Marine Biology (1995) 122: 605–617

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Physiological ecology of a mussel with methanotrophic endosymbionts at three hydrocarbon seep sites in the Gulf of Mexico

Received: 8 November 1994/Accepted: 12 January 1995

Abstract In situ growth rates were determined, using two, 1-yr mark/recapture experiments, conducted between September 1991 and July 1993, for an undescribed mytilid, Seep Mytilid Ia, at three hydrocarbon seep sites in the Gulf of Mexico. The sites are located at depths of 540 to 730 m, approximately 27°45′N; 91°30′W, and are separated by distances of 6 to 18 miles. These seep mytilids harbor methanotrophic endosymbionts and use methane as both a carbon and energy source. The mussel habitats were chemically characterized by analysis of water samples taken from precisely located microenvironments over, among and below the mussels, using small-volume, interstitial water samplers and the "Johnson Sea Link" submersible. Substantial differences were found in habitat conditions, growth rates, and population structure for the mussels at the three sites examined. The growth rate of these seep mytilids reflects the methane concentration in their immediate habitat. Mussels at sites with abundant methane had growth rates that were comparable to shallow water mytilids at similar temperatures (5 to 8 °C) with increases in shell length up to 17 mm yr^{-1} documented for smaller mussels (< 40 mm shell length). In conjunction with measurements of growth rates, three condition indices (glycogen content, tissue water content, and the ratio of ash-free dry weight to shell volume) were used to determine the relationship between the condition of the mussels, their growth rates, and their habitat chemistry. The three condition indices were correlated with growth rate and were often significantly different between mussels in different samples.

Communicated by J.P. Grassle, New Brunswick

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Introduction

The communities which inhabit the soft-bottom deep-sea environment are typically characterized by low population densities, low biomass, and high species diversity. Growth and colonization in the deep sea are generally slow in comparison with these same processes for shallow water habitats (Grassle and Sanders 1973; Turekian et al. 1975). It has been suggested that a major limiting factor for growth and productivity in most deep-sea environments is food (Neter et al. 1990). In 1977, dense communities of organisms with low species diversity were discovered around hydrothermal vents along the Galapagos Rift (Corliss et al. 1979). It has since been shown that the dominant macrofauna harbor chemoautotrophic endosymbionts [reviewed by Fisher (1990)]. In 1984, organisms closely related to those around deep-sea hydrothermal vents were discovered at hydrocarbon seep sites in the Gulf of Mexico at depths between 500 and 1000 m (Kennicutt et al. 1985). These seep communities are similar to hydrothermal vent communities in their low species diversity and high biomass of a few symbiont-containing species (vestimentiferan tubeworms and mussels) (Hessler et al. 1985; Carney 1994).

There are several important differences between the hydrocarbon seeps and most hydrothermal vents. Seepage of reduced compounds at the seeps is diffuse and includes high levels of dissolved CH_4 (Kennicutt et al. 1985). This is in sharp contrast to the vents, where the channeling of hydrothermal fluids, often relatively low in CH_4, limits the release to relatively small point sources (Lilley et al. 1983; Welhan and Craig 1983; Merlivat et al. 1987). Another notable difference between seeps and most hydrothermal vents (cf. Guymas basin) is that relatively high levels of potentially toxic

hydrocarbons are often present in seep fluid (Kennicutt et al. 1985).

The most common species of mussel associated with hydrocarbon seeps in the Gulf of Mexico (Seep Mytilid Ia, Fisher 1993) harbors methanotrophic endosymbionts (Childress et al. 1986; Fisher et al. 1987) and the vestimentiferans, like the vent tubeworms, harbor sulfur-oxidizing endosymbionts (Brooks et al. 1987; Fisher et al. 1988). A variety of evidence, including the stable carbon isotope ratios of the tissues (Childress et al. 1986; Brooks et al. 1987), the ability to grow on methane as sole carbon and energy source (Cary et al. 1988), and the transfer of methane carbon to the host tissue through digestion of the symbionts (Childress and Fisher 1992), indicates that the mussels derive the bulk of their nutritional carbon from their symbionts.

Since the initial discovery of the hydrocarbon seep communities, roughly 24 communities with mussels and/or vestimentiferans have been visited by submersibles, and the existence of 12 others have been inferred from trawl collections along the Gulf of Mexico continental slope, at depths of 500 to 1000 m (Kennicutt et al. 1988; MacDonald et al. 1990a; MacDonald et al. 1992). Mussel beds at three sites, separated by 6 to 18 miles, were chosen for the present study. These are all found within the Green Canyon (GC) Lease Area and are referred to as Bush Hill (GC184/185, named for the abundant fields of vestimentiferans present), GC 234, and GC 272.

A primary purpose of the present study was to determine the range of growth rates of Seep Mytilid Ia in the Gulf of Mexico. Three sites were characterized with respect to mussel population structure and water chemistry. These data are used, in conjunction with results from laboratory experiments on these mussels, to estimate rates of community productivity and methane oxidation. The physiological state of the mussels at each site was determined, using the growth rates and several condition parameters, in order to determine the suitability of different habitat types for the mussels.

Materials and methods

Site descriptions

Bush Hill

The Bush Hill site is located at 27°46.96′N; 91°30.46′W in water depths of 540 to 580 m. The central portion of this site is ~10 000 m^2 in area, is characterized by numerous dense assemblages of tube worms (hence its name), and at least six substantial, and several smaller beds of Seep Mytilid Ia (personal observation, MacDonald et al. 1989). The mussel beds are irregular in shape, ranging in area from 1 to 20 m^2 and are often associated with substantial tube worm clumps. Streams of methane bubbles (Brooks et al. 1989; MacDonald et al. 1989) are present at many beds, and disturbance of the bottom often causes the release of large oil globules. Numerous tubeworm clumps and occasional mussel beds can be found over an additional area of about 120 000 m^2 on the eastern and southern flanks of the main site (MacDonald et al. 1989). The underlying sediment structure has been deformed by rising gas bubbles, and numerous carbonate outcroppings are scattered over the site (Brooks et al. 1986; Brooks et al. 1989). Two mussel beds were sampled at Bush Hill, one for the in situ growth study and the other for the quantitative ring collections. The "growth bed" was located at the base of an individual tube worm bush and the "ring collection bed" was in the middle of a tube worm clump (a large collection of bushes).

GC 234

The GC 234 site is located at 27°44.7′N; 91°13.3′W, at a depth of ~540 m. ["Mussel Beach" (MacDonald et al. 1990b) is in another area of the same lease block.] The two beds sampled are about 100 m apart and are both in close proximity to large aggregations of tubeworms, one of which completely covers an area of >200 m^2. Like Bush Hill, tube worms are the dominant fauna at this site. There are several other large mussel aggregations (5 to 15 m^2) in this area that are associated with tube worm clumps. Both white and orange bacterial mats were found in close proximity to mussel and tubeworm aggregations. As at Bush Hill, streams of gas bubbles were commonly observed in mussel beds, sediment often appeared to be heavily oil-stained, and carbonate outcroppings were common. This central area is surrounded by numerous aggregations of tubeworms which extend ~100 m to the north and 1 km to the east. The mussel bed used for the in situ growth study was surrounded by tube worms and is approximately 5 m in diameter. Areas within the bed were densely populated by small mussels (<40 mm in shell length). The ring collection bed was located at the periphery of some tube worm clumps and was ~3 m in diameter.

