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Environment drives physiological variability in the cold seep mussel *Bathymodiolus childressi*

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Abstract

The ability of an organism to respond to changes in its environment depends upon its short-term physiological plasticity and the constraints of its genetic makeup. At hydrothermal vents and cold seeps, the spatially variable physiological characteristics of symbiont-bearing animals are often assumed to reflect short-term physiological adjustments to a patchy and dynamic chemical environment. However, the extent to which these spatially variable responses represent fixed characteristics unique to animals inhabiting the different environments (such as might arise from genetic differentiation) has not been tested. The seep mussel *Bathymodiolus childressi* depends upon methanotrophic bacteria for the bulk of its nutrition and inhabits a range of environments where it displays varying growth and body condition. In this study, we first investigated the multiscale environmental and physiological variability of *B. childressi* by measuring dissolved gas concentrations and mussel body condition in 12 mussel beds at four geographically distinct sites. Brine-dominated seeps tended to have higher methane and sulfide concentrations and host mussels of better body condition than petroleum-dominated sites. Then, using two transplant experiments, we evaluated whether local environmental conditions or stock effects determined the observed differences in growth and body condition of *B. childressi.* In all cases, mussels transplanted to new sites acquired or nearly acquired the characteristics of their host population, illustrating the primary role of the environment in determining the physiological characteristics of resident mussels. However, mussels from different sites sometimes responded differently to the same environment, suggesting stock-related effects also play a role in the spatial variation observed in the physiology of chemosynthetic fauna.

The response of an organism to environmental perturbation depends both upon its morphological and physiological plasticity and upon the constraints of its genotype. Marine bivalves alter many characteristics (including metabolic rate, growth, and biomass) in response to a range of environmental factors including temperature (Widdows 1973; de Vooys 1976), nutrient availability (Riisgard and Randlov 1981; Frechette and Bourget 1985), pollution (Viarengo and Canesi 1991), competition and predation (Seed 1969; Peterson and Beal 1989; Ardisson and Bourget 1991), and parasitic infection (Perez Camacho et al. 1997). However, different bivalve populations often respond differently to a given set of environmental conditions, reflecting the effects of genetic differentiation on organism response. Cold hardiness, growth, mortality, and biomass of bivalves have all been shown to be under some level of genetic control (Dickie et al. 1984; Mallet et al. 1986, 1987).

At deep-sea hydrothermal vents and cold seeps, heterogeneity of the chemical and physical environment has been cited almost exclusively to explain the patchy distributions and the variable physiological responses of resident chemosynthetic fauna (Hessler et al. 1985; Smith 1985; Fisher et al. 1988; MacDonald et al. 1990*a*; Nix et al. 1995; Barry et al. 1997; Shank et al. 1998; Smith et al. 2000). On the upper Louisiana slope of the Gulf of Mexico, the bivalve *Bathymodiolus childressi* is one of the most abundant and widespread species colonizing patchily distributed hydrocarbon seeps. *B. childressi* depends upon methanotrophic endosymbionts for the bulk of its nutrition (Childress et al. 1986; Fisher and Childress 1992; Streams et al. 1997) and can grow with methane as a sole carbon and energy source (Cary et al. 1988). Nix et al. (1995) found positive correlations between methane concentrations and *B. childressi* growth rates in situ and further suggested that toxic sulfide and hydrocarbon levels may negatively influence the growth and body condition of this mussel. Smith et al. (2000) suggested that seeps dominated by supersaturated brine express higher methane concentrations, lower toxic sulfide concentrations, and lower abundances of hydrocarbons and so support mussels with greater growth and better body condition than seeps dominated by petroleum. Although these studies have shown strong correlations between environmental characteristics and the physiological responses of *B. childressi*, no study has determined whether the observed variation represents short-term physiological responses to current conditions (here environment effects) or fixed responses unique

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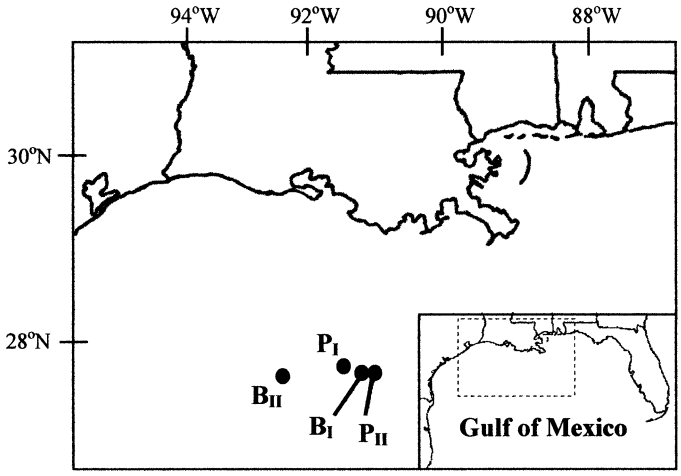

Fig. 1. Map of the Gulf of Mexico showing the locations of the four seep sites examined in this study.

to the mussels inhabiting the different environments such as might arise from genetic differentiation (here stock effects).

This study evaluates the environmental and physiological variability of *B. childressi* and determines the importance of environment and stock to the growth and body condition of this dominant seep species. First, we investigated whether environmental characteristics (primarily methane) and mussel body condition vary significantly between two different seep types (brine dominated vs. petroleum dominated). The level of genetic differentiation among *B. childressi* from different geographic locations remains unknown. However, based upon the results of previous investigations and the first part of this study, we identified two putative mussel stocks: one fast growing with a better body condition and one slow growing with a worse body condition, inhabiting brine-dominated and petroleum-dominated sites, respectively. In the second part of this study, we transplanted mussels between petroleum-dominated and brine-dominated sites in order to test whether environment or stock was responsible for the observed differences in mussel growth and body condition.

