was then centrifuged at 16,000 *g* for 5 min at 4°C, and the supernatant was frozen at −85°C until used.

All enzymes were assayed using a Pharmacia Ultrospec 4000 spectrophotometer. Temperature was maintained at 20°C using an Isotemp recirculating water bath connected to the spectrophotometer. Unless noted otherwise, 5 μL of tissue extract was used and absorbance was measured at 340 nm to monitor changes in the rate of oxidation/reduction of NAD(P)H/NAD(P)$^+$. Specific assay conditions were as follows: citrate synthase (CS): 0.1 M Tris-HCl (pH 8.1), 1 mM 5,5'-dithio-bis[2-nitrobenzoic acid], 10 mM acetyl-CoA, 10 mM oxaloacetate (absorbance was monitored at 412 nm [$\epsilon = 13.6$]); hexokinase (HK): 45 mM HEPES (pH 7.5), 3.7 mM glucose, 7.5 mM $MgCl_2$, 11 mM α-monothioglycerol, 35 mM NADP, two units glucose-6-phosphate dehydrogenase, and 0.22 mM ATP; β-hydroxyacyl-CoA dehydrogenase (HCoADH): 12.5 mM sodium pyrophosphate (pH 7.3), 25 mM S-acetoacetyl-CoA, and 5 mM NADH; glutamate dehydrogenase (GDH): 50 mM triethanolamine (pH 7.5), 2.5 mM EDTA, 0.2 mM NADH, 100 mM NH_4Cl, and 7 mM α-ketoglutarate; arginine kinase (AK): 100 mM Tris/HCl (pH 8.0), 750 mM KCl, 250 mM magnesium acetate, 100 mM ATP, 25 mM phosphoenolpyruvate, 5 mM NADH, five units pyruvate kinase, five units lactate dehydrogenase, and 200 mM arginine.

Regression analysis was used to examine the scaling of gill weight with body weight. Two-way ANOVA was used to test for effects of size and salinity on enzyme activities and for interactions of the two, independently for anterior and posterior gills. One-way ANOVA was used to test for effects of size among enzyme posterior gill/anterior gill activity ratios. Where significant effects were detected, Student's *t*-tests were used for pairwise comparisons. Results were considered significant at $P < 0.05$.

Results

Crab carapace width (CW) and weights, as well as gill weights for the three size classes are presented in Table 1. Crab CW ranged from 10 to 150 mm, while animal body mass encompassed a range greater than 2,000-fold, from 0.1 g to more than 200 g. Gill weight was related to body weight by the allometric scaling equation, $Y = aM^b$, where Y is gill weight, a is a constant, M is body mass, and b is the scaling exponent (Schmidt-

Table 1: Carapace widths (CW), body weights, and gill weights of *Callinectes sapidus* used for enzyme assays

	N Total (Pooled)	Crab CW (mm)	Crab Weight (g)	Anterior Gill Weight (g)	Posterior Gill Weight (g)
35 ppt:					
Small	57 (6)	19.2 ± 2.3 (14.2, 27.1)	.75 ± .29 (.25, 1.92)	.021 ± .010	.057 ± .029
Medium	5 (4)	62.2 ± 1.8 (59.7, 66.8)	20.32 ± 1.68 (18.27, 24.47)	.057 ± .016	.119 ± .039
Large	4 (4)	143.7 ± 3.2 (138.2, 151.4)	211.73 ± 17.97 (167.80, 240.40)	.423 ± .080	.857 ± .184
17 ppt:					
Small	82 (7)	18.1 ± 2.0 (11.5, 24.8)	.60 ± .20 (.10, 1.44)	.013 ± .005	.077 ± .039
Medium	6 (4)	50.6 ± 3.0 (45.5, 57.2)	11.84 ± 2.16 (7.65, 16.50)	.031 ± .009	.078 ± .020
Large	4 (4)	143.8 ± 3.0 (138.8, 149.7)	207.58 ± 17.59 (177.00, 244.20)	.282 ± .031	.609 ± .200
5 ppt:					
Small	80 (6)	17.3 ± 2.1 (11.1, 23.4)	.57 ± .24 (.11, 1.58)	.025 ± .017	.033 ± .010
Medium	5 (4)	45.7 ± 2.9 (39.2, 50.9)	9.17 ± 1.78 (5.64, 13.13)	.021 ± .006	.064 ± .011
Large	4 (4)	137.8 ± 5.6 (137.7, 148.9)	200.18 ± 13.48 (181.00, 225.90)	.435 ± .044	.814 ± .124