GC 272

The GC 272 site is located at 27°41.28′N; 91°32.45′W, at a depth of 730 m. This site is relatively small (roughly 1000 m^2) and consists of several aggregations of tubeworms and a single 15 m^2 mussel bed. The surrounding area (several km^2) has been surveyed fairly extensively over the past 3 yr (1991 to 1993). Two species of vesicomyid clams occur there, either singly or in aggregations of a few individuals. Only a few scattered mussel beds and small tubeworm aggregations have been seen in the area. The in situ growth experiment and ring collection were made on the same mussel bed, within 1 m of each other. This bed was adjacent to a large (15 m^2) aggregation of tubeworms, and dead mussel shells were scattered around the periphery.

Mussel collection

Mussels used in our study were collected, from depths of 540 to 730 m, in September 1991, August 1992, and June 1993, using the deep-sea submersible "Johnson Sea Link" (Harbor Branch Oceanographic Institution). They were placed in a temperature-insulated retrieval box mounted on the front of the submersible. On the deck of the ship, the mussels were immediately transferred to buckets of 8 °C sea water and placed in a cold room (8 °C) for all subsequent handling. Shell length and width of all mussels were measured with calipers to the nearest 0.1 mm.

A subset of each collection (6 to 20 mussels, depending on the purpose) were processed for determination of morphometric relations and condition indices. Following shell measurements, the shells were drained of sea water by prying them open a few mm. The adductor muscles were then severed over an open, pre-weighed, specimen bag, so that all internal fluid was collected. Gill, mantle, and foot tissue subsamples were taken, placed in pre-weighed

cryovials, and immediately frozen in liquid nitrogen. The remaining tissue ("bulk tissue") was removed from the shell, added to the specimen bag, and stored at −20 °C. The shells were cleaned, dried and marked for future shell volume measurements. Tissue samples were transported to Pennsylvania State University in liquid nitrogen or on dry ice and upon arrival stored in a −70 °C freezer.

Mussel densities and size distributions were determined from collections made from within a 0.5-m diameter ring placed firmly in a bed of mussels. The ring consisted of a 20-cm high band of 2-mm thick stainless steel, with three 15-cm spikes protruding below the ring to anchor it in place, and a handle for manipulation by the submersible. Mussels had been collected from the beds used for the growth studies several times in the years previous to the ring collections. To avoid making quantitative collections from impacted beds, the ring collections at Bush Hill and GC 234 were made from nearby, undisturbed beds, which were visually similar. The similarity in water samples and size ranges of mussels collected from the beds within each site support the linked interpretation of the sites as presented here. In the case of the bed at GC 272, the ring collection was made from a relatively inaccessible and therefore undisturbed portion of the same bed as that used for the growth study. Again, the similarity in water samples and size ranges support the joint interpretation of the two data sets. Placement of the collection ring was not random within the patchy, small mussel beds, but efforts were made to sample "typical" areas within each bed (the extreme periphery, obvious areas of dead shells, and mounds of mussels were avoided). After placement of the ring, essentially all mussels within the ring were collected (first by grab sampler, then with a suction sampler), and a video record was made of the ring to document the occasional mussel not collected. The mussels from each collection were placed in separate sections of the temperature insulated collection box and processed as described below. Between 0.7 and 16% of the mussels were damaged during a given collection. These were counted and measured with as much accuracy as possible.

Water sampling and analysis

Water samples were collected using specially designed samplers which allowed collection of undiluted, small volume (1 to 5 ml) samples from specific areas above, within, or below the mussel beds. Samples were taken from three levels within the mussel beds; at the top of the shells near the siphons, 5 cm below that level, and 10 cm below that level. The three water samplers used were constructed of 316 stainless steel. Water was drawn through 2-μm fritted filters (the interstitial samplers employ stainless steel filters and the "wand" sampler a plastic filter). The "wand" sampler consisted of a filter mounted on the end of a 70-cm tube fitted with a handle for the submersible. The interstitial water samplers were essentially large thumbtacks with the filters mounted below 14 cm diameter plates, above a sharpened point, and isolated from internal dead spaces by O-rings. The water was collected from a 1-cm length of filter centered either 5 or 10 cm below the plates with a flow rate of about 2 ml min^{-1}.

The filters were connected to 7 m of 0.025 cm i.d. polyetheretherketone tubing using low dead volume fittings and were plumbed into the rear compartment of the submersible through stainless steel swagelock fittings (and a stainless ball valve) such that the tubing completely penetrated the hull of the submersible and was connected to a plastic 0.15-cm diameter valve and leur lock (syringe) fitting on the inside of the compartment. The entire sampling apparatus, from filter to syringe fitting, had a dead volume of < 1.8 ml. Tubing from all three water samplers entered the rear compartment through the same ball valve which served as an emergency cut off should an inner valve fail. All three samplers were carried in quivers mounted on the work platform of the submersible and could be picked up by the submersible manipulator and used at will.

The samplers were positioned by the submersible pilots, the lines bled (2 ml), and the samples collected in 1-ml glass syringes fitted with low volume gas-tight valves. After collection of an 0.5-ml sample, the sample was stored on ice until analyzed. To minimize the spontaneous oxidation of sulfide in the water samples before analysis, the sampling syringes were primed with 0.1 ml of a degassed pickling solution. In 1992 a solution of 1*M* Na_4EDTA in 1*N* HCl was used, which inhibited the initial rate of the oxidation reaction by 65%. In 1993 this solution was replaced with a basic zinc acetate solution [5:1, 2.6% ZnAcetate (W/V):6% NaOH (W/V), (Gilboa-Garber 1971)], which precipitated the sulfide and inhibited the oxidation reaction much more effectively (>90% of the initial sulfide and oxygen were recovered after 20 h of refrigerated storage in laboratory tests). Therefore, only the 1993 environmental sulfide data are presented here. Methane data from both years are given, as these data were not affected by the change in protocol. All water samples were analyzed on board ship for ΣCO_2, CH_4, O_2, ΣH_2S, and N_2 by gas chromatography (Childress et al. 1984) within 10 h of collection.

Growth studies

Mussels were collected as previously described and the collection location was marked with a numbered float. A representative size range (~150 mussels) from each location was set aside for marking, and an additional 20 were sampled as controls. In 1991, three different marking methods were tested: (1) notching the outer margin of the shell with a file; (2) gluing a commercial color-coded, numbered larval fish tag (6.9 × 3.2 mm), near the umbo of the shell; and (3) gluing a hand-made color-coded polypaper tag (9.8 × 3.3 mm), near the umbo. Before attaching the tags, shell length and width were measured. A small area of the shell was quickly dried and lightly abraded with #220 sand paper. The tags were attached with cyanoacrylate glue (Loctite #447 and/or #401), allowed to dry for 60 s, and rinsed in two changes of cold sea water. This entire procedure required the mussels to be removed from sea water for less than 2 min. All manipulations were conducted in the cold room (8 °C). Both types of tags persisted until 1992. Only the larval fish tags were used in subsequent years. Notching was found to be unnecessarily invasive and was also discontinued after 1991. The mussels used in our study were never kept on board ship for more than 15 h.

For deployment, the mussels were placed in insulated acrylic containers filled with chilled sea water, returned to the site of collection, and released within 0.5 m of the marker float.

Marked mussels were retrieved in August 1992 and in June 1993. Twenty marked mussels and 20 unmarked mussels from each collection were processed as described above, with tissue subsamples removed from the first six. All other marked mussels were measured and re-released with additional newly marked mussels from the same collections (only the latter are presented in the growth analysis).

