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Bacterial recolonization of deposit-feeder egesta: In situ regrowth or immigration?

Abstract—The related processes of disturbance, recolonization, and succession can play major roles in structuring communities and in generating spatial heterogeneity. Nonequilibrium processes have been little studied with respect to microbial community dynamics. Here we report experimental efforts to identify the mechanisms of recolonization of egesta employing two intertidal deposit feeders, *Balanoglossus aurantiacus* and *Nereis succinea.* Using direct microscopic counts and Biolog plates, we compared the quantitative and qualitative patterns of recovery in egesta isolated from underlying sediments on ''latrines'' to recolonization in naturally incubated fecal casts. Significant recovery over the 2-h observation periods was never seen on latrines. Rapid recovery in metabolic potential was observed in the naturally incubated fecal casts of both animal species. Likewise, a rapid numerical recovery was seen in the egesta of *N. succinea* casts on sediments. In contrast, no numerical recovery was evident in any fecal casts of *B. aurantiacus* over 2 h, regardless of treatment. For these two species, recolonization appears to be dominated by migration of bacteria from underlying sediments as opposed to repopulation by survivors of ingestion. These findings indicate that renewal of available (digestible) microbial resources to deposit feeders is more rapid than would be predicted if regrowth was the dominant process of recolonization. Furthermore, because recovery time is short relative to the interval between disturbances, deposit feeding is unlikely to play a major role in structuring benthic microbial communities.

Sedimentary bacteria are important to the functioning of ecosystems in that they play key roles in food webs and are primary remineralizers of organic matter (e.g., Blackburn 1988; Deming and Baross 1993). Although macrofaunal organisms frequently ingest and efficiently digest (e.g., see review of Kemp 1990) bacteria associated with sediments, their influence relative to benthic bacterial production has usually been shown to be of little significance (e.g., Plante et al. 1989; Kemp 1990).

It is not so clear, however, that there are not important qualitative effects of deposit feeding. In fact, it has been documented that the microbial communities of deposit-feeder egesta vary from those of surrounding sediments and ingesta (Dobbs and Guckert 1988; Wilde and Plante pers. comm.). Further, the influences of diverse deposit feeders may vary as the strength (Plante and Mayer 1994; Plante and Shriver 1998), and physical location within the gut (Plante and Mayer 1994) of bacteriolytic agents have been shown to differ among taxonomic groups. The influence of community shifts associated with digestive passage on the community structure of bulk sediments, however, is equivocal. Although microbial communities of fecal casts are distinct from those of sediments, in situ, numerical differences disappear quickly (Plante and Jumars 1993; van de Bund et al. 1994).

The rapid return to a community resembling that in sediments (at least numerically) could be due to regrowth of the dominant groups of bacteria surviving gut passage. Alternatively, migration of bacteria from adjacent sediments could account for these observations. Given the swimming speeds of bacteria (>50 μm s^{-1}; Vaituzis and Doetsch 1969), even large fecal pellets or castings could be colonized in a matter of minutes.

The rate of microbial recolonization of feces may be important to resource renewal for deposit-feeding invertebrates (Newell 1965). Fecal materials are eventually degraded and reingested but not until after microbial conversion of inorganic to organic forms of nitrogen (e.g., Newell 1965; Findlay and Tenore 1982). The importance of locally produced microbial food will vary with environmental conditions—under conditions of significant sediment transport, for instance, advected supply likely is relatively more important than in situ microbial growth.

The nature of recolonization will also be important. Numerous studies illustrate that bacterial assemblages of fresh deposit-feeder egesta differ markedly from ambient sediments (Dobbs and Guckert 1988; Ward-Rainey et al. 1996; Wilde and Plante pers. comm.). With respect to subsequent ingestion by deposit feeders, if recolonization of feces is primarily due to regrowth (of either enteric or those ingested bacteria surviving the digestive gantlet), these bacteria will be of lesser nutritional value as they are more likely to be resistant to digestion. More importantly, because benthic detritivore feces are foci of microbial activity (e.g., Hargrave 1976; Juniper 1981), lasting differences in community structure between egesta and bulk sediments would have important geochemical implications.

In the present article, we test the hypothesis that bacterial recolonization of fecal materials is via rapid migration from ambient sediments. We employ a simple method of isolating freshly extruded fecal coils from surrounding sediments and compare both quantitative and qualitative recovery of bacterial assemblages with that of controls.