Note. Gills from crabs in the smaller size classes were too small to be used individually. Therefore, gills from these animals were pooled to yield the *N* value in parentheses. The pooled gills were blotted, weighed, and divided by the number of animals to determine the mean anterior and posterior gill weights. Anterior and posterior gills were removed from both the left and right side of each crab. Data are means ± standard error of the mean. For crab carapace width and weight, the minimum and maximum values are given in parentheses.

Nielsen 1984; Fig. 1). The *b* value obtained for posterior gills was 0.89, and for anterior gills it was 0.99.

Enzyme activities for each size class and salinity are presented in Figure 2. The anterior and posterior gill data are presented together in Figure 2 to facilitate comparison of the two gill types (note that the data were analyzed independently). Two-way ANOVA indicated a significant effect of body size on activity in all cases, with the exception of GDH in both anterior and posterior gills and AK in anterior gills (Fig. 2). All of the enzymes with size-dependent activities scaled negatively with body size. However, gills from the medium-size crabs had the same mean activities as gills from the smallest crabs, with the exception of two cases, where medium-size crabs had significantly higher mean activities than those of the small crabs (CS posterior gills at 17 ppt, and AK posterior gills at 17 ppt; Fig. 2). In contrast, the large crabs had significantly lower mean activities than the small and/or medium size classes in 16 of the 21 comparisons made for enzymes with size-dependent activities (Fig. 2).

A significant effect of salinity on enzyme activity was detected only for CS, in both anterior and posterior gills. In both types of gills, the mean CS enzyme activities for each size class were higher at lower salinities, and this pattern was more dramatic in posterior gills. The posterior gills from the smallest animals had significantly higher mean CS activity at 5 ppt than at the other two salinities, while anterior gills from the largest animals had significantly higher mean CS activities at 5 ppt and 17 ppt than at 35 ppt (Fig. 2*A*). There were no significant interactions of salinity and size class on activity for any of the enzymes assayed.

The mean activities across all size classes of CS, HCoADH, and AK were significantly higher in posterior gills than anterior gills (*t*-test), while HK and GDH mean activities were the same in the two types of gills. CS and HCoADH had mean activities in posterior gills that were approximately threefold higher than in anterior gills, while mean AK activities differed by a factor of two. The difference in mean activities between anterior and posterior gills was greater for crabs in the small and medium size classes than for those in the large size class. This effect is demonstrated in Figure 3*A*, which shows the posterior/anterior gill activity ratio for each size class. One-way ANOVA indicated a significant size effect for each enzyme, and *t*-tests revealed

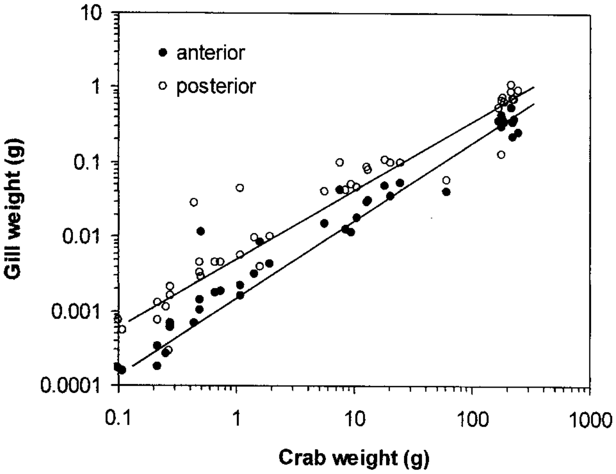

Figure 1. Scaling relationship of gill wet weight on crab weight. The data were fit to the allometric scaling equation, $Y = aM^b$. The equations for the lines are gill weight = 0.002 × crab weight$^{0.99}$ (r^2 = 0.94, $P < 0.05$) for anterior gills, and gill weight = 0.006 × crab weight$^{0.89}$ (r^2 = 0.92, $P < 0.05$) for posterior gills.