Methods

Study sites and sampling design—This study included sampling at four geographically distinct seep sites within the Minerals Management Service (MMS) Green Canyon and Garden Banks leasing blocks on the upper Louisiana slope of the Gulf of Mexico (Fig. 1). Two of these sites were designated petroleum sites and two were designated brine-pooling sites based upon descriptions in MacDonald (1998) and are hereafter referred to as petroleum-dominated and brine-dominated seep types, respectively.

Bush Hill, a petroleum-dominated site (hereafter referred to as P_I for petroleum-dominated site I) as indicated by the active release of methane bubbles and large oil globules from the sediment, is located at 27°47′N, 91°30′24″W at a depth of 540 to 580 m at the border of the MMS Green Canyon leasing blocks 184 and 185 (Brooks et al. 1989). The main portion of P_I (~10,000 m^2) supports a large number of vestimentiferan tubeworm assemblages and numerous mussel beds ranging in size from 1 to 20 m^2. Additional scattered mussel beds and tubeworm clumps can be found in the 120,000 m^2 surrounding the main site. The second petroleum-dominated seep (P_{II}) is located at 27°44.7′N, 91°13.3′W at a depth of ~540 m within the MMS Green Canyon leasing block 234 (Brooks et al. 1989). P_{II} covers an area of perhaps several square kilometers, and the central portion of this site supports an abundance of vestimentiferan aggregations and several mussel beds. Actively bubbling methane has commonly been observed in these mussel beds, and much of the sediment is oil stained (Fisher pers. obs.).

Brine Pool NR1, a brine-dominated seep (hereafter referred to as B_I for brine-dominated site I), is located at 27°43′24″N, 91°16′30″W and a depth of 650 m within the MMS Green Canyon leasing block 233 (MacDonald et al. 1990*b*). B_I is characterized by a large pool of brine ~22 m in length and 11 m wide with a salinity of 120 g kg^{-1} (MacDonald et al. 1990*b*). The pool is surrounded by a single continuous mussel bed, varying in width from 3 to 7 m from the inner edge of the brine and covering an area of ~540 m^2 (MacDonald et al. 1990*b*). The second brine-dominated seep (B_{II}) is located at 27°37′N, 92°11′W and a depth of ~670 m within the MMS Garden Banks leasing block 425 (MacDonald 1998). B_{II} supports a patchy distribution of high-density mussel beds and very few vestimentiferans. Unlike B_I, this site does not have a distinct well-developed brine pool but rather appears to be in the early stages of development (MacDonald 1998).

Differences between seep types, sites, and beds—Water among mussel beds from each of the four sites was sampled using the Johnson Sea Link II manned submersible (Harbor Branch Oceanographic Institute) in July 1997 and July 1998. Prior to the animal collections at each mussel bed, three to five water samples were collected within the bed, 2.5 cm beneath the top surface of the mussel shells, using equipment and methodology described by Nix et al. (1995) and Smith et al. (2000). On board the ship, all samples were processed using a modified gas chromatograph that allowed the simultaneous quantification of methane, total sulfide (the sum of S^{2-}, HS^-, and H_2S), and oxygen (Childress et al. 1984). Owing to impurities in the carrier gas used in 1997, oxygen and sulfide quantification were unreliable, but these values should be internally consistent since they were all obtained using the same carrier gas. One-way analysis of variance (ANOVA) was used to investigate the influence of seep type (brine-dominated, petroleum-dominated) and site (within each seep type) on methane concentration. Nonparametric Kruskal–Wallis tests were used to investigate whether methane concentration varied among beds within each site. Because sulfide and oxygen concentrations were not quantifiable, these data have been summarized but not formally analyzed.

Following the completion of water sampling, mussels were collected by placing a square stainless steel ring with 23-cm high sides and a collection area of 0.164 m^2 in a position that the submersible pilot judged to be representative of the mussel bed, undisturbed, and amenable to collection (Smith et al. 2000). Once the ring was positioned, all mussels within it were collected by scooping them into a

temperature-insulted box on the front of the submersible. Five mussel beds were sampled at P_I, and two mussel beds were sampled at each of P_{II} and B_{II}. B_I hosts a single continuous mussel bed, but due to the very large size of this bed, samples were collected from four different areas that, for the purposes of this study, are considered independent of each other.

On board the ship, the shell lengths of all living mussels were measured, and six to twelve mussels from each collection were prepared for determination of two measures of body condition: condition index and water content. Condition index (a measure of size-specific mass) and water content are often used as indicators of bivalve body condition because tissue mass is free to fluctuate within the relatively fixed volume of the shell in response to environmental quality (Crosby and Gale 1990). Here we analyze the residuals of the mass : volume regression as a measure of condition index instead of calculating the commonly used mass : volume ratio (Crosby and Gale 1990) because the latter often varies with animal size (Jakob et al. 1996).

In the lab, the solid tissue of each individual was homogenized, and three subsamples of the tissue homogenate were weighed wet and dried to a constant mass at 60°C (approximately 48 h). The subsamples were then combusted at 500°C, and the total ash-free dry weight (AFDW) of solid tissue for each individual was then calculated from the total wet mass of its original homogenate. Tissue water content was calculated as the proportion of total individual wet weight accounted for by internal water. Shell volumes were calculated from the mass of sand needed to fill the shells (Nix et al. 1995; Smith et al. 2000).

Mass was log transformed and water content was arcsine transformed ($2 \times \arcsin [y^{1/2}]$) prior to analysis to normalize and homogenize error variances. Mussel masses from collections were compared using a fully nested mixed model analysis of covariance (ANCOVA) (JMP, SAS Institute) with seep type, sites of a seep type, and mussel bed within site as factors and shell volume (log transformed) as a covariate. For simplicity, the body condition measure using mass is hereafter referred to as condition index because volume was used as a covariate in the model to adjust all comparisons for animal size. Water content was analyzed similarly using mixed model ANOVA.