Samples were collected at Breach Inlet, a protected sandy beach on the north end of Sullivans Island, South Carolina, U.S.A. The intertidal sand flat is situated between a rock jetty to the south and the rocky foundation of a bridge to the north. The dimensions of the flat area are ~30 × 100 m.

The golden acorn worm, *Balanoglossus aurantiacus* (Enteropneusta: Ptychoderidae) and *Nereis succinea* (Polychaeta: Nereidae) are common deposit feeders here (~1.0 ± 0.4 m^{-2} and 141.4 ± 48.3 m^{-2} for *B. aurantiacus* and *N. suc-*

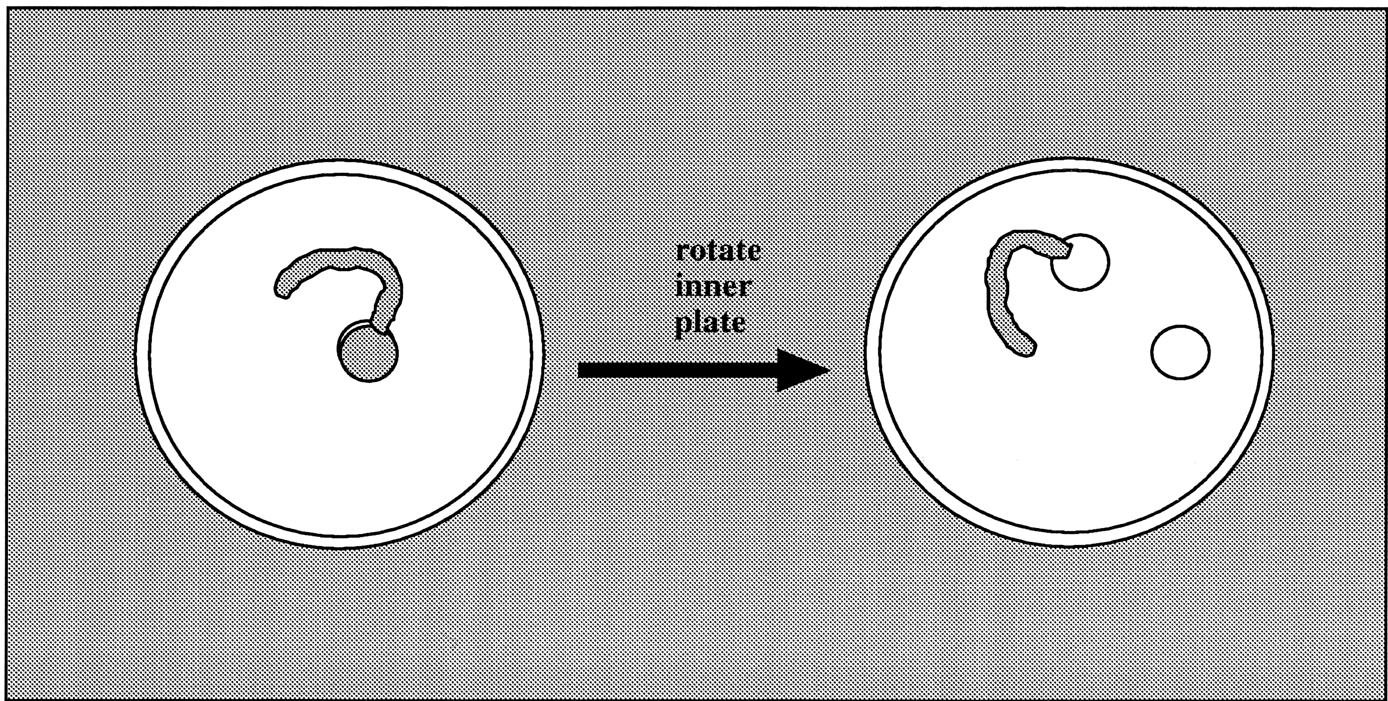

Fig. 1. Latrines were made by flipping the bottom portion of 100 × 15 mm sterile polystyrene petri plates, then drilling 10-mm holes through both sections. After subjects defecated through these aligned holes, the inner section was rotated to isolate egesta from underlying sediments.