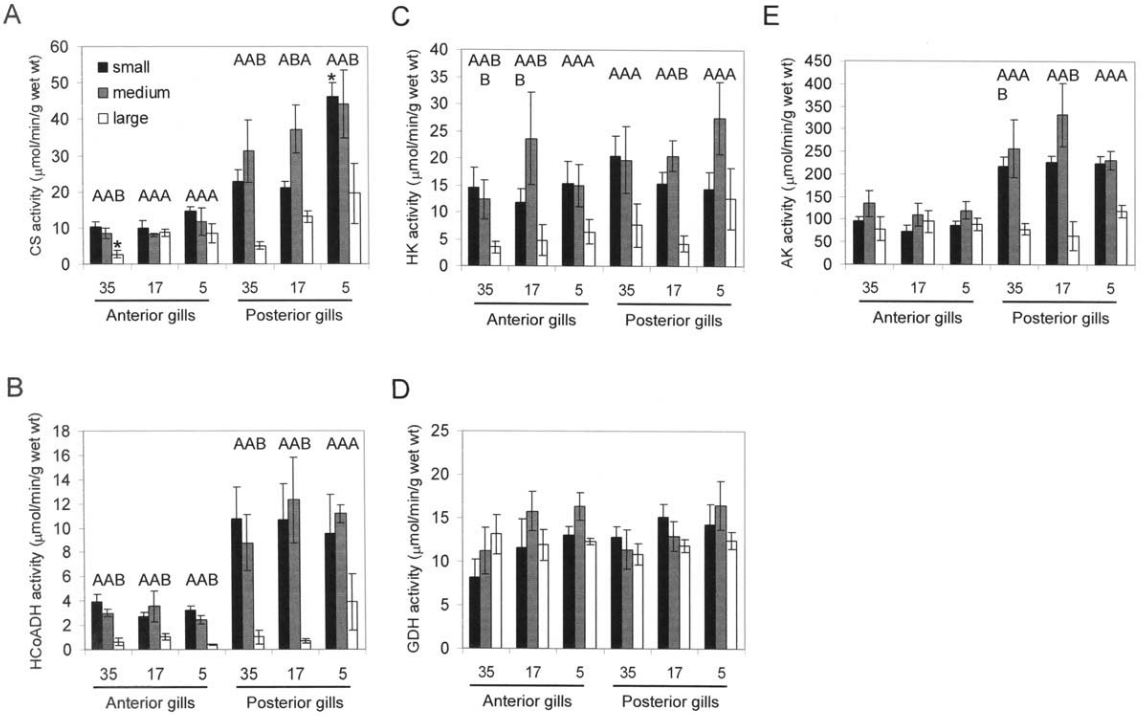

Figure 2. Mean enzyme activities in anterior and posterior gills from crabs of different size classes and acclimation salinities (5, 17, 35 ppt). The bar shading codes are indicated in *A* and are the same for all enzymes. *A*, Citrate synthase; *B*, β-hydroxyacyl-CoA dehydrogenase; *C*, hexokinase; *D*, glutamate dehydrogenase; and *E*, arginine kinase. Two-way ANOVAs were conducted on anterior and posterior gills independently to test for effects of size, salinity, or an interaction of the two. Significant salinity effects were demonstrated only for citrate synthase, and pairwise comparisons were made for this enzyme. The asterisk indicates the salinity treatment that differed from the other two salinities within that size class. Significant size effects were found in most cases, and the letters above each bar represent the results of pairwise comparisons between size classes. Within a salinity treatment, histogram bars that have the same letter above them are not significantly different from each other.

that the large crabs had significantly lower posterior/anterior activity ratios than did small- and/or medium-size crabs. The posterior/anterior activity ratio also varied with salinity. A two-way ANOVA revealed significant effects of size for all three enzymes, as well as a significant effect of salinity on the posterior/anterior activity ratio for AK (the salinity effect on HCoADH posterior/anterior activity ratio was nearly significant at $P = 0.06$). However, all three of these enzymes tended to have higher posterior/anterior activity ratios at lower salinity, and when the ratios for the three enzymes were pooled to yield a single average, a significant effect of size and salinity was detected (Fig. 3*B*).