Environment versus stock experiments—Based on the results of two previous studies (Nix et al 1995; Smith et al. 2000), we identified two potentially different stocks for further investigation: (1) the apparently fast growing, better body condition mussels associated with brine-dominated sites and (2) the apparently slow growing, worse body condition mussels associated with petroleum-dominated sites. To evaluate the contribution of environment and stock effects to the observed differences between brine-dominated and petroleum-dominated sites, two transplant experiments were conducted (one between P_I and B_I and one between P_{II} and B_I) (Fig. 2). At each of the two petroleum-dominated sites (P_I and P_{II}), a mussel bed was chosen haphazardly to act as a donor of mussels transplanted to and as a host to mussels transplanted from the brine-dominated site (B_I). Because the brine-dominated site (B_I) hosts a single continuous

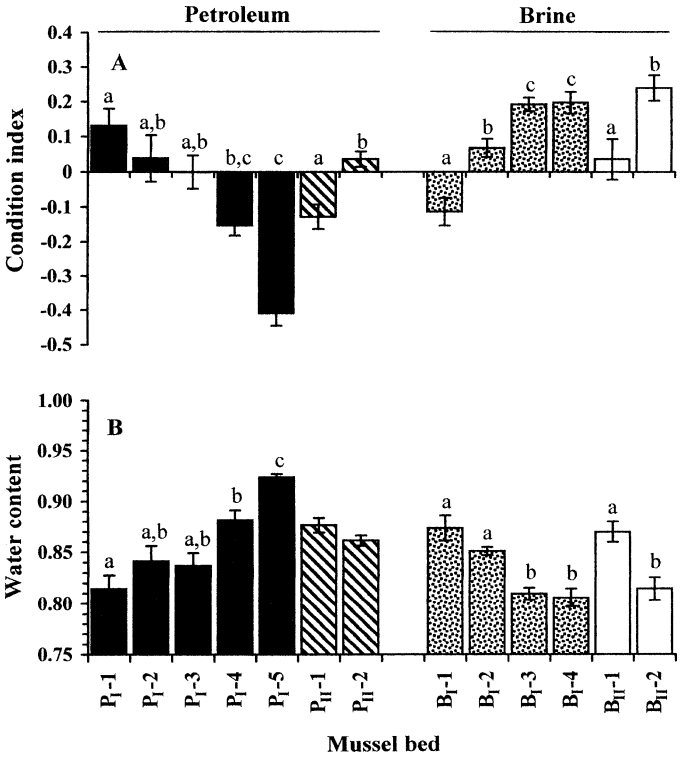

Fig. 2. Two measures of mussel body condition in 12 mussel beds from four sites: (A) condition index (residuals of the log–log regression between mass and volume) and (B) water content as a proportion of whole animal shell-free wet weight. For purposes of comparing condition index, residuals of the mass : volume regression (log [mass] = 1.02 log [volume] − 1.07) were calculated. Different letters indicate beds having significantly different condition indices or water contents within each of the four sites based on Tukey's pairwise comparisons.

bed of mussels, two different areas within the bed were chosen haphazardly to act as a donor of mussels transplanted to and as a host to mussels transplanted from each of the petroleum-dominated sites (P_I and P_{II}). That portion of the mussel bed at B_I used in transplants with P_I is hereafter referred to as just B_I and that portion used in transplants with P_{II} is hereafter referred to as B_I'.

Each reciprocal transplant included four classes of individuals: tagged controls, tagged transplants, 1997 untagged controls, and 1998 untagged controls. Tagged controls were individuals collected from a bed, measured, tagged, and returned to their bed of origin. Tagged transplants were those individuals collected, measured, tagged, and deployed at a new host bed. Untagged controls were additional individuals collected from a host bed in 1997 or 1998 that were never deployed, but rather were used to estimate nonmanipulated mussel body condition at each location used in the experiments.

In July 1997, mussels were collected by scooping them into a temperature-insulated collection box for transport to the surface. In a cold room (~8°C) on board the ship, 300 live individuals from each mussel bed were measured and then marked by gluing (Loctite 441) color-coded, numbered larval fish tags to the umbo. A subset of six to eight addi-

Table 1. Mean (SE) dissolved gas concentrations and mean mussel length and mass (ash-free dry weight) in 13 mussel beds from four sites.

Site	Bed	Methane (mmol L^{-1})	Sulfide (mmol L^{-1})	Oxygen (mmol L^{-1})	Length (mm)	Mass (g)
P_I	1	0.038 (0.021)	0.001 (0.001)	0.057 (0.021)	59.2 (7.7)	2.40 (0.85)
	2	0.000 (0.000)	0.000 (0.000)	0.051 (0.003)	79.4 (5.2)	5.34 (1.41)
	3	0.003 (0.003)	0.004 (0.001)	0.106 (0.013)	75.2 (5.7)	3.30 (0.85)
	4	0.000 (0.000)	0.000 (0.000)	0.226 (0.048)	65.1 (5.0)	1.39 (0.31)
	5	0.000 (0.000)	0.000 (0.000)	0.276 (0.101)	68.9 (4.5)	0.98 (0.17)
P_{II}	1	0.000 (0.000)	0.000 (0.000)	0.081 (0.030)	53.8 (4.0)	0.88 (0.23)
	2	0.035 (0.016)	0.001 (0.001)	0.129 (0.012)	62.4 (3.8)	1.69 (0.37)
B_I	1	0.019 (0.001)	0.000 (0.000)	0.086 (0.005)	88.6 (13.3)	4.79 (1.38)
	2	0.794 (0.069)	0.042 (0.027)	0.235 (0.066)	75.0 (10.4)	3.92 (1.25)
	3	1.483 (0.351)	0.100 (0.075)	0.111 (0.056)	75.4 (5.5)	5.08 (1.00)
	4	0.433 (0.253)	0.001 (0.001)	0.126 (0.007)	82.3 (4.3)	5.78 (1.06)
B_{II}	1	0.452 (0.173)	0.000 (0.000)	0.101 (0.012)	104.3 (5.9)	7.46 (0.61)
	2	—	—	—	72.8 (11.3)	7.15 (2.46)

tional individuals representative of the size range of animals in the collection (1997 untagged controls) was processed for determination of two measures of body condition: condition index and water content. Immediately prior to the launch of the submersible (less than 18 h after collection), 300 marked mussels (150 tagged controls and 150 tagged transplants) were placed in temperature-insulated deployment containers filled with chilled seawater (~8°C). At the host site, the lids were removed from the containers and the mussels were emptied into the space cleared by the original collection.