Transplant experiment

A transplant experiment (with two treatment levels, Transplant 1 and Transplant 2) was conducted at Bush Hill. Mussels were collected in September 1991 and marked as described above. For deployment, 20 mussels were placed in each of two 42 × 19 cm coated wire cages and placed in the insulated collection box, in chilled 8 °C sea water. The transplant cages were returned to the area of collection and deployed approximately 2 m (Transplant 2) and 3 to 4 m (Transplant 1) away from the collection (control) bed (the same bed used for the in situ growth study). Cages were retrieved in August 1992, and the mussels were treated as described for the growth studies. The 20 unmarked mussels from their original collection site (Bush Hill growth site, 1991 and 1992 collections) served as controls for the transplant experiment.

Measures of condition

Bulk tissue was thawed and homogenized in a 10× dilution of distilled, deionized water using a Brinkman PT3000 Polytron with a PT-DA 3020/2TM generator. Replicate samples (10 ml) were taken from this primary homogenate for determination of ash-free dry weight. A 1-ml aliquot was removed and further homogenized, in a hand-held glass tissue grinder, then diluted an additional 50× for glycogen determination. This glycogen assay is a modification of several previous protocols (Raabo and Terkildsen 1960; Keppler and Decker 1974; Passonneau and Lauderdale 1974; Clark and Keith 1988). Briefly, glycogen was degraded to glucose by amyloglucosidase, and glucose was quantified spectro-photometrically following a dye-linked enzymatic oxidation (Sigma Diagnostics kit #510). Modifications included the use of phosphate buffer (0.067*M*, pH 5.8), which was found to be conducive to the action of all enzymatic steps, and a 2-h pre-incubation with amyloglucosidase (0.5 unit activity ml^{-1}). This protocol yielded >90% recovery of spikes and allowed quantification in tissue homogenates so that prior isolation of glycogen was not needed (Keppler and Decker 1974). Free glucose concentrations in the tissue were determined in separate aliquots (without the addition of amyloglucosidase) and were subtracted for determination of tissue glycogen levels. Replicate glycogen and glucose standards were run with each assay and the standard curves used to calculate the tissue glycogen levels.

Condition index (CI), the ratio of the ash-free, dry mass of soft tissue to internal shell volume (Smith 1984), is a commonly used measure of relative condition in bivalves and is a sensitive indicator of condition in related deep-sea mussels (Smith 1984; Fisher et al. 1988). Soft-tissue dry weight was determined after drying replicate 10-ml samples of the homogenate described above to constant weight at 60 °C. Ash weight was determined after ashing these samples in a muffle furnace, at 500 °C, for 12 h. Internal shell volume was calculated based on the weight of sand contained in each valve. Each shell valve was filled with sand, leveled off across the open edge, and the weight of sand recorded, three times per valve. Sand weight was converted to volume based on the empirically determined relation between the two. Tissue water content was also calculated from the ash-free dry weights. It provides an easily obtained measure of condition but, unlike CI, it is sensitive to residual mantle cavity water.

Statistical methods

Due to the nature of the condition indices data (percentages and ratios), an arcsine transformation ($y' = 2\arcsin\sqrt{y}$) was applied to all three condition parameters before testing for significant differences between sites and years (Gilboa-Garber 1971). The parameters were normally distributed (Shapiro–Wilk's test, Minitab 1989), and the variability of the condition parameters was analyzed with respect to site, year, and length by ANCOVA using a General Linear Model (GLM) procedure (SAS program, version 607). If significant differences ($p < 0.01$) in CI, water content, glycogen content, or length were supported by one-way analysis of variance, then multiple comparison tests (Fisher PLSD and Scheffé tests, Statview II) were used to demonstrate significant differences between specific sites and years.

Since the growth rate varied with initial length, statistical comparisons between sites and years were conducted on the size-specific growth rates [$\Delta L/L_i$, where ΔL = final length − initial length and L_i = initial length (Bayne et al. 1982; Wootton 1991)] and were compared by ANCOVA using a reduced model GLM procedure. Ages were estimated using the von Bertalanffy growth model and the variances of the age estimates were calculated using the following formula:

$$[\partial g/\partial K]^2 \operatorname{var}(K) + 2\operatorname{cov}(K, L_{max})[\partial g/\partial K][\partial g/\partial L_{max}] + [\partial g/\partial L_{max}]^2 \operatorname{var}(L_{max}), \quad (1)$$

where g = age, L_{max} = the extrapolated point of no growth, K = the rate at which L_{max} is approached, L_i = initial length and $[\partial g/\partial K] = (1/K^2)[\ln(1 - L_i/L_{max})]$ and $[\partial g/\partial L_{max}] = -L_i/[K \times L_{max} \times (L_{max} - L_i)]$. This formula was derived by M. Ghosh-Dastidar (Pennsylvania State University) using point estimates and standard errors for both K and L_{max} and the results from an asymptotic Kramer-delta procedure (Arnold 1990).

Results

Site characterizations

Bush Hill mussel beds

Sulfide was undetectable in the water samples from both experimental mussel beds (ring collection and growth) in 1993. Methane levels in the samples from the two beds spanned a similar, relatively low range of undetectable to <60 μ*M* in both 1992 and 1993 (Table 1). Streams of bubbles (presumably methane) as well as large globules of oil were observed within both beds during the mussel collections. The retrieval container mounted on the submarine, as well as the personnel handling the organisms, were always covered in an orange oil residue after recovery of mussels.

Larger mussels predominated in these collections, with the majority >55 mm in length and none <9 mm (Fig. 1A). Mussel density in the Bush Hill bed was calculated to be 881 mussels m^{-2} based on the ring collection. Using the density and size frequency data from the ring collection, and the allometric equation derived by Fisher (1993) from the logarithmic regression of tissue wet weight and shell length in 120 mussels between 12 and 110 mm shell length {tissue wet weight (g) = 2.88×10^{-5} [shell length (mm)]$^{3.162}$}, the soft tissue biomass for this bed was calculated to be 11.3 kg m^{-2}. Since the dissolved gas concentrations, and size ranges and distributions of mussels from both beds were similar, the beds are considered together in the discussion of "Bush Hill" mussels.

GC 234 mussel beds

Both sulfide and methane levels in the water samples from the GC 234 beds were relatively high, especially in the samples taken 5 cm below the top of the mussels (between and below the shells), where sulfide and methane concentrations approached 8 and 11 m*M*, respectively (Table 1). Oil globules and streams of bubbles were also observed within these beds during sampling. The size distributions of mussels from the two beds sampled were similar to each other and dramatically different from the other two sites in that most of the mussels here were 15 to 40 mm long, with only a few larger mussels (Fig. 1B). Mussel density calculated from the ring collection at this site was 3560 mussels m^{-2}, with biomass calculated to be 7.3 kg m^{-2}. Since

Table 1 Methane and sulfide concentrations in water samples taken from each site in 1992 and 1993. Depth of 0 cm is at the height of the siphons of larger mussels in the bed, and depths of 5 to 10 cm are distances below that level (between and below the mussels). (*BH* Bush Hill; *GC* Green Canyon; *ND* non-detectable; – not sampled)