In contrast to gill mass, enzyme activities did not scale according to the above allometric equation (Fig. 4), based on the fact that log-log plots of activity against body weight indicated a nonlinear relationship. For all of the enzymes, including those that scaled negatively with body size (Figs. 4*A*, 4*B*, 4*F*), activity was independent of crab size for juvenile animals with weights less than 25 g (Fig. 4). The size-independent scaling in crabs less than 25 g encompasses a 250-fold change in body mass. This pattern was apparent in both anterior and posterior gills.

Discussion

This study examined the scaling of enzymes of energy metabolism for the purpose of addressing whether low acclimation salinity leads to an increase in gill metabolic capacity and whether such an increase is greater in smaller crabs. We hypothesized that the oxidative enzymes would scale negatively according to the allometric equation $Y = aM^b$ and that the negative slope would be steeper at lower acclimation salinities. The lack of fit to the above equation prevented a log-linear analysis of the scaling pattern. However, if our hypothesis was correct, then we would expect the two-way ANOVA to detect a significant effect of size, salinity, and an interaction of size and salinity. We did not observe the expected global pattern of enzyme upregulation at low salinity. In fact, each enzyme behaved differently with respect to size, salinity, and gill type. The activities of CS and HCoADH were size dependent and higher in posterior than anterior gills; however, only CS was inducible by low salinity. The activity of HK was size dependent but not different in anterior and posterior gills, while GDH activity was neither size dependent nor different in the two types of gills.

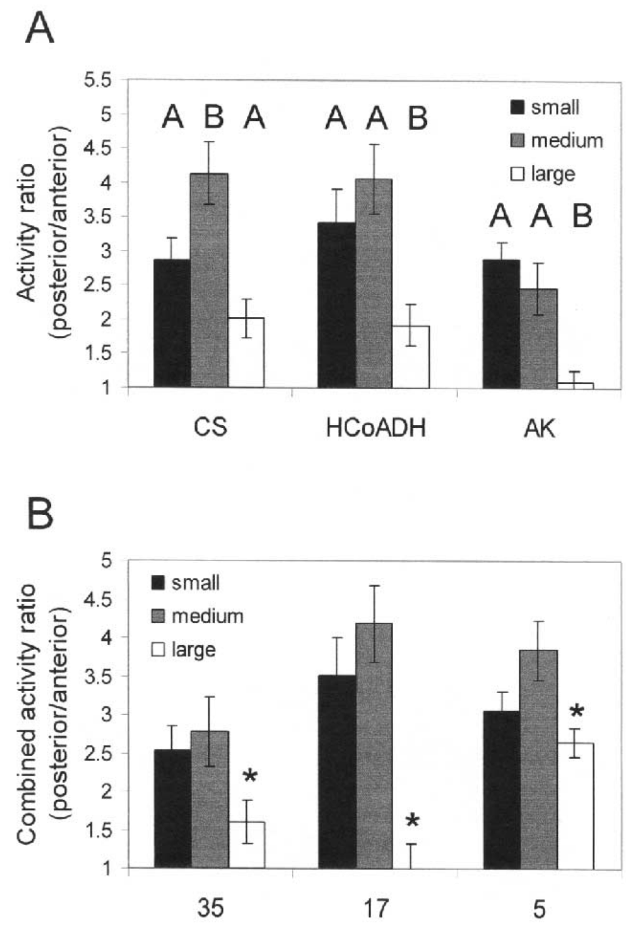

Figure 3. *A*, Activity ratio of the posterior to anterior gills for each size class. One-way ANOVA indicated a significant effect of size for each enzyme. Pairwise comparisons were conducted among size classes for each enzyme, and histogram bars that have the same letter above them were not significantly different from each other. *B*, Effect of acclimation salinity on the combined mean activity ratio for the enzymes citrate synthase (CS), β-hydroxyacyl-CoA dehydrogenase (HCoADH), and arginine kinase (AK). Two-way ANOVA indicated a significant effect of size, salinity, and an interaction of size and salinity. It can be seen that within each size class, the highest activity ratios occurred at the lower salinities. Pairwise comparisons indicated significant salinity differences only for the large size class, where the asterisk indicates that the values at each of the three salinities were significantly different from the other two.