In July and August 1998, deployed mussels were recollected (recovery rate: 24–46%) and their shell lengths were measured. Shell growth was calculated as the change in length between 1997 and 1998 and was standardized to yearly growth based on the length of time between deployment and collection (0.926–0.995 yr). A subset of six to eight unmarked individuals (1998 untagged controls) and six to eight each of the tagged controls and the tagged transplants was processed for determination of body condition as above. All retrieved tagged shells containing no tissue were measured and recorded as having died during the deployment period.

In each of the two transplant experiments, three basic tests were performed using full two-way ANOVA or ANCOVA on those individuals collected alive and intact: (1) the effect of collection year and site on mussel body condition (1997 untagged controls vs. 1998 untagged controls), (2) the effect of tagging and site on body condition (1998 untagged controls vs. tagged controls), and (3) the simultaneous effects of site and stock on mussel growth and body condition (tagged controls vs. tagged transplants). For condition index, mass (log transformed) was analyzed using ANCOVA with log(volume) as a covariate. Water content (arcsine transformed) was analyzed using ANOVA. Growth was transformed using the function $\log_{10}(y + 1)$ and was analyzed using ANCOVA with initial shell length (log transformed) as a covariate. If the main effects of collection year or tagging or their interaction with site were significant in the first and second tests, pairwise comparisons between years or between tagged and untagged controls were performed within each site using *F*-tests. If a main effect of site or stock or their interaction was significant in the third test, planned comparisons were made between transplanted mussels and the tagged controls of their host bed and between transplanted mussels and the tagged controls of their origin bed using *F*-tests. Within each experimental replicate (P_I/B_I transplant or P_{II}/B_I' transplant) *p* values were adjusted for multiple comparisons using the sequential Bonferroni method (Rice 1989). In the P_{II}/B_I transplant, growth of tagged transplants could not be directly compared to their origin populations using ANCOVA because the interaction between length and site was significant ($p = 0.001$). To compare the P_{II}/B_I transplant growth to origin populations, data were divided into 10-mm size classes based on initial length and compared within size classes using paired *t*-tests.

To determine whether host environment had a size-specific effect on the recovery of different stocks, chi-square tests were used to determine whether the numbers of recovered versus nonrecovered animals were independent of whether the animals were large or small. Nonrecovered individuals in this analysis include animals that were collected dead, that moved between 1997 and 1998, and those missed during recovery in 1998. Animals of each stock at each host site were assigned large and small designations based upon whether they fell above or below the median size at the time of deployment in 1997. To investigate environment and stock effects on patterns of mortality, chi-square tests were used to compare the number of dead and living transplanted individuals collected in 1998 to that for tagged controls from the host and the origin site. Fisher's exact test was used to determine whether the numbers of dead and living mussels (of each stock at each site) were independent of whether the individuals were large or small.

Results

Spatial variability—Observed environmental methane concentrations were not significantly different between seep types (weighted ANOVA: $F_{1,2} = 12.07$, $p = 0.074$); however, petroleum-dominated sites displayed consistently lower methane concentrations than brine-dominated sites (Table 1).

Methane concentrations were not significantly different among petroleum-dominated ($F_{1,5} = 0.40$, $p = 0.556$) or among brine-dominated ($F_{1,3} = 0.11$, $p = 0.762$) sites. Methane concentrations were significantly different between different beds within P_I ($H = 12.3$, df = 4, $p = 0.015$) and P_{II} ($H = 4.35$, df = 1, $p = 0.037$) and between different collection locations within B_I ($H = 9.49$, df = 3, $p = 0.023$). Sulfide tended to be more abundant at brine-dominated sites (detected in 6 of 14 water samples) than at petroleum-dominated sites (detected in 5 of 22 water samples), and oxygen tended to be similar between brine- and petroleum-dominated sites (Table 1). Sulfide was particularly abundant at B_I, where it was detected in 6 of 11 water samples.

Fully nested, mixed model ANCOVA/ANOVA showed that body condition differed between the mussels at the two seep types (condition index, $F_{1,83} = 3.68$, $p = 0.0808$; water content, $F_{1,86} = 18.96$, $p = 0.0345$) and between mussel beds within individual seep sites (condition index, $F_{9,83} = 19.19$, $p < 0.0001$; water content, $F_{9,86} = 15.93$, $p < 0.0001$) but not between different sites of a seep type (condition index, $F_{2,83} = 0.38$, $p = 0.6590$; water content, $F_{2,86} = 0.06$, $p = 0.9377$) (Table 1; Fig. 2). Estimates of the variance components indicate differences between different beds within individual sites account for 66.5% and 66.3% of the variability in condition index and water content, respectively. Differences between sites of a seep type accounted for 0% of the variability in both parameters. These results support the two previous studies of Nix et al. (1995) and Smith et al. (2000) and indicate that our designations among these three sites (fast growing, better body condition mussels of B_I, slow growing, worse body condition mussels of P_I and P_{II}) were robust.