Mussel bed	Sampling depth (cm)	Methane concentration (μM)		Sulfide concentration (μM)
		1992	1993	1993
BH:ring	0	56	ND	ND
	5	ND	22	ND
	10	–	–	–
BH:growth	0	–	20	ND
	5	ND	ND	ND
	10	10	–	–
GC 234:ring	0	–	72	ND
	5	772, 869, 75	10744, 9466	6746, 7956
	10	986	–	–
GC 234:growth	0	–	832	179
	5	15, 16	802	1150
	10	ND	–	–
GC 272:ring	0	–	2	ND
	5	–	4	ND
	10	–	–	–
GC 272:growth	0	–	2	ND
	5	35, 141, 290	4	ND
	10	822, 27	–	–

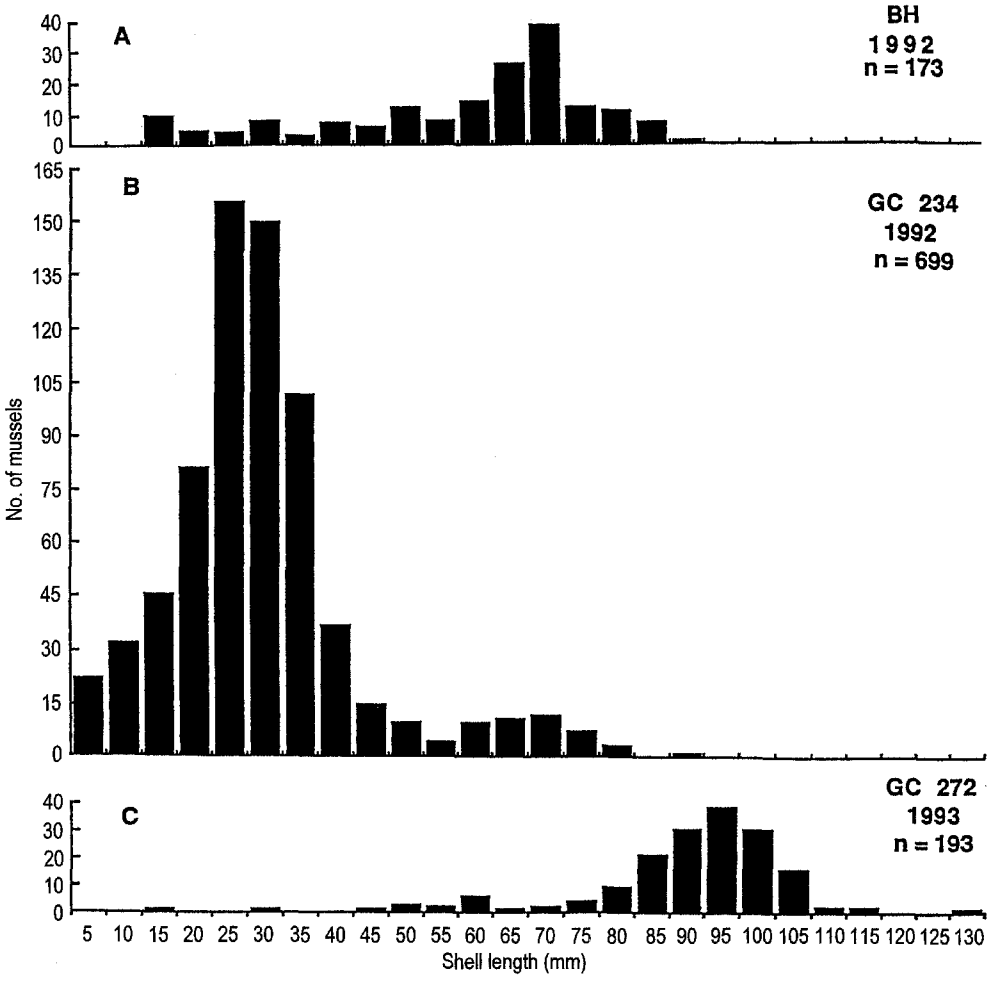

Fig. 1 Seep Mytilid 1a. Size frequency distributions for mussels at three hydrocarbon seep sites. **A** Bush Hill (*BH*) (in 1992); **B** Green Canyon (*GC*) 234 (in 1992); **C** GC 272 (in 1993)

the dissolved gas concentrations, and size ranges and distributions of mussels from both beds were similar, the beds are considered together in the discussion of "GC 234" mussels.

GC 272 mussel bed

Sulfide was not detected in any water samples from this mussel bed, and methane levels decreased dramatically from 1992 (35 to 822 μM) to 1993 (2 to 4 μM). In contrast to the other two sites, no oil globules were released while collecting and neither the submersible nor investigators were oil-stained after handling the mussels. Gas bubbles were not observed in this mussel bed. This bed was dominated by large mussels, 78 to 105 mm long (Fig. 1C). Mussel density in the bed was calculated to be 876 mussels m^{-2}, with calculated mussel biomass of 37.7 kg m^{-2}.

In situ growth

Change in length as a function of initial shell length of the marked mussels was determined for the periods September 1991 to August 1992 and August 1992 to June/July 1993 for each of the three beds sampled (Fig. 2). The relationship between growth rate and length was evident at sites with smaller mussels.

No significant difference was found between the average specific growth rates of mussels at Bush Hill and GC 234 in 1991/1992, but the average rate in GC 234 was higher in 1992/1993 ($p < 0.016$). Comparisons of GC 272 to Bush Hill and GC 234 indicated no significant difference in 1991/1992; however, in 1992/1993, the average specific growth rate at GC 272 fell sharply and was significantly lower than GC 234 ($p < 0.05$) yet not significantly different from Bush Hill (Table 2).

Bivalve ages are commonly estimated by means of either the von Bertalanffy or the Gompertz growth equations (Bayne and Worrall 1980; Rhoads et al. 1981; Craeymeersch et al. 1986). The von Bertalanffy equation, $L_t = L_{max}[1 - ((L_{max} - L_o)/L_{max})e^{-kt}]$ (where L_t is the shell length at age t, L_{max} is the extrapolated point of no growth, L_o is the length at age 0, and K is the rate at which L_{max} is approached), in this case can be reduced to $L_t = L_{max}[1 - e^{-kt}]$ because L_o is so small and $(L_{max} - L_o)/L_{max} \approx 1$. This equation provides a better description of growth for a population of more mature bivalves (Powell and Somero 1986; Seed and Richardson 1990), and the Gompertz equation $\{\log_{10}L_t = \log_{10}L_{max}[1 - e^{-k(t-t_o)}]\}$ gives better descriptions of growth of juveniles (Theisen 1973). Due to the size disparity between the sites, ages were estimated for each population using both techniques. The age estimates from these two equations were not notably different at GC 234. However, at Bush Hill, the Gompertz equation yielded much greater ages (up to 350 yr) than did the von Bertalanffy equation. This is likely a reflection of the poor representation of smaller mussels at this site. Therefore, the results shown (Fig. 3A and B) are age estimations based on the von Bertalanffy growth equation. The parameters, L_{max} and K, are