The only enzyme that had a different scaling pattern in the two different gill types was AK, which had greater size-dependent activity in the posterior gills, while the anterior gills were independent of size.

A significant effect of salinity was found only for CS, where mean activity in gills from the smallest crabs was twofold greater at 5 ppt acclimation salinity than at 17 or 35 ppt (Fig. 2*A*). Since CS is a citric acid cycle enzyme that participates in the oxidation of many metabolic fuels, it may be a good index of the overall energy cost associated with osmoregulation in gills. A recent study examined the Na^+-K^+ ATPase in gills of blue crabs over a range in body mass comparable to that in the present study (Li and Roer 2001). As expected, the highest activities were found in posterior gills from small juveniles that had been acclimated to low salinity (5 ppt). However, Li and Roer (2001) observed an 11-fold salinity-induced increase in Na^+-K^+ ATPase activity in these crabs, while our study found that CS activity increased by only twofold under the same conditions (Fig. 2*A*). The fact that other enzymes did not demonstrate salinity effects is somewhat surprising, particularly since low salinity leads to a proliferation of ion-transporting epithelial cells in blue crab gills (Aldridge and Cameron 1982). It is possible that our relatively short exposure times (7 d) did not allow for complete acclimation (see Guerin and Stickle 1997), and longer times at low salinities may lead to a further upregulation of metabolic enzymes to facilitate hyperosmoregulation. The significant salinity effect on CS activity in anterior gills also was not predicted (Fig. 2*A*), although it is thought that anterior gills in adult blue crabs contribute somewhat to osmoregulation (Engel et al. 1975; Neufeld et al. 1980; Piller et al. 1995). Despite the fact that only CS activity had significant salinity effects, several enzymes had higher activities at low salinity in posterior gills from large animals. The smaller size classes did not show this pattern. This is contrary to our prediction, and while total activity was always higher in smaller animals, this result suggests that enzyme activity is induced by salinity to a greater extent in adults. The extremely low CS activities in posterior gills from adult animals acclimated to 35 ppt probably reflects the fact that osmoregulatory costs are minimal at salinities that are in the osmoconforming range of *Callinectes sapidus* (i.e., >25 ppt).

The much higher activities found in posterior gills than anterior gills for CS, HCoADH, and AK indicate that the posterior gills have a greater capacity for ATP production in all size classes (Fig. 3*A*). This pattern is consistent with the elevated activity of ion-transport ATPases found in this tissue (Lucu 1990; Péqueux 1995). The largest differences in activity between the anterior and posterior gills for these three enzymes were found in the small- and medium-size crabs, which had significantly higher posterior/anterior gill activity ratios than large crabs (Fig. 3*A*). This suggests that in juveniles, the respiratory and osmoregulatory functions of gills are partitioned in a manner similar to that of adults, and this functional division may be more extreme in small crabs. The data presented in Figure 3*B* shows that the posterior/anterior activity ratio is significantly altered by salinity. In fact, at low salinities the ratio for adult crabs is nearly as high as that of the small and medium size classes. This is a reflection of the overall increase in posterior gill activity (mean of CS, HCoADH, and AK) at low salinity for this size class, and it is consistent with the notion that salinity induction is more dramatic in adults.