Transplant experiments—Significant differences between collection years were found only in the P_I/B_I transplant. In the P_I/B_I transplant, individual condition index was significantly higher in 1998 than in 1997 in the overall model (Table 2), but the between year difference was only significant at B_I ($t = 7.69$, $p < 0.0001$) (Fig. 3). Water content was significantly lower in 1998 than in 1997 at B_I ($t = 3.59$, $p = 0.0049$) but not at P_I (Table 2; Fig. 3). The difference between years at B_I but not P_I produced the significant interaction between site and year. Significant differences between tagged and untagged controls were found only in the P_{II}/B_I' transplant beds where condition index was significantly higher in untagged mussels (Table 2; Fig. 3). When this apparent tagging effect was investigated within each site, untagged animals had a higher condition index at P_{II} ($t = 2.47$, $p = 0.0357$), but tagged and untagged animals were not significantly different at B_I' ($t = 1.33$, $p = 0.2117$).

Site showed a significant effect on growth and body condition in both transplants (Table 3; Figs. 3 and 4). Stock also influenced mussel response to transplantation, but its effect was not consistent between the two transplant experiments. In the P_I/B_I transplant, stock significantly influenced condition index and water content but not growth, while in the P_{II}/B_I' transplant, stock significantly influenced growth and condition index but not water content (Table 3; Figs. 3 and 4). In all cases, however, site accounted for far more of the overall variance than did stock. In general, mussels transplanted from brine-dominated to petroleum-dominated sites tended to show lower growth, lower condition index, and higher water content such that they acquired the characteristics of the host population or fell in between the host and origin populations (Tables 4 and 5; Figs. 3 and 4). Mussels transplanted from petroleum-dominated to brine-dominated sites tended to show greater growth, higher condition index, and lower water content such that they fell in between or performed better than the host and origin populations (Tables 4 and 5; Figs. 3 and 4).

In analyses of size-specific recovery and mortality rates, large and small designations were used instead of specific size classes because low recovery rates (24–46%) made application of chi-square tests to size class data impossible

Table 2. Results of the two-way ANOVA/ANCOVAs analyzing the effect of collection year and site and the effect of tagging and site on the condition index and water content of mussels used in the two transplant experiments. The simultaneous effects of year and site were determined using the untagged control mussels collected in 1997 and 1998. The simultaneous effects of tagging and site were determined using the tagged and untagged controls collected in 1998.

	B_I/P_I transplant experiment				B_I'/P_{II} transplant experiment			
	Condition index		Water content		Condition index		Water content	
Source	df	*p*	df	*p*	df	*p*	df	*p*
Year and site								
Year	1	0.0002	1	0.0576	1	0.5242	1	0.5926
Site	1	0.3199	1	0.0779	1	<0.0001	1	<0.0001
Year × site	1	0.0021	1	0.0017	1	0.9778	1	0.7770
Volume	1	<0.0001	—	—	1	<0.0001	—	—
Error	22		23		22		23	
Tagging and site								
Tag	1	0.9376	1	0.6690	1	0.0085	1	0.1130
Site	1	0.0128	1	0.1096	1	<0.0001	1	<0.0001
Tag × site	1	0.8401	1	0.5362	1	0.0894	1	0.5605
Volume	1	<0.0001	—	—	1	<0.0001	—	—
Error	20		21		21		22	