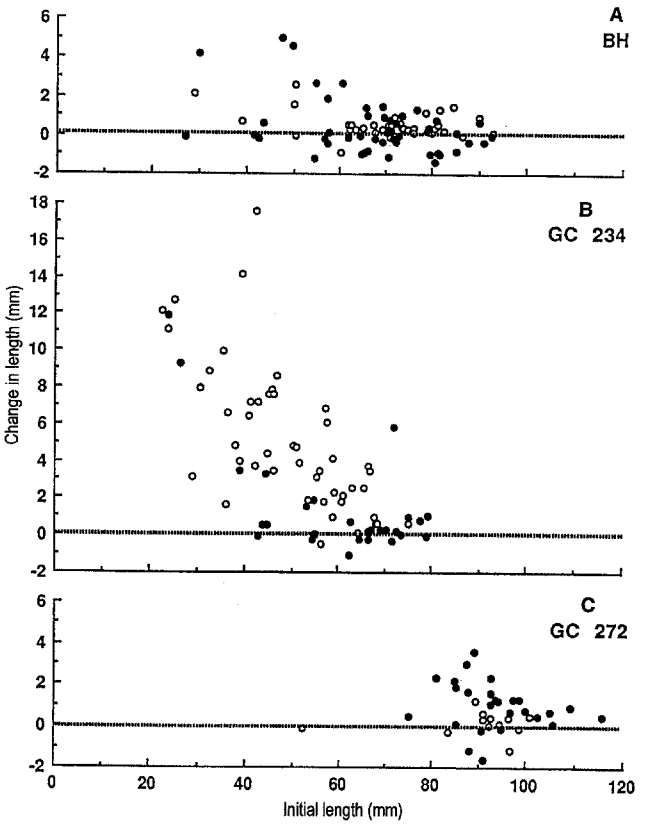

Fig. 2 Seep Mytilid 1a. Change in length as a function of initial shell length for mussels from three hydrocarbon seep sites. [*Filled circles* change in length from 1991 to 1992 (~326 d) as a function of 1991 length; *open circles* change in length from 1992 to 1993 (~321 d) as a function of 1992 length.] **A** Bush Hill (*BH*); **B** Green Canyon (*GC*) 234, **C** GC 272

Table 2 Seep Mytilid Ia. Mean specific growth rate of mussels from three hydrocarbon seep sites and a transplant experiment. Rates determined as length-specific growth, the measured change in length (mm) per time (between 340 and 320 d) per initial length (mm) [specific growth rate = $(\Delta L/L_i)$] and normalized to a per year basis. SD of the mean given in parentheses. Underlined value is significantly different from the other two sites ($p < 0.05$)

Site	1991–1992 growth	*n*	1992–1993 growth	*n*
Bush Hill	0.010 (0.031)	47	0.010 (0.015)	38
Transplant 1[a]	0.029 (0.036)	20	–	
Transplant 2	−0.005 (0.006)	20	–	
GC 234[b]	0.041 (0.108)	34	0.132 (0.135)	47
GC 272	0.011 (0.013)	26	0.001 (0.005)	10

[a] Growth at Transplant 1 significantly different from Bush Hill over same time period ($p < 0.01$)
[b] Significantly different between years ($p < 0.05$)

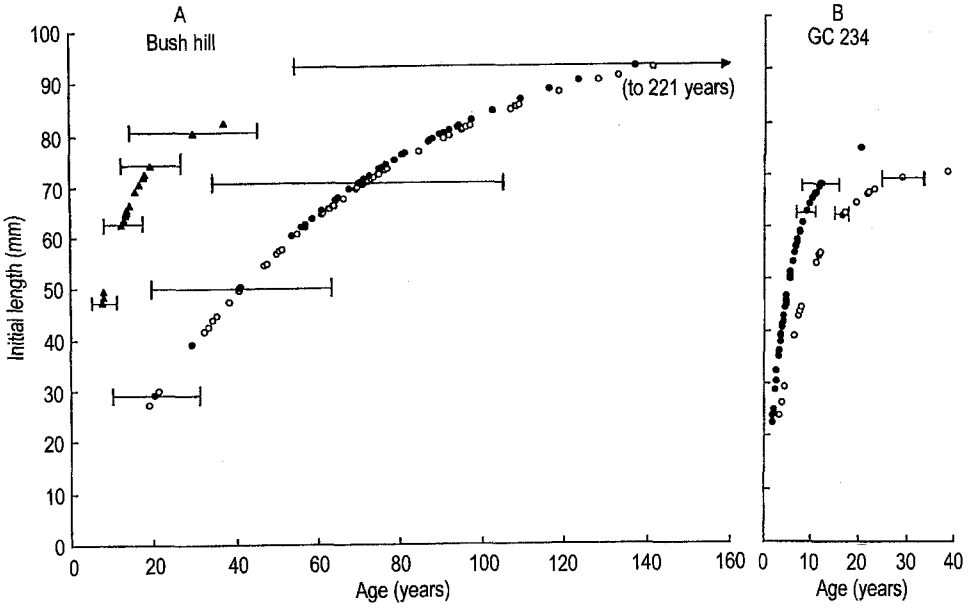

Fig. 3 Seep Mytilid 1a. Age estimations for mussels from **A** Bush Hill (*open circles* 1991; *filled circles* 1992; *filled triangles* Transplant I); and **B** Green Canyon (*GC*) 234 (*open circles* 1991; *filled circles* 1992). Estimates calculated using the von Bertalanffy growth equation, $L_t - L_{max}(1 - e^{-kt})$. Parameters for the equation, derived from a Ford–Walford plot, given in Table 3

Table 3 Seep Mytilid Ia. Growth parameters derived from Ford–Walfords plots, with SD given in parentheses

Site	K ($mm\,yr^{-1}$)		L_{max} (mm)	
	1992	1993	1992	1993
GC 234	0.205 (0.024)	0.210 (0.032)	74.37 (3.82)	74.26 (4.43)
Bush Hill (BH)	0.016 (0.013)	0.016 (0.007)	103.14 (6.26)	104.40 (15.83)
BH Transplant I	0.108 (0.023)	–	83.74 (4.24)	–

estimated from a Ford–Walford plot (Craeymeersch et al. 1986; Seed and Richardson 1990) and are given in Table 3. These parameters are obtained by plotting the length at a given time against the length a year before (Bayne and Worrall 1980). Age estimations for GC 234 and Bush Hill are shown in Fig. 3A and B and were not calculated for GC 272 due to the absence of mussels < 50 mm in length (Fig. 2C).

Based on these age estimations, a 30-mm mussel at Bush Hill would be about 20 yr and a 65-mm mussel about 65 yr old, whereas mussels of the same shell length at GC 234 are about 2.5 and 18 yr old, respectively.

These estimates suggest that the oldest mussels at the two sites are 160 yr (85 mm) and 38 yr old (70 mm), respectively. It is important to note that these calculations are based *on current growth rates* at the sites and do not reflect the potentially changing conditions within a bed.

Growth rates were determined for each of the transplants at Bush Hill (Table 2). The average specific growth rate of Transplant 2 mussels was $0.029\,mm\,yr^{-1}$ (significantly higher than in the source bed). For Transplant 1 mussels, the average specific growth rate was not significantly different than zero

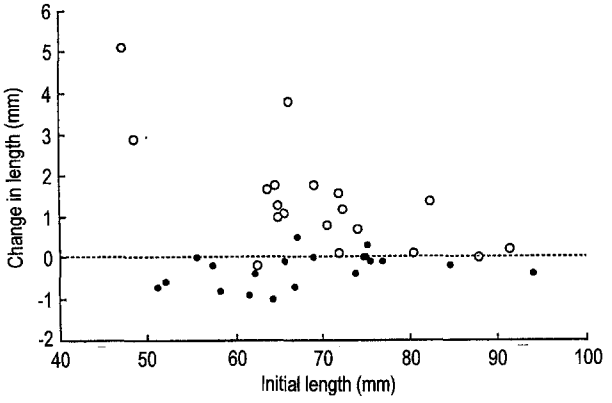

Fig. 4 Seep Mytilid 1a. Change in length as a function of initial shell length for two Bush Hill transplant experiments. (*Open circles* Transplant 1, located 3 m away from the original Bush Hill mussel bed; *filled circles* Transplant 2, located 2 m away from the original Bush Hill mussels bed.) Duration of present study was 325 d

(Fig. 4). The von Bertalanffy parameters were estimated for Transplant 1 using a Ford–Walford plot (Craeymeersch et al. 1986; Seed and Richardson 1990), and the resultant age estimates are also shown (Fig. 3A).