cinea, respectively) and are dominant representatives of their guild at this site because of size and abundance, respectively. To follow bacterial colonization of the egesta of these deposit feeders, fecal and ambient, surficial sediment samples were collected over a 2-h period by use of a 1-ml syringe (with the Luer end cut off). Samples were taken while emersed during both ebb (t = 0 h samples) and flood tide (t = 2 h samples). To collect fresh egesta, existing fecal mounds or coils were flagged and numbered to mark their locations, then brushed away. After the next egestion (t = 0 h), 100 mm diameter round polystyrene plates (cut from petri dishes) were inserted at a 45° angle into the sediment beneath the egesta to block further addition of fecal material (cf. Findlay et al. 1990). To test whether recolonization was due to in situ growth versus migration from underlying sediments, we placed "latrines" (cf. Kermack 1955; Fig. 1) over burrow exits to capture and isolate egesta from sediments. Latrines and control areas were covered with 5.5-cm high × 11.5-cm deep cylindrical glass culture dishes to inhibit evaporation. Preliminary tests showed that fecal casts on latrines lost significantly more water relative to naturally incubated egesta (7.8% vs. 2.6% h^{-1}, P = 0.001). With the glass covering, water loss was not significantly different than that for unmanipulated *B. aurantiacus* fecal casts (P = 0.776). All samples, sediment (SED), naturally-incubated fecal casts (NF), and fecal casts on latrines (LF) were transferred to sterile 50-ml centrifuge tubes and held on ice until return to the laboratory.

Initially, 20-ml nine salt solution (NSS) (Westerdahl et al. 1991) was added to each sample. Bacteria were dislodged from the sediment by use of a short burst (20 s) of sonication with a 3-mm sonic probe (Branson Sonifier 250) at setting four on the output control (amplitude = 306 μm, power output = 65 W). Sonication optimization studies demonstrated that the highest average well color development (AWCD; index of cell respiration, see below for details) in the Biolog plates occurred with this duration and intensity of sonication. Aliquots from these dilutions were used for the 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) reduction assay (see below). Serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}) from these samples were used for Biolog plates, CTC assays, and total counts. Samples were then dried so as to normalize to dry g $sediment^{-1}$. Values for blanks (tubes of 20 ml NSS) were subtracted for each measurement.

Biolog GN microtiter plates (Biolog Inc.) were used to provide community-level physiological profiles. Each plate is loaded with 95 different carbon sources and a redox dye (tetrazolium violet). Bacterial respiration reduces the tetrazolium dye to formazan in active cells, so that the pattern of colored wells (different carbon sources) represents a metabolic fingerprint. To achieve the optimal dilution for the Biolog inoculations, 20 ml more NSS was added to each sample and vortexed for 30 s at high speed. Biolog plates were then inoculated with 150 μl $well^{-1}$ of the supernate by use of a multipipettor and incubated aerobically at room temperature. Color formation in microplate wells was analyzed at t = 0, 24, 48, and 72 h (and 96 h for *B. aurantiacus* samples) on a Titertek Multiskan Plus (Titertek) microplate reader at 570 nm. The AWCD for each plate was calculated as the average absorbance of the 95 test wells after subtracting the absorbance of the control well (blank) and setting any negative values equal to 0.

We enumerated metabolically active bacteria through time using the fluorogenic redox dye CTC (Polysciences, Inc.; Rodriguez et al. 1992). This tetrazolium salt is used as an artificial electron acceptor in the electron transport system, indicating oxidative cell metabolism. Aliquots (2 ml) were taken from the diluted, sonicated sample and transferred to 15-ml centrifuge tubes for the CTC reduction assay. CTC (200 μl of 25 mM) was added to each tube and incubated (4 h) at 28°C in the dark while shaking (200 rpm). Duplicate aliquots (1 ml) were retrieved from this incubation and fixed in formalin (4% final concentration) for direct counts of active and total bacteria.

Total counts were made with DAPI by use of a modified version of the protocol of Hymel and Plante (1998). Briefly, fixed samples were centrifuged at 4,000 $\times$ g for 15 min then resuspended in Trizma buffer (0.05 M, pH 8.10) and a dispersing agent (0.5% Triton-X 100) and sonicated for 20 s with a 3-mm sonic probe at 65 W. Samples were then stained with DAPI (5 μl ml^{-1}) for 20 min, recentrifuged to remove stain, and concentrated onto 0.2-μm black polycarbonate membranes (Poretics).

Both total and active counts were made from each slide with use of a Nikon epifluorescence scope at $\times$1,250. DAPI-staining bacteria were counted with use of a UV filter set (Omega XFO2, 330WB80 exciter, 400EFLP emitter). Active bacteria (CTC staining) were counted with use of a rhodamine filter set (Omega 605DF55, center wavelength = 605 nm, discriminating filter, full bandwidth at half-maximum transmission = 55 nm). For each sample and filter set, 20 grids (or more) were counted (for active or total counts) to include >200 cells per slide.