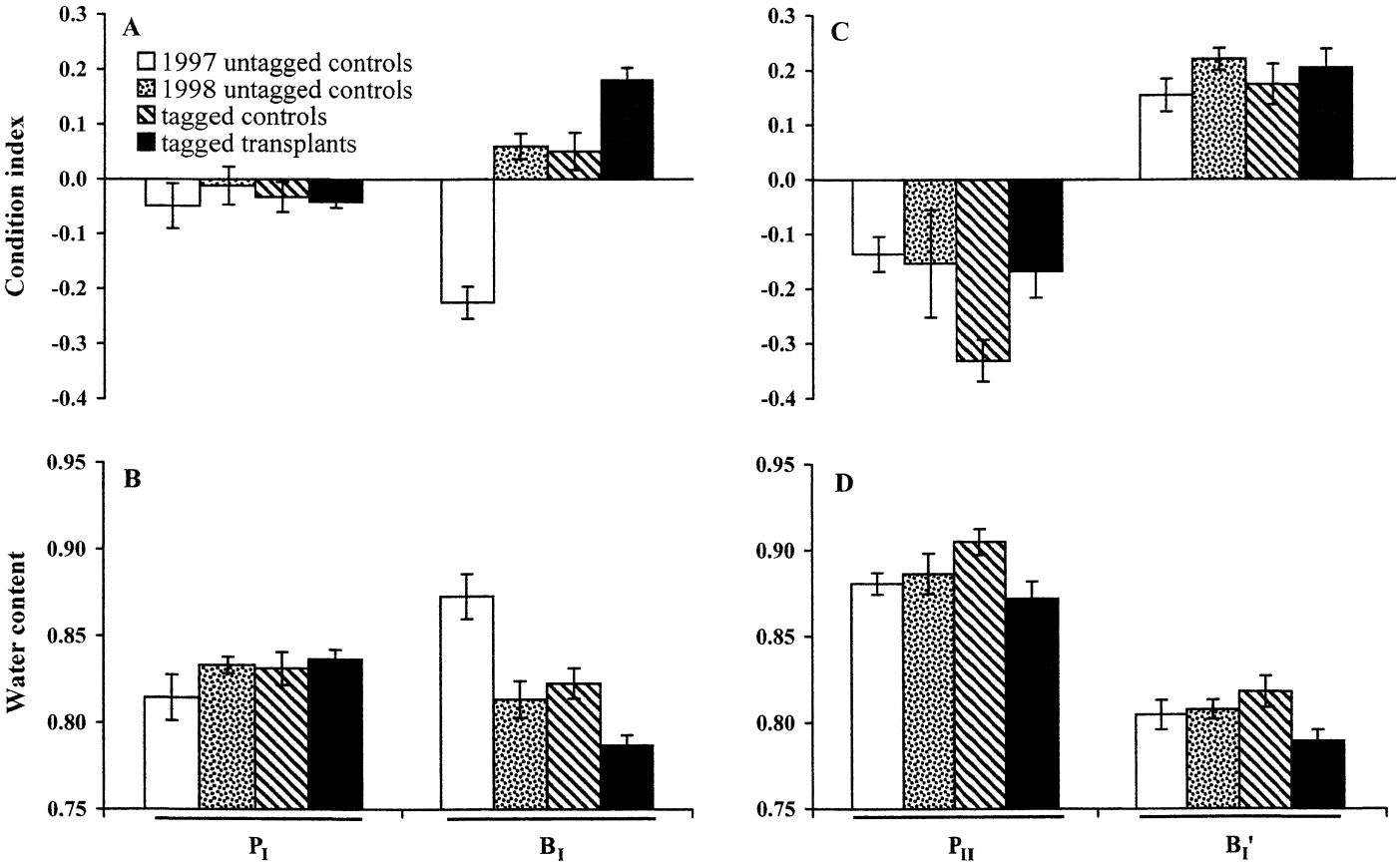

Fig. 3. Condition index and water content in 1997 and 1998 untagged controls, mussels tagged and placed back at their site of origin (tagged controls), and mussels tagged and placed at a new host site (tagged transplants) from two transplant experiments: (A,B) P_I–B_I and (C,D) P_{II}–B_I'. For purposes of comparing condition index, residuals of the mass : volume regression were calculated from a pooled data set including all untagged controls, tagged controls, and tagged transplants from both host sites for each of the two transplant experiments (P_I/B_I transplants, log [mass] = 0.959 log [volume] − 0.788; P_{II}–B_I' transplants, log [mass] = 0.990 log [volume] − 1.02).

Table 3. Results of two-way ANOVA and ANCOVA analyses for the simultaneous effects of environment and stock on the growth, condition index, and water content of transplanted mussels. These effects were determined simultaneously using tagged controls and tagged transplants. For ANCOVA analyses of growth, the covariate size was initial mussel length; for ANCOVA analyses of condition index, the covariate ''size'' was mussel shell volume.

	Growth		Condition index		Water content	
Source	df	*p*	df	*p*	df	*p*
B_I/P_I transplant experiment						
Environment	1	<0.0001	1	<0.0001	1	0.0009
Stock	1	0.7089	1	0.0128	1	0.0139
Environment × stock	1	0.3833	1	0.0506	1	0.0588
Size (covariate)	1	<0.0001	1	<0.0001	—	—
Error	147		21		22	
B_I'/P_{II} transplant experiment						
Environment	1	<0.0001	1	<0.0001	1	<0.0001
Stock	1	<0.0001	1	0.0063	1	0.5576
Environment × stock	1	<0.0001	1	0.0019	1	0.0020
Size (covariate)	1	<0.0001	1	<0.0001	—	—
Error	110		22		23	

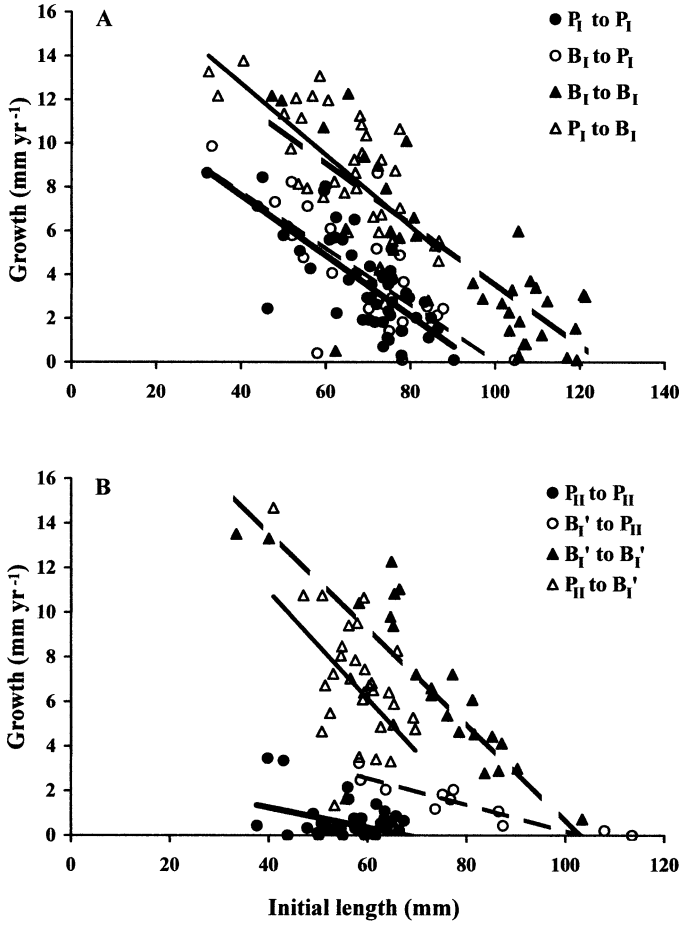

Fig. 4. Relationship between growth and initial length in tagged controls and tagged transplants in (A) the P_I–B_I transplant and (B) the P_{II}–B_I' transplant. Mussels originating from petroleum-dominated sites (P_I or P_{II}) are represented by solid lines, and mussels originating from brine-dominated sites (B_I or B_I') are represented by broken lines. Mussels tagged and placed back at their site of origin are represented by heavy lines, and mussels tagged and placed at a new host site are represented by thin lines.