Measures of condition

No significant differences were found between marked and unmarked mussels in terms of glycogen, water content, and CI in any collection from any site during the present study. We conclude that any differences between sites or years are site-specific and not the result of the marking procedure.

Three indices of condition were used to characterize the physiological state of the mussels. As a check on the reliability of each parameter (in terms of agreement among the indices) correlation analysis was conducted on each pair of indices. The correlation coefficients for each pair of indices were significant (glycogen:water content $= -0.68$; glycogen:CI $= 0.73$; CI:water content $= -0.89$, $n = 183$).

The effect of size on variation in the condition parameters was analyzed by ANCOVA using a GLM procedure. Shell length (size) does not contribute significantly to variation in the condition parameters and we conclude that variation among parameters was due to differences between sites (mussel beds) and between years.

Table 4 shows the mean values ($\pm$SD) of each of the condition parameters in each year for the three growth sites. Significant differences were found between GC 234 and Bush Hill for water content and CI in 1991 ($p < 0.01$); no significant differences were found, for these parameters, between the sites in 1992 and 1993 or in glycogen content in any year. GC 272 mussels had significantly higher glycogen contents ($p < 0.05$), higher condition indices ($p < 0.01$) and lower water contents ($p < 0.01$) than mussels from the other two sites in both 1991 and 1992, but not in 1993. Mean shell lengths of mussels from all sites were significantly different in each year ($p < 0.05$), but mean lengths within a site were not significantly different between years. Any significant differences in pairwise comparisons (between sites in a given year, or between years at a single site) were corroborated by all three indices, with one exception: Bush Hill and GC 234 in 1991, where a significant difference was not found in glycogen content but was for the other two parameters.

Additionally, all three condition parameters were significantly related to the growth rates measured in adults at GC 272 (Fig. 5). This data set was chosen because of the range in growth rates among adults there and to avoid a data set with a large size range of individuals (because of the allometric effects on growth rate and not on the other parameters). The largest r^2 and smallest relative 95% confidence interval on the slopes of these regressions was that relating growth and CI. This is likely due to the fact that determination of tissue water content is sensitive to residual mantle water and, therefore, to the draining technique (which is accomplished in a cold van on board ship), whereas CI is not and to the fact that glycogen content may be more sensitive to reproductive state and hydrocarbon exposure (Bayne 1976; Lowe and Pipe 1986; Hummel et al. 1989; De Zwaan and Mathieu 1992). The significant relationship between the growth rates recorded for individual mussels, and their condition as measured by our indices, corroborates the efficacy of our measures of physiological condition: the "healthy" mussels are growing faster.

Table 4 Seep Mytilid Ia. Condition indices and size range for mussels at three hydrocarbon seep sites and a transplant experiment. Values for each site and year are averages ($\pm$SD). Underlined values were significantly different ($p < 0.005$) from collections at the same site in other years. Values underlined with dashed line (% water in GC 234 in 1991 and 1992) were significantly different from each other ($p < 0.01$). (*WW* wet weight; *BH* Bush Hill; *GC* Green Canyon; *TP* Transplant)

Collection		Glycogen (% WW)	Condition index ($g\,ml^{-1}$)	Water (% WW)	Length (mm)		*n*
Site	Year				Range	Mean ($\pm$SD)[a]	
BH	1991	0.88 (0.63)	0.037 (0.014)[b]	90.4 (2.03)[b]	58–83	73 (8.3)	17
BH	1992	1.13 (0.88)	0.053 (0.018)	90.8 (2.28)	27–93	68 (15.7)	32
BH	1993	0.73 (0.44)	0.051 (0.010)	90.5 (1.25)	59–92	74 (10.5)	12
GC 234	1991	1.55 (0.82)	0.061 (0.018)[b]	88.1 (1.84)[b]	47–75	67 (7.9)	20
GC 234	1992	1.50 (0.96)	0.062 (0.019)	90.3 (1.92)	36–81	60 (13.9)	29
GC 234	1993	1.33 (0.73)	0.062 (0.020)	89.3 (2.84)	45–83	63 (12.1)	12
GC 272	1991	2.94 (1.19)[b]	0.079 (0.024)[b]	86.0 (2.61)[b]	72–105	90 (9.6)	20
GC 272	1992	3.58 (1.24)[b]	0.105 (0.025)[b]	85.3 (2.43)[b]	53–116	91 (15.3)	30
GC 272	1993	1.07 (0.66)	0.056 (0.011)	90.0 (1.45)	64–110	92 (11.5)	11
TP I	1992	1.47 (0.73)	0.061 (0.13)	89.9 (1.6)	52–92	70 (10.6)	6
TP II	1992	0.41 (0.31)	0.036 (0.012)[c]	93.3 (1.28)[c]	50–94	68 (11.1)	6

[a] Mean lengths between sites were significantly different every year ($p < 0.01$ between GC 272 and the other two sites and $p < 0.05$ between BH and GC 234). Mean lengths were not significantly different within the sites between years

[b] Significantly different from values at the other sites in the same year ($p < 0.01$)

[c] Significantly different ($p < 0.05$) from BH-values in the same year

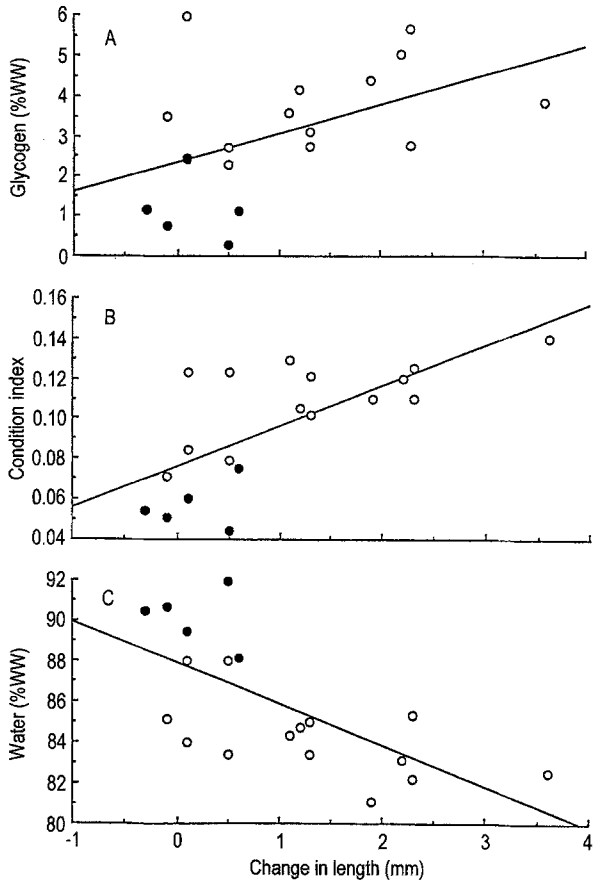

Fig. 5 Seep Mytilid 1a. Relation between growth rate and condition indices in mussels from Green Canyon (*GC*) 272; data for 1992 (*open circles*) and 1993 (*filled circles*) combined for the linear regression. **A** Glycogen as a function of change in length, regression equation: $y = 0.73x + 2.313$ ($p < 0.05$), 95% CI on slope is ± 0.67; **B** condition index as a function of change in length, regression equation: $y = 0.020x + 0.076$ ($p < 0.001$), 95% CI on the slope is ± 0.01; **C** Water content vs change in the length (mm), regression equation: $y = -2.03x + 87.88$ ($p < 0.005$) 95% CI on slope is ± 1.15. Note: data for 1992 are significant on their own (and therefore the regression equations above are not being driven by the 1993 data), with the exception of the 1992 % glycogen vs growth