Although our field experiments followed a simple repeated-measures design, because covariance matrices of all error terms satisfied the Huynh-Feldt condition (Huynh and Feldt 1970; Milliken and Johnson 1984) we used ANOVA for the split-plot design to test for effects of time and sample type (or ''location'': sediment or isolated or naturally incubated fecal casts) on total bacterial abundance, numbers of active bacteria, and percentage of active bacteria. Prior to analysis, data were tested for normality (Lillefor's test) and heteroscedasticity (Levene's test) and natural log or square-root transformed as required (and retested). If main effects were significant, post hoc multiple comparison tests employed Fisher's LSD correction at an experimentwise α level of 0.05; if main effects were not significant, the more conservative Bonferroni's adjustment was used (Milliken and Johnson 1984).

Community-level physiological comparisons were conducted looking at two components: overall metabolic rate and resource utilization patterns (Garland 1997). To analyze the overall intensity of color development, AWCD after 72 h incubation for *N. succinea* and after 96 h for *B. aurantiacus* was contrasted among sample types by use of ANOVA, as described above for bacterial count data.

Similarities in carbon source use patterns of microbial communities were investigated by use of multivariate analysis. In order to visualize differences in community respiration patterns between sampling types, we used principal components analysis (PCA) with the Biolog data (Garland 1997). Additionally, with the first five principal components as variables, we used MANOVA to test for differences among the sample types (Glimm et al. 1997). All statistical analyses were conducted by use of SYSTAT for the Macintosh.

Epifluorescence microscopy—Total bacterial numbers in *N. succinea* samples showed a significant location effect ($P < 0.001$), whereas there was no effect of time ($P = 0.364$) but the time $\times$ location interaction term was highly significant ($P < 0.001$). At $t = 0$, LF and NF were statistically indistinguishable ($P = 0.799$), whereas both were significantly lower, by ~75% ($P < 0.001$ for both LF and NF), than SED samples (Fig. 2a). By $t = 2$ h, total bacterial numbers had increased in both NF ($P = 0.051$) and SED ($P = 0.014$) samples, such that NF numbers more closely resembled those of SED (although still statistically distinct; $P < 0.001$), and had become statistically greater than those of LF ($P < 0.001$) (Fig. 2a). Numerical patterns for the fraction of active bacteria was similar in that sample location ($P < 0.001$) and the interaction term ($P = 0.014$) were significant, whereas time was not ($P = 0.187$). Again, as for total counts, LF and NF values for active bacteria were similar to one another ($P = 0.708$), yet distinct from SED ($P = 0.001$ and 0.004 for LF and NF, respectively), at $t = 0$ h. Over the 2 h of incubation, again, significant numerical increases were observed in NF ($P = 0.047$) and SED ($P = 0.041$) samples, with no significant changes in LF ($P = 0.085$). By $t = 2$ h, all locations were distinct, with NF values intermediate to LF and SED (Fig. 2b). No significant differences were discernible over time ($P = 0.105$) or among locations ($P = 0.264$) with respect to percent active cells (Fig. 2c).

Trends were somewhat different for *B. aurantiacus.* Overall, the main effects of location ($P = 0.003$) and time ($P < 0.001$) were both significant with respect to total bacterial numbers, whereas the interaction was not ($P = 0.511$). Initially, total numbers were not different among location ($P = 0.087$ for NF vs. SED and $P = 0.528$ and 0.269 for LF vs. NF and SED, respectively) (Fig. 3a). After 2 h, significant decreases were noted in both fecal cast types ($P = 0.002$ and 0.001 for LF and NF, respectively) (Fig. 3a), resulting in significantly higher bacterial counts at 2 h in SED versus LF ($P = 0.019$) and NF ($P = 0.002$) (Fig. 3a). Counts of active bacteria revealed no significant temporal changes ($P = 0.367$), nor was location ($P = 0.115$) or interaction (0.406) found to be significant (Fig. 3b). Similar results, with no significant differences ($P = 0.624$, 0.057, and 0.321 for location, time, and interaction, respectively), were seen with percent active cells (Fig. 3c). The marginally significant overall time effect was primarily due to the increase in NF samples ($P = 0.028$ with LSD correction and not significant with Bonferroni's), whereas changes in LF or SED were not significant ($P > 0.340$ for both, by use of LSD or Bonferroni's correction).