Of the enzymes that had higher activities in posterior gills,

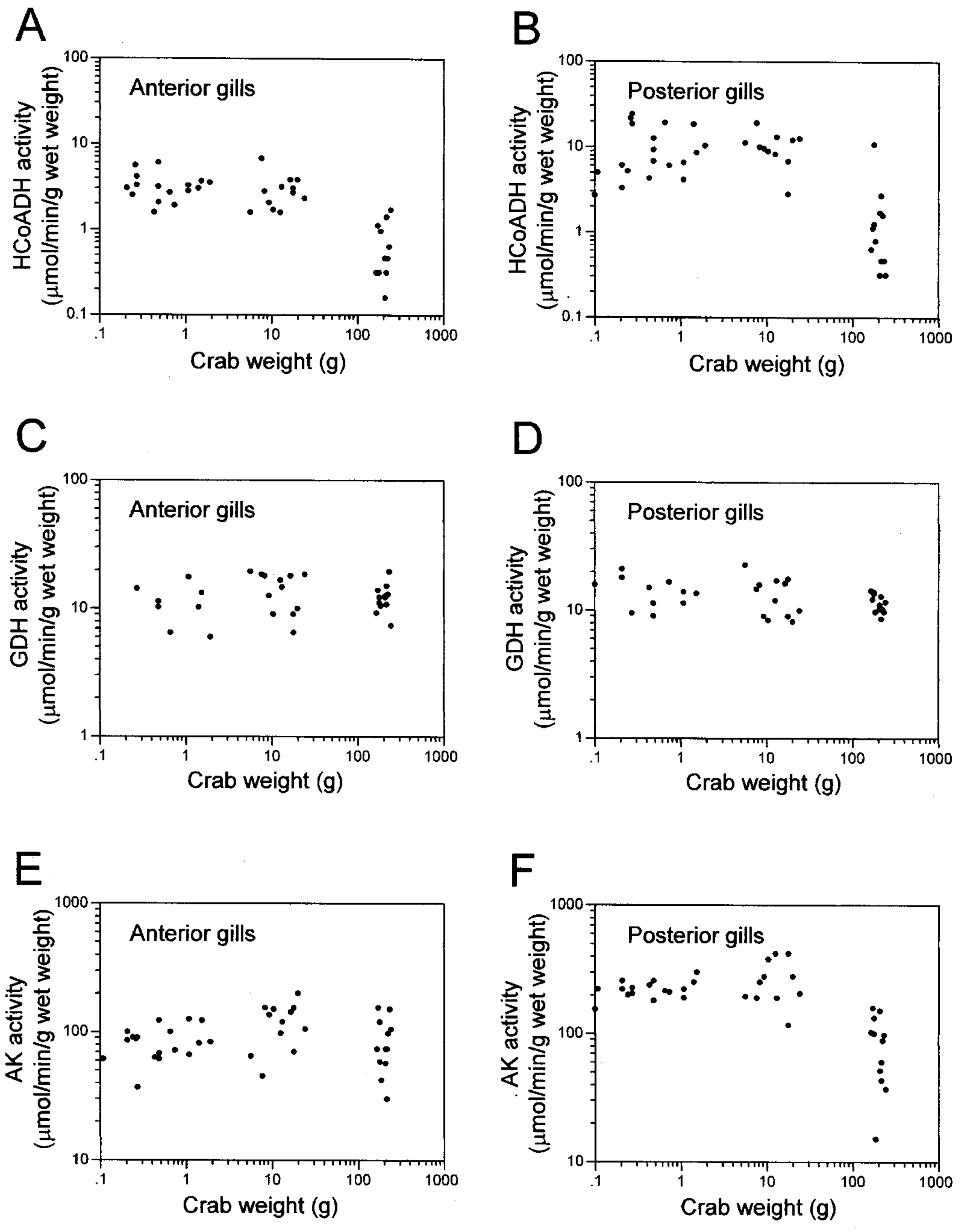

Figure 4. Examples of enzyme activity scatterplots showing the scaling relationships of enzyme activity on crab weight for all of the activity data (not separated by salinity treatment). HCoADH (β-hydroxyacyl-CoA dehydrogenase) activity scaled negatively with crab weight in both anterior (*A*) and posterior (*B*) gills. This activity is representative of the pattern observed for CS (citrate synthase) and HK (hexokinase; not shown), with the exception that HK did not have significantly higher activities in posterior gills than anterior gills. Note the similar values of activity for all crabs with a mass less than approximately 25 g. GDH (glutamate dehydrogenase) activity was the only enzyme that did not scale with crab weight in anterior (*C*) or posterior (*D*) gills. AK (arginine kinase) was the only enzyme that scaled differently in the two gill types, and its activity was independent of body mass in anterior gills (*E*) while scaling negatively with body mass in posterior gills (*F*).