Discussion

The average specific growth rates of Seep Mytilid Ia (measured as increments in shell length) at active seep sites are equal or slightly higher than those reported for healthy mussels from littoral environments at similar temperatures (5 to 8 °C) (Theisen 1973; Forster 1981; Sukhotin and Kulakowski 1992). The growth rates of the related vent mytilid, *Bathymodiolus thermophilus*, are also similar to littoral mytilids from comparable habitat temperatures (ca. 1 cm yr^{-1} at 10 to 20 °C) (Rhoads et al. 1981; Stromgren and Cary 1984; Lutz et al. 1985). However, these rates (Table 2) are orders of magnitude higher than rates reported for a small deposit-feeding deep sea bivalve [0.084 mm yr^{-1} (Turekian et al. 1975)]. Unlike most deep sea environments, vents and seeps are very productive (as a result of chemoautotrophy and methanotrophy), and thus, as long as the sites remain active, the fauna is not as nutrient limited as the species in the surrounding deep sea (see reviews by Fisher 1990; Tunnicliffe 1991; Lutz and Kennish 1993).

Like most animals, the younger individuals (<40 mm shell length) grow much more rapidly, although mussels over a year old are apparently still growing at rates over 1 cm yr^{-1} at some sites (Figs. 2B and 3B). This is likely affected by allometric changes in size specific respiration rates and resource allocation to reproductive effort (Thompson 1984; Reiss 1989; Seed and Suchanek 1992). The fast growth rates of young individuals may also be of ecological importance to the species. There are several possible predators at the seeps, including several decapods, starfish, and fish (Carney 1994). These types of predators forage selectively on smaller mussels in temperate habitats (Seed and Suchanek 1992) and likely behave similarly at seeps. The shells of the seep mytilids are relatively delicate (especially those <15 mm shell length), and the mussels are therefore potentially easy prey. Rapid early growth would shorten the period of maximum vulnerability to selective predation (Seed and Suchanek 1992). On the other hand, the slow growth rates of adults suggest that these bivalves may live a long time after reaching reproductive competence, even under suboptimal conditions.

The use of the new water sampling tools described here allowed collection of discrete water samples from specific points in and over the mussel beds, without the extensive dilution by ambient water which had plagued previous efforts of this sort (personal observation CRF; Fisher 1993). It is clear in evaluating the present chemical data (and confirmed by subsequent sampling) that the presence of "seep fluid" is patchy, even on scales of decimeters within a mussel bed, and that there are substantial vertical gradients on scales of centimeters as well. Since it is not known which pools of near bottom and interstitial waters the mussels tap for methane (from their siphons only or through the pedal gape as well), it is not clear exactly what methane concentrations they are actually exposed to. However, large differences in methane and sulfide concentrations among the waters bathing the mussels at the different sites were evident, as was a change in conditions at GC 272 between 1992 and 1993.

Methane concentrations measured in the two experimental beds at Bush Hill were consistently in the range 0 to 56 μM over both years (Table 1). Based on the laboratory studies of Kochevar et al. (1992), methane concentrations between 20 and 60 μM, with methane as the major carbon and energy source, are barely adequate for growth of this mussel. Although growth was easily detectable in the population as a whole (Table 2), the lack of detectable growth in over 30% of the

mussels <60 mm in shell length (Fig. 2A) supports the suggestion that nutrient levels (mainly methane) were barely sufficient for maintenance of this population. The marginal environmental methane levels are also reflected in the physiological condition of the mussels from Bush Hill. Although the only year in which they were significantly different from the other sites was in 1991, the average values for the condition indices, for the Bush Hill collections were the lowest of any site every year.

At the other end of the spectrum, methane concentrations in 5-cm depth interstitial water from the GC 234 beds were as high as 10744 μM, and a concentration of 832 μM was documented around the siphons of mussels in 1993 (Table 1). The highest specific growth rates were found in the GC 234 mussels for the 1992–1993 period (Table 2, Fig. 3B). The average values of the condition parameters were in general intermediate between those of the Bush Hill and GC 272 mussels with the GC 272 mussels having significantly higher CI, glycogen content and significantly lower water content, in both 1991 and 1992. These data imply that factors other than methane may be affecting the health of the mussels. Certainly the sulfide levels measured in the GC 234 beds were the highest measured in our study, and all collections from this site were oil-stained.

At the single bed investigated at GC 272, the five water samples taken in 1992 ranged from 27 to 822 μM CH_4 and the four taken in 1993 from 2 to 4 μM CH_4. This indicates a substantial difference in the microhabitat of the mussel bed in the 2-yr period. The reduced growth rates and significant drop in the physiological condition of the population in 1993 (Fig. 2C, Tables 2, 4) suggest that the measured microhabitat chemistry reflects conditions which had persisted for some time and not short-term fluctuations in chemistry. In 1991 and 1992 all three condition indices, indicated that the mussels from GC 272 were in the best condition of any of the collections, although methane levels were higher at GC 234 in 1992. No crude hydrocarbon seepage was apparent within the GC 272 bed. The bioaccumulation of hydrocarbons in tissue of shallow-water mussels is known to adversely affect growth rates, reproductive processes, and nutritive storage capabilities (Lowe and Pipe 1986; Stromgren et al. 1986; Stromgren and Nielsen 1991; reviewed by Williams et al. 1993). As previously mentioned, crude hydrocarbons are abundant in both the GC 234 and Bush Hill mussel beds. Wade et al. (1989) found that hydrocarbon loading (mostly polycyclic aromatics) of several invertebrates at the seeps is high, especially for the sessile organisms. Among mussels examined from several beds in all three sites, McDonald (1990) found that the tissue hydrocarbon concentrations were highest at the GC 234 site. McDonald (1990) demonstrated relatively high activities of a cytochrome P450-dependent enzymatic detoxification mechanism in Seep Mytilid Ia. This adaptation to their potentially toxic environment may exert a substantial metabolic cost on seep mussels inhabiting areas with high hydrocarbon levels and explain the better condition of the GC 272 mussels.

The population structure of mussels from the three sites was strikingly different. Clearly, the beds sampled at GC 234 had recently settled juveniles in 1992, and the bed at GC 272 had not had substantial recruitment in many years (the Bush Hill beds showed an intermediate pattern). It is not clear why there would not have been recruitment to GC 272 prior to 1992 as all measurements there (chemistry, growth rates, and condition indices of large mussels) suggest that this was an excellent habitat at that time. The mussels there are currently relatively isolated from other mussel beds, and this may have been a factor. Alternatively, if the variation in conditions documented during our study are characteristic of the recent history of this bed, this may explain the lack of juveniles at GC 272, as they would be much more sensitive to significant perturbations in nutrient supply due to their higher metabolic demands. When one considers the spatial and temporal variation in seepage and the reliance of the mussels on methane in the seeping fluid, even large differences in population structure of mussel beds which are only meters apart are not surprising (personal observation; MacDonald et al. 1990b).