Biolog—AWCD of Biolog plates inoculated with fresh ($t = 0$ h) *N. succinea* fecal samples from latrines or sediment surface were indistinguishable from one another ($P = 0.822$) but were significantly lower than SED readings, ($P < 0.001$, for both LF and NF) (Fig. 4a). In contrast, aged (2 h) fecal samples incubated naturally on ambient sediment resembled sediment samples ($P = 0.234$), whereas those feces incu-

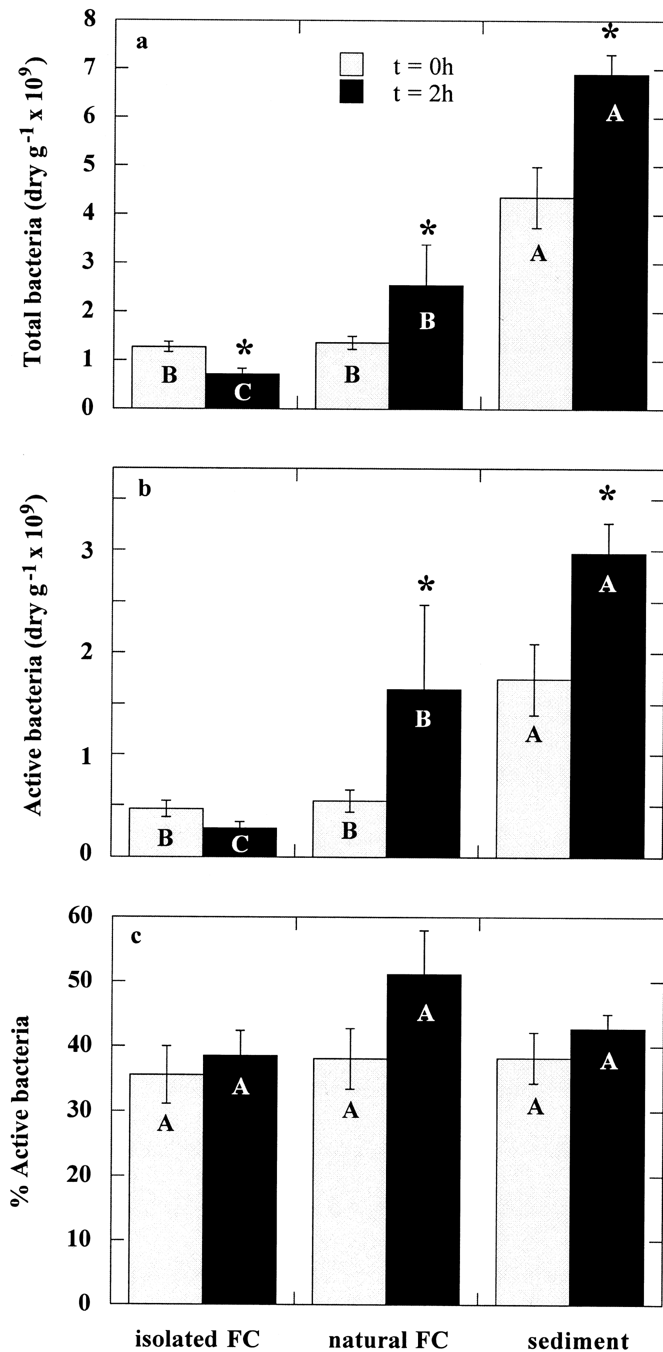

Fig. 2. Mean ± SEM of (a) total bacterial densities, (b) active bacteria, and (c) percentage of active cells through time in the egesta of *N. succinea* and surrounding sediment. A change in letter indicates a significant difference: black letters for $t = 0$ h, and white for $t = 2$ h. Asterisks indicate significant temporal change. FC, fecal casts.