(numerous blocks with expected counts less than 1). Within each stock at each site, the number of recovered versus non-recovered individuals was independent of whether the animals were large or small ($p \geq 0.148$ in all cases). Similarly, the number of dead versus living individuals of each stock at each site was independent of whether the animals were large or small ($p \geq 0.110$). Individuals transplanted from the brine-dominated site to the petroleum-dominated sites displayed significantly higher proportions of dead individuals than host or origin tagged controls (Fig. 5). Those transplanted from petroleum-dominated sites to the brine-dominated site showed no significant difference in proportions of dead individuals when compared to both the host and origin tagged controls (Fig. 5). It must be noted that these measures of mortality allow only a rough comparison since all individuals deployed were not collected. Differences in mortality may actually reflect differences in mobility between stocks, since deployed animals were free to move away from the deployment site. High estimated mortality among brine-dominated stocks at petroleum-dominated sites may result from healthier individuals moving away from the deployment area, leaving only the most unhealthy to die. Alternatively, low relative mortality estimated in petroleum-dominated stocks overall may result from individuals moving away from the deployment site before dying.

Table 4. Results of contrasts between tagged transplants and the tagged controls at the new host site and at the origin site. Growth, condition index, and water content of animals transplanted to a new site were compared to the tagged controls at that new host site and the tagged controls at their site of origin using ANCOVA (condition index and growth) or ANOVA (water content). Shown are *p* values for treatment effect. + = transplant (listed first) higher than control; − = transplant lower than control. Bold print indicates those *p*-values significant after correction for multiple comparisons using the sequential Bonferroni method.

Comparison	Growth	Condition index	Water content
B_I to P_I vs. P_I to P_I	0.267 (+)	0.636 (−)	0.600 (+)
B_I to P_I vs. B_I to B_I	**<0.001** (−)	0.016 (−)	0.222 (+)
P_I to B_I vs. B_I to B_I	0.964 (+)	0.010 (+)	**0.004** (−)
P_I to B_I vs. P_I to P_I	**<0.001** (+)	**<0.001** (+)	**0.001** (−)
B_I' to P_{II} vs. P_{II} to P_{II}	**<0.001** (+)	0.026 (+)	0.027 (−)
B_I' to P_{II} vs. B_I' to B_I'	—	**<0.001** (−)	**0.002** (+)
P_{II} to B_I' vs. B_I' to B_I'	**<0.001** (−)	0.865 (+)	0.036 (−)
P_{II} to B_I' vs. P_{II} to P_{II}	—	**<0.001** (+)	**<0.001** (−)

Discussion

The cold seep mussel *Bathymodiolus childressi* responded physiologically to the differing environments of brine-dominated and petroleum-dominated sites. Brine-dominated sites favored higher mussel growth and better body condition than did petroleum-dominated sites. When transplanted from one

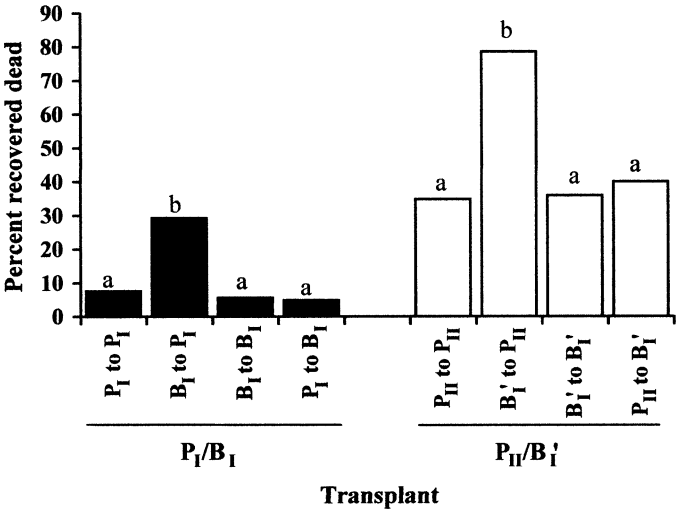

Fig. 5. Percent of tagged transplants and tagged controls retrieved dead in 1998. Within each transplant experiment (P_I–B_I or P_{II}–B_I'), different letters indicate values found to be significantly different using chi-square tests after correction for four multiple comparisons (Rice 1989).

Table 5. Mean growth (SE) of tagged transplants and tagged controls in each 10-cm size class compared using *t*-tests. Comparisons were not made within a size class if fewer than two individuals were present in both the transplant and origin populations.

Size class (mm)	Growth (cm) of			Growth (cm) of		
	P_{II} to B'_I	P_{II} to P_{II}	*p*	B'_I to P_{II}	B'_I to B'_I	*p*
40.0–49.9	12.7 (2.0)	1.0 (0.6)	0.0002	—	—	—
50.0–59.9	6.5 (0.5)	0.5 (0.1)	<0.0001	—	—	—
60.0–69.9	5.0 (0.6)	0.4 (0.1)	<0.0001	—	—	—
70.0–79.9	—	—	—	1.7 (0.2)	5.0 (1.1)	0.0271
80.0–89.9	—	—	—	0.8 (0.3)	4.1 (0.5)	0.0012

of these environments to the other, *B. childressi* consistently acquired characteristics more similar to the mussels native to their new environment than of their original environment. Like studies performed on shallow water mussels (Dickie et al. 1984; Mallet et al. 1987), this demonstrated that short-term adjustments to current environmental conditions account for a majority of the physiological variability observed among mussels inhabiting these seep sites.