The variable nature of methane seepage (Behrens 1988; Roberts 1990) makes evaluation of the calculated ages problematic. Although this variability is on longer temporal scales than that documented around hydrothermal vent fauna where significant differences in the chemical environment can occur within seconds to minutes (Johnson et al. 1988), it can certainly be significant over time periods relevant to age calculations. For example, if one assumes that conditions have been constant at the Bush Hill site for the past 160 yr, then there are likely mussels present there at least that old. However, the strong possibility of changing conditions within a bed (as clearly documented for GC 272 within 3 yr) suggests that the mussels there may have reached a significant portion of their present size under more favorable environmental conditions and that the calculated ages are therefore an overestimation. This possibility was empirically demonstrated by one of the transplant experiments where mussels from the Bush Hill site were moved <3 m and left for 1 yr. Maximum ages calculated from the shell growth of the transplanted Bush Hill mussels dropped to 40 from 160 yr for the source population (Fig. 3A). Similarly, the age calculations based on growth rates observed at GC 234 in two succeeding years are quite different (Fig. 3B), although the error bars derived from the variances in the von Bertalanffy estimates imply a higher level of confidence in the estimations. Thus the high probability of changing conditions within a bed must be considered when evaluating age estimates based on growth during current conditions. Based on the high potential growth rates, their ability to grow very slowly and

survive marginal conditions, and the size of the largest mussels at GC 272, it is reasonable to conclude that Seep Mytilid Ia can live for at least 50 yr, and perhaps much longer.

The transplant experiments provided additional insight into the ecology of the mussels. The initial purpose of these experiments was to deprive the mussels of methane by placing them away from their food source in unoccupied mud and to follow the effects on condition and growth. The results from Transplant 2 (2 m from the original site) were as expected. The average specific growth rate was $-0.005\ mm\ yr^{-1}$ (but was not significantly different from zero, $p > 0.3$), all condition indices fell dramatically from the already low levels at Bush Hill, and in fact six of the 20 mussels were dead upon recovery. However, the mussels from Transplant 1 (located a little further away from the original bed and about 2 m from Transplant 2) were in better condition and grew at a faster rate (Tables 2 and 4) than mussels in their bed of origin, and none of the 20 mussels died. The differences in growth rates in mussels which were only 2 m apart indicate that very localized effects are responsible. These differences are most likely differences in exposure to seeping fluid and since the Transplant 1 mussels fared better than in their bed of origin, site chemistry alone is insufficient for establishment of a bed. This observation implies that Seep Mytilid Ia, like intertidal mussels, requires a hard substrate upon which to colonize. Thus, the authigenic carbonates, which are often associated with seepage in the Gulf of Mexico (Brooks et al. 1990), form habitats suitable for colonization by mussels.

Community oxidation and production rates were determined for each of the beds sampled by ring collections. According to the model of Kochevar et al. (1992), freshly collected seep mussels have a basal metabolic rate of $1\ \mu mol\ O_2\ g^{-1}\ h^{-1}$ and methane oxidation rates between 4 and $10\ \mu mol\ g^{-1}\ h^{-1}$ under conditions of non-limiting methane. It should be noted that these rate determinations were made on mussels with an average tissue wet weight of 23 g. Since metabolic rate varies allometrically with wet weight in *Mytilus edulis* (Bayne 1976; Reiss 1989), the allometric equation $BMR = aW^b$ (Reiss 1989) (where BMR = basal metabolic rate, W = the wet weight of the mussel, and a and b are derived parameters; $a = 0.102$, $b = 0.71$) was applied to the above rates and the minimum community oxidation rates necessary to sustain basal metabolism and community methane consumption rates under optimal conditions were estimated for each site using the quantitative ring collection data [the allometric relationship between length and wet weight from Fisher (1993) was used to determine wet weights for individual mussels in each ring collection]. The parameters a and b were taken from published estimates for the well-studied *M. edulis* (Bayne and Worrall 1980). Minimum calculated community oxidation rates range from $2.9\ g\ m^{-2}\ d^{-1}$ at GC 234 to $49.4\ g\ m^{-2}\ d^{-1}$ at GC 272 (Bush Hill = $7.2\ g\ m^{-2}\ d^{-1}$). Under optimal conditions (of O_2 and CH_4 concentrations), maximal calculated methane consumption rates range from $14.6\ g\ m^{-2}\ d^{-1}$ at GC 234 to $197.7\ g\ m^{-2}\ d^{-1}$ at GC 272 (Bush Hill = $36.2\ g\ m^{-2}\ d^{-1}$). These values are in the same range as those calculated by (Fisher 1993) and are several orders of magnitude higher than for any other aquatic system (Kuivila et al. 1988; Frenzel et al. 1990; Fisher 1993).

Summary

When all data and observations are considered together, one can make some general conclusions concerning each site: the Bush Hill beds have been active for at least 40 yr, with appropriate conditions for larval settlement over most of that period. However, methane concentrations were low in 1993 (although a stream of methane bubbles issued from one point in the bed), growth rates were low, and the population was dominated by large mussels. A plausible explanation, which is supported by the mussel condition indices, is that there had been a recent decline in the quality of the habitat within this particular bed. At the beds in GC 234, there was a preponderance of young mussels, indicating a recent period of active settlement, and methane concentrations were high, as were growth rates. However, the condition indices were significantly higher than for Bush Hill mussels only in 1991 and significantly lower than GC 272 in both 1991 and 1992. Thus, although there were plenty of young, rapidly growing mussels, there may have been some factor detrimental to condition. We suggest that this factor was exposure to high hydrocarbon levels and that this may have exerted a considerable cost to the seep mussels. At GC 272 on the other hand, oil was never apparent during the collections. The mussels here were growing at appreciable rates in 1992 and were exposed to moderate to high levels of methane. Condition of mussels in both 1991 and 1992 was excellent by all criteria. However in 1993, growth rates, condition, and methane concentrations in the bed had all decreased. The almost complete absence of small mussels at GC 272 cannot be explained by the environmental measurements. We conclude that none of the beds in the present study provided "perfect" conditions for the mussels and that sites with higher growth rates and productivity are likely present elsewhere.

Acknowledgements The present study would not have been possible without the excellent support of the Captains and crew of the R.V. "Seward Johnson" and R.V. "Edwin Link" and the pilots and crew of the D.S.R.V. "Johnson Sea Link". We thank the Harbor Branch Oceanographic Institute engineering group for assistance in the design and for construction of the water samplers and insulated mussel deployment containers. We thank P. Goans for laboratory assistance, and M. Simpkins, S. MacKenzie, K. Kelley, M. Streams, L. Lussier and R. Nikalous for assistance in the cold room at sea. We also thank M. Ghosh-Dastidar, M. Horne, S. Warner and C. Rowe

for statistical assistance, and Drs. B. Carney, W. Dunson, I. MacDonald, F. Williams and an anonymous reviewer for critically reviewing the manuscript. These studies were supported by subcontract L10094 to Mineral Management Service contract 14-12-0-0001, Gulf of Mexico OCS region, to J.M. Brooks, NSF-EAR-9158113 and NSF-OCE-9114386 to CRF, the NOAA National Undersea Research Center at UNCW, and the Harbor Branch Oceanographic Institution.

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