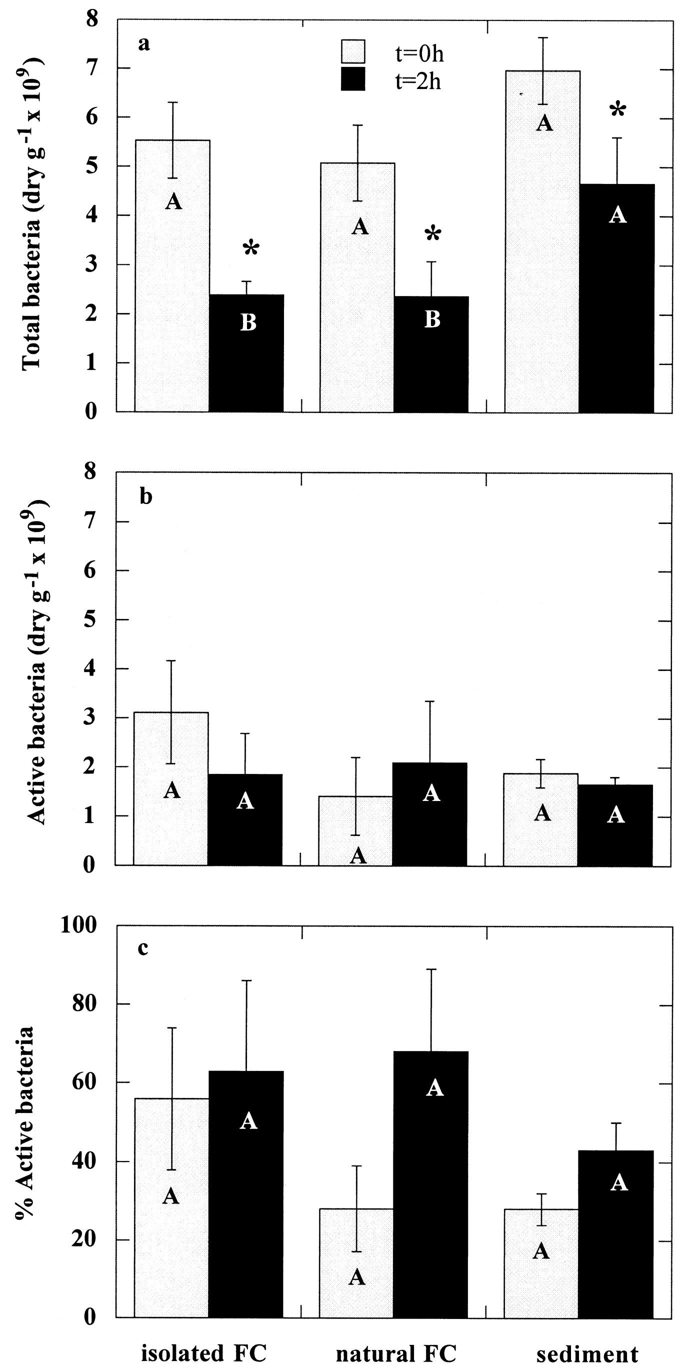

Fig. 3. Mean ± SEM of (a) total bacterial densities, (b) active bacteria, and (c) percentage of active cells through time in the egesta of *B. aurantiacus* and surrounding sediment. A change in letter indicates a significant difference: black letters for $t = 0$ h, and white for $t = 2$ h. Asterisks indicate significant temporal change.

bated on latrines did not differ significantly from the 0 h readings ($P = 0.894$) but were lower than both the NF ($P = 0.056$) and SED ($P = 0.003$) samples (Fig. 4b).

PCA on the individual well responses of Biolog plates revealed differences in the catabolic profiles between fecal and sediment samples at $t = 0$ h; a plot of the first two principle components illustrates that isolated and natural fecal samples were similar but that both were distinct from surrounding sediments (Fig. 5a). Two-sample comparisons employing the first five PCs statistically corroborate these results in that SED samples differed from fecal samples ($P = 0.001$ and 0.014 vs. LF and NF, respectively; MANOVA), whereas LF and NF did not differ significantly ($P = 0.494$).