The differing growth and body condition of *B. childressi* among sites and beds likely reflect complex interactions between various physical (such as methane availability, sulfide concentrations, hydrocarbons, and brine) and biological factors (such as competition, predation, and parasitism). Smith et al. (2000) assessed the physiological and environmental characteristics of mussels from a brine-dominated site (B_I in this study) and compared the results to a similar study at several petroleum-dominated sites (Nix et al. 1995). The authors proposed that, unlike mussels inhabiting petroleum-dominated sites, mussels inhabiting brine-dominated sites are not methane-limited and so grow faster, attain larger adult sizes, and display a better body condition. Smith et al. (2000) also showed that portions of the Brine Pool NR1 mussel bed with high methane concentrations generally lacked sulfide and supported mussels of greater growth and better body condition, while portions with low methane availability had sulfide present and supported mussels of lower growth and worse body condition. Nix et al. (1995) associated greater growth and better body condition with mussel beds having greater methane concentrations but lacking potentially toxic sulfide and crude oil. Our data support previous observations that associate greater mussel growth and better body condition with areas of higher methane levels, but in the current study, sulfide tended to be more abundant in those areas with higher methane concentrations.

At these cold seeps, *B. childressi* faces high parasitic infection intensities (e.g., *Bucephalus* and chlamydia/rickettsia) and infections that vary significantly in kind and intensity among sites and among beds (Powell et al. 1999). Trematode (*Bucephalus*) infection intensities reached such high levels within several beds at two petroleum-dominated sites (P_I and P_{II} in this study) that the bulk of the tissue of many mussels was displaced by this parasite and the hosts were reproductively inactive (Powell et al. 1999). However, this parasite was not observed to occur at brine-dominated sites (B_I and B_{II} in this study). Certainly, the striking differences in parasite infections could account for some of the variation in *B. childressi* growth and body condition found in this study; however, like the influence of other biological factors (competition, predation, etc.) this remains to be explicitly investigated.

Individual *B. childressi* from brine-dominated sites are likely to have a higher fitness and contribute more to the maintenance of upper Louisiana slope *B. childressi* populations than individuals from petroleum-dominated seeps. The greater growth of mussels at brine-dominated seeps would tend to increase their fitness through effects on size-dependent reproduction and mortality; for example, number and size of offspring, competitive ability, and predation pressure are all often correlated with body size in marine mollusks (Paine 1976; Peterson 1986; Petraitis 1995). The better body condition of these mussels can also be linked to fitness through its influence on reproductive capabilities, foraging success, and reactions to environmental stress (Jakob et al. 1996). Additionally, the fitness of the petroleum-dominated seep mussels should be further depressed by apparent parasitic castration of at least some of its members. However, because *B. childressi* is capable of responding rapidly (within 1 yr in some cases) relative to its lifespan of at least several decades (Nix et al. 1995), the fitness of mussels at petroleum-dominated seeps may increase if environmental conditions improve.

We currently have no data concerning the genetic similarity of *B. childressi* at the different seep sites, so the significant effects of stock on *B. childressi* growth and body condition found in this study may or may not reflect actual genetic differences. The stock effects may, alternatively, reflect the rate of response of individuals to environmental change, previous exposure to environmental conditions at the bed of origin (Peterson and Black 1988), or environmentally induced changes in strategy early in development (''developmental conversion'' of Smith-Gill 1983). Some evidence suggests that 1 yr was not enough time for transplanted individuals (particularly in the P_{II}/B'_I transplant) to equilibrate with their new environments. Overall differences in mussel growth and body condition between the two host environments were greater in the P_{II}/B'_I transplant than in the P_I/B_I transplant, so individuals in the P_{II}/B'_I transplant would have had to change more in order to fully acquire the characteristics of the host bed. In the P_I/B_I experiment (where the starting difference was less), transplanted individuals largely adopted the characteristics of the host bed, except that those moved from the less favorable petroleum-dominated to the brine-dominated environment attained a better body condition than the mussels native to the brine-dominated site. In the P_{II}/B'_I experiment (where the starting difference was greater), transplanted individuals did not fully

acquire the characteristics of the host, showing growth intermediate between but significantly different than both the host and origin and body condition not significantly different than that of the host (although still intermediate between host and origin). Those transplanted from B_I' to P_{II} likely used storage compounds to maintain growth for a short time, while those transplanted from P_{II} to B_I' likely accumulated tissue before growing their shell.

As designed, this study cannot explicitly resolve the contribution of historical factors or developmental conversion to the observed stock differences. Several site-specific comparisons do suggest historical stress effects did not play a substantial role here. In the P_I/B_I transplant experiment, mussels from the petroleum-dominated site were capable of performing better in brine-dominated environments than those mussels native to the brine-dominated environment. Were historical factors driving this system, one would expect that the performance of individuals transplanted from the less favorable environment of petroleum-dominated sites (lower methane, higher sulfide, greater parasite infection intensities) would be hindered relative to the performance of individuals originating at the brine-dominated site.

Two lines of evidence suggest a genetic basis for the stock effects found in this study. First, mussels from the petroleum-dominated sites tended to rapidly acquire the better body condition of the host mussels when transplanted to the brine-dominated site and, in the case of P_I mussels transplanted to B_I, even perform better than the host. Second, in both transplants, mussels from brine-dominated sites suffered heavy mortality when transferred to petroleum-dominated sites, while mussels originating in petroleum-dominated site suffered similar mortality in both environments. Petroleum-dominated sites represent a more stressful environment likely favoring more efficient energetic pathways, while brine-dominated sites, representing a less stressful environment, likely allow less efficient individuals to persist. This would produce a greater variance in energetic efficiencies among mussels at brine-dominated sites with a lower average response in terms of tissue and shell growth. Postsettlement selective mortality provides a likely mechanism for maintaining the observed stock effects. Like other marine mussels, *B. childressi* possesses a planktotrophic, and presumably widely dispersing, larval phase (Gustafson and Lutz 1994) that would minimize the effects of geographic isolation on the genetic makeup of different populations. Under these circumstances, the environmental conditions of specific seep sites or individual beds may select for a subset of broadly available individuals of different genetic makeup. Considering the fitness differences that likely exist between brine-dominated and petroleum-dominated seep mussels, the genotypes colonizing petroleum-dominated seeps probably represent a subset of those present at brine-dominated seeps.

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