relative to anterior gills, only HCoADH is specific to a class of metabolic fuel molecules. This may indicate that fatty acids are oxidized specifically to support osmoregulation in posterior gills. This is supported by evidence that adult blue crab gills have a greater potential for fatty acid oxidation than does heart or locomotor muscle, but the latter tissues have a higher potential for glucose and amino acid oxidation (Walsh and Henry 1990). The activity of HK and GDH was not different in anterior or posterior gills, which suggests that the contributions of glucose and amino acid oxidation to energy metabolism are the same in respiratory and ion-transporting gills. However, it has generally been thought that at low salinity, elevated amino acid oxidation in gills is important in the regulation of both blood osmolarity and cell volume (Gilles 1979). In concordance with this idea, Pressley and Graves (1983) demonstrated that rates of glucose and amino acid oxidation were increased in posterior gills of the blue crab at reduced salinity. Despite the fact that the posterior gills are principally responsible for active ion transport, some studies have found that anterior and posterior gills show similar increases in rates of oxygen consumption following transfer to low salinity (Engel et al. 1975; Piller et al. 1995). It is possible that under hyposmotic conditions, both gill types oxidize glucose and amino acids, while posterior gills preferentially use fatty acids as metabolic fuels, perhaps to help facilitate active ion pumping.

Arginine kinase was the only enzyme that had a different scaling pattern in anterior and posterior gills (Fig. 2*E*). AK catalyzes the reversible transfer of a phosphoryl group from arginine phosphate to ADP, forming ATP, and it is known to serve as a temporal ATP buffer during transitions from low to high ATP demand (reviewed in Ellington 2001). In this role, AK operates near equilibrium, meaning that the vast majority of ATP formed will pass through the AK reaction. We examined this enzyme because AK has been shown to be upregulated twofold at low salinity in adult posterior gills of blue crabs (Kotlyar et al. 2001). We also observed a nearly twofold increase in AK activity in posterior gills of adult blue crabs at low salinity, but this increase was not significant, and there were no salinity effects in smaller crabs (Fig. 2*E*).

Although salinity induction of AK was not apparent, the different scaling pattern in anterior and posterior gills warrants further discussion. In anterior gills, AK was independent of body size, meaning that the amount of enzyme necessary to temporally buffer ATP was the same in juveniles and adults. However, AK activity in juveniles is much higher in posterior gills than anterior gills, while in adults AK activity is similar in the two types of gills (Figs. 2*E*, 3*A*). Near-equilibrium enzyme activities are often considered to be noninformative as indicators of metabolic flux. However, Suarez (1998, 2000) has pointed out that while maximal activities of these enzymes must be in great excess in order to maintain a given metabolic flux rate, maximal activity is still matched to flux rate. Thus, the scaling pattern indicates that in juveniles, the osmoregulatory posterior gills have a much greater potential for ATP flux than do the respiratory anterior gills. An alternative explanation for the posterior gill AK data stems from the similarity of this enzyme's scaling pattern to that of other oxidative enzymes (HCoADH and CS). This may indicate that AK flux is functionally coupled to oxidative phosphorylation and serves a spatial ATP buffering role in this tissue (Ellington et al. 2001). An energy transport function of AK has been previously examined, but this role is usually thought to be important when the sites of ATP consumption are distant from the sites of production, such as in the flagella of primitive sperm (Ellington and Kinsey 1998) or protozoans (Noguchi et al. 2001).

An unexpected feature of the observed scaling pattern was that all of the enzymes had activities that were constant in juvenile crabs across at least a 250-fold range in body mass (0.1–25 g). While aerobic metabolism typically scales negatively with body size (Schmidt-Nielsen 1984), activities of enzymes involved in aerobic pathways have scaling patterns that are tissue and species specific. Mass-specific activities often decrease with body size and have a scaling exponent with an absolute value that is less than or equal to the value of -0.25 usually observed for specific metabolic rate (Somero and Childress 1980, 1990; Emmett and Hochochka 1981; Schmidt-Nielsen 1984; Goolish 1991; Mayrand et al. 1998). However, size-independent activities of aerobic enzymes have also been observed in fish brain (Somero and Childress 1980, 1990) and fish muscle (Burness et al. 1999; Norton et al. 2000). Those enzymes that were size dependent in crab gills were not linear on log-log plots, but rather had a dramatic decrease in activity in adult crabs (Fig. 4*A*, 4*B*, 4*F*). This scaling pattern has also been observed for Na^+-K^+ ATPase activity in *C. sapidus* gills over a similar range of body mass (Li and Roer 2001), suggesting that the capacity for ATP production, as measured in this study, is matched to the ion-transporting ATP demand.