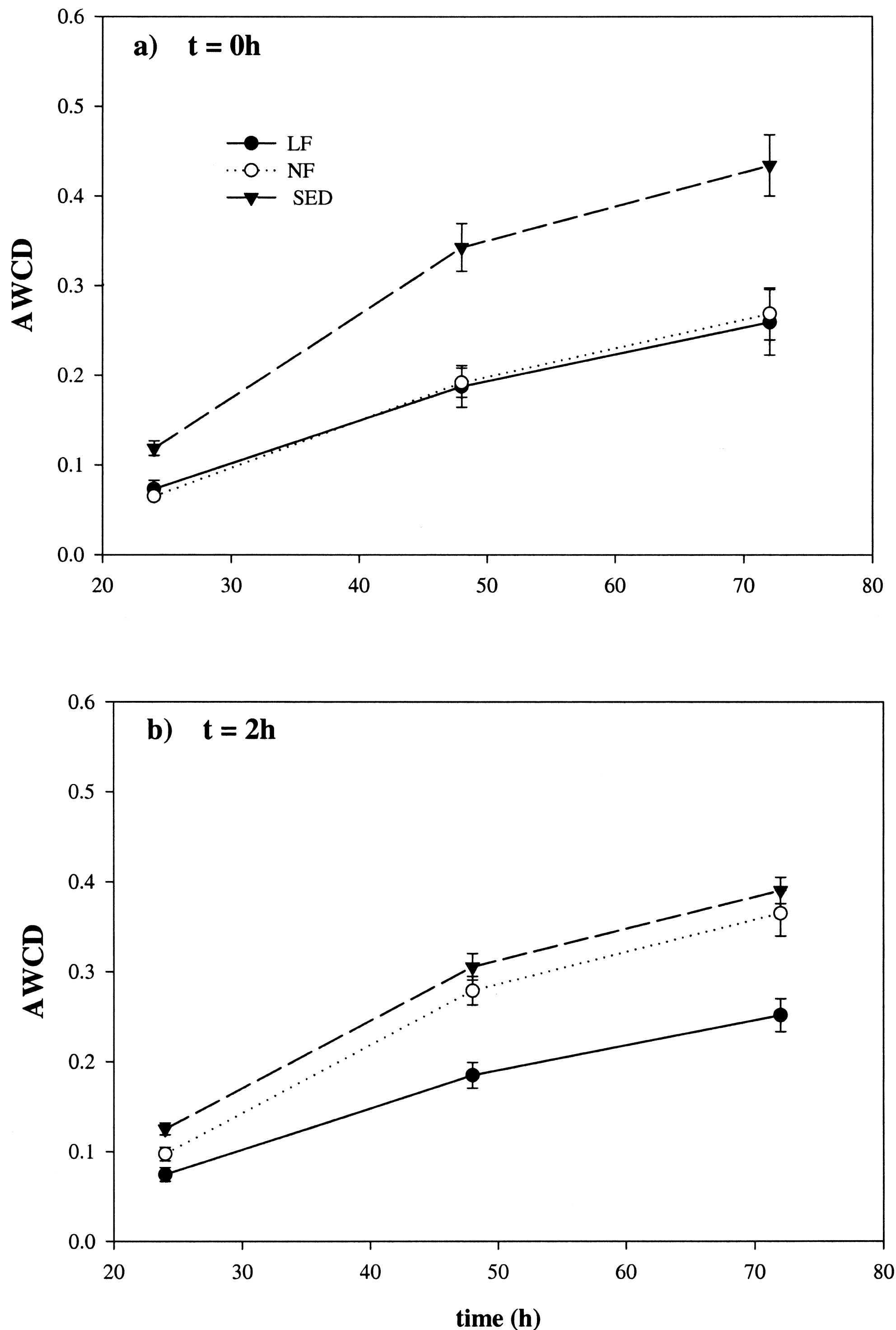

Fig. 4. Time course for AWCD (mean ± SEM) in Biolog plates inoculated with *N. succinea* (a) fresh and (b) aged fecal coils and adjacent sediment.

After 2 h of incubation, however, natural fecal samples and surrounding sediments were less distinguishable (Fig. 5b; $P = 0.023$, MANOVA with first five PCs), whereas isolated and natural fecal samples diverged (Fig. 5b; $P = 0.004$, MANOVA with first five PCs). SED and LF remained statistically different ($P = 0.001$; MANOVA). Comparisons of individual substrate usage were also performed via ANOVA. For those substrates that differed significantly (which generally coincided with correlates of PCs 1 and 2), pairwise comparisons (with use of Fisher's LSD correction) revealed that, with the one exception of α-ketobutyric acid, metabolic response was always (10 of 11 significant differences) great-