However, we do not know the physical basis for the unusual scaling pattern. Crab gill weight scaled with body weight according to the above allometric equation (Fig. 1), although to our knowledge, the scaling of blue crab gill surface area, and specifically, the osmoregulatory area, has not been examined. Detailed morphometric analyses have shown that fish gill surface area increases with body mass with a scaling exponent ranging from about 0.8 to 1.0 (Schmidt-Nielsen 1984). We therefore do not believe that the scaling pattern is the result of a dramatic reduction in relative gill surface area in the adult crabs. This does not preclude the possibility that the difference in activities between large and small animals may reflect changes in the density or size of "patches" of transport epithelia (Aldridge and Cameron 1982; Towle and Kays 1986) instead of a difference in the enzyme activity per cell. However, the reduction of enzyme activities in adult crabs occurred in anterior and posterior gills (except for AK), suggesting that the reduction is not associated strictly with a change in transporting epithelial cells, since these cells are principally found in posterior gills. In addition, some enzymes were size independent, which is unlikely if there was a dramatic change in the composition of gill cell types as crabs grew to adult size. Although the data here were presented as activity units per wet weight, the same pattern was found for the Na^+-K^+ ATPase when expressed in enzyme units per (mg) protein (Li and Roer 2001). This suggests that size dependence of gross morphological variables that may bias measurements of wet weight, such as cuticle thickness, would not change the general pattern observed.

In summary, our data suggest that acclimation to low salinity does not induce drastic increases in the metabolic potential for ATP production. Juvenile crabs have higher posterior/anterior gill activity ratios, which indicates that osmoregulatory costs may be somewhat greater in smaller crabs. However, the ability to increase activity to meet osmoregulatory metabolic needs appears to be slightly more pronounced in gills from adult crabs. Perhaps small crabs maintain a high metabolic potential in gills because their high SA : V makes them particularly susceptible to the short-term salinity changes that are common in estuaries. In adult crabs transferred to hyposaline water, the reduction in blood osmolarity occurs over a protracted time course of 24 or more hours (Tagatz 1971). Adult crabs may therefore have sufficient time to upregulate metabolic enzymes in response to osmotic stress, which allows them to maintain relatively low activities at higher salinities. The expectation is that fluctuations in blood osmolarity would be much faster in juveniles, based on SA : V, which may mandate that juveniles always possess a high capacity for ion pumping.

In this context, juveniles could be considered to be preacclimated to hyposmotic salinities. While we focused on the ATP production capacity, it is debatable whether ion pumping associated with hyperosmoregulation at low salinity represents a sizable energetic challenge to the organism as a whole. The posterior, ion-transporting gills (6 and 7) of the blue crab comprise only about 1% of the total body weight in small juveniles and approximately 0.3% of the adult body weight. In addition, the activities of metabolic enzymes tend to be considerably higher in other tissues, such as muscle and heart, than in gill tissue (Walsh and Henry 1990). Therefore, it is probably unlikely that a tissue that constitutes a small fraction of the total body weight and has relatively low metabolic potential would make a substantial contribution to whole-body oxygen consumption.

A number of previous studies have shown increases in whole-animal oxygen consumption and other respiratory parameters in blue crabs following exposure to hyposmotic salinities (King 1965; Laird and Haefner 1976; Sabourin 1984). However, recent work by McGaw and Reiber (1998) has shown that the greatest increase in cardiac output associated with exposure to low salinity in the adult blue crab is directed through the sternal artery, which supplies the legs and mouth parts. This enhanced perfusion is related to behavioral responses to low salinity, such as increased flicking and cleaning of the antennae, and not to osmoregulatory ion pumping per se. So, while the whole-organism response to low salinity may be energetically costly, the active uptake of ions via the posterior gills may not represent a substantial energetic challenge.

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