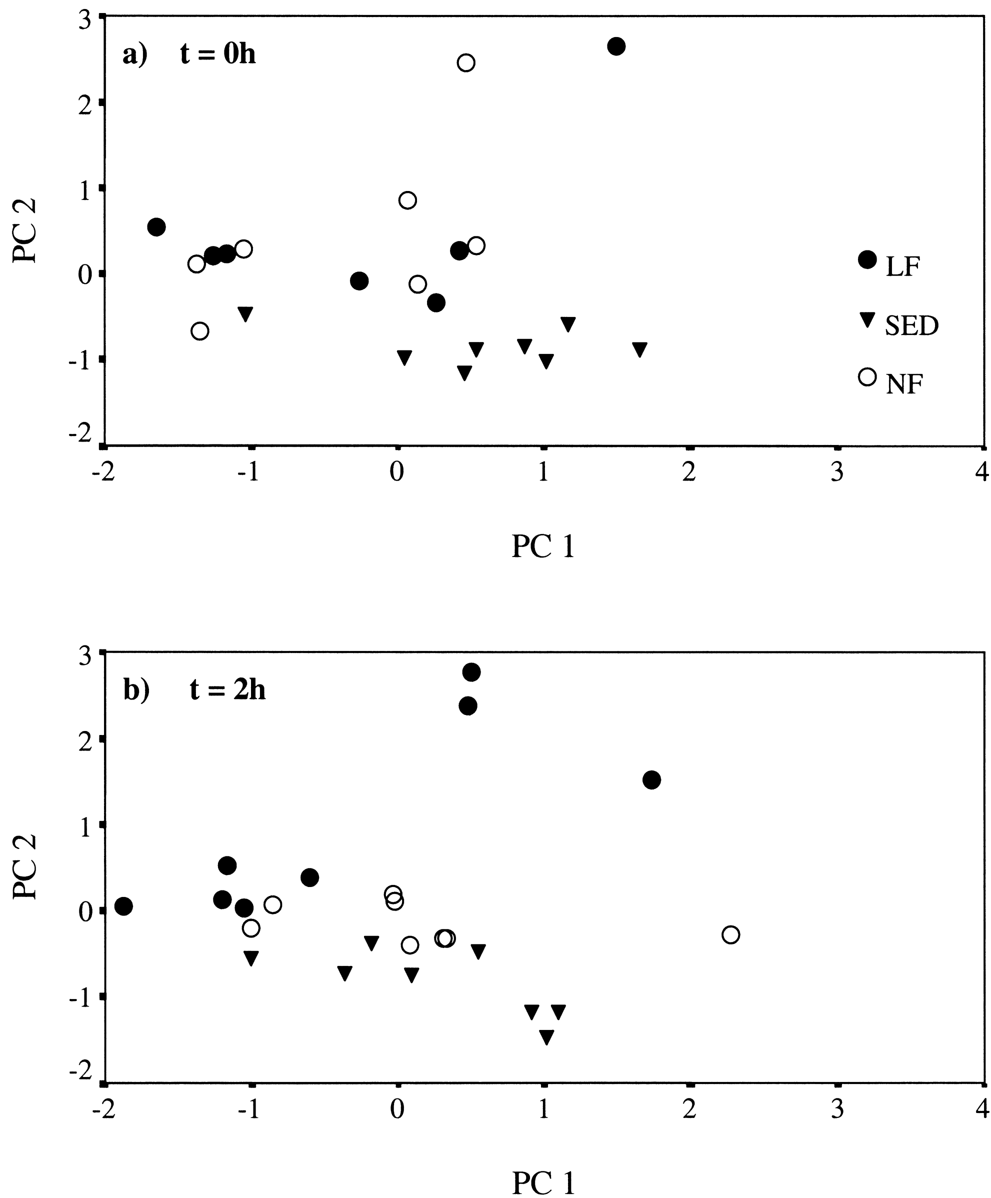

Fig. 5. Comparison of catabolic profiles from Breach Inlet sediment and fecal matter of *N. succinea*; plot of first (PC 1) and second (PC 2) principal components derived from PCAs of 72-h Biolog readings.

er in SED than in fecal samples at $t = 0$ h (data not shown). Similarly, at $t = 2$ h, 12 substrates were used more in SED samples (comparing now only to LF), whereas only glycyl-L-glutamic acid showed a significantly stronger response with LF inoculum (data not shown). Bacterial assemblages in *N. succinea* egesta appear to be a subset of ingested surficial sediments, with little indication of novel activities or strains associated with these microhabitats.

AWCD trends for *B. aurantiacus* were similar to those for *N. succinea* in that the two types of fecal samples exhibited a significantly weaker overall response than sediment samples at $t = 0$ h ($P < 0.001$ for both), yet were quite similar to one another ($P = 0.961$) (Fig. 6a). By 2 h, LF and SED samples resembled those taken at $t = 0$ h ($P = 0.151$ and 0.565, respectively), whereas the NF sample responses increased with age ($P = 0.002$) and gave AWCD that were significantly different than either LF ($P < 0.001$) or SED ($P < 0.001$), and were intermediate between them (Fig. 6b).

PCA results mirror those of the AWCD in that sediment samples appear to be distinct ($P < 0.001$ vs. both NF and LF, MANOVA) from the indistinguishable ($P = 0.304$) fecal samples at $t = 0$ h (Fig. 7a), but, by 2 h, all three sample types were distinct, with NF samples apparently intermediate (Fig. 7b). MANOVA employing the first five PCs corroborate these results ($P = 0.023$ for SED vs. NF, $P < 0.001$ for LF vs. both SED and NF). Closer examination of individual well responses presents a different picture for *B. aurantiacus* compared with *N. succinea.* ANOVA revealed that at $t = 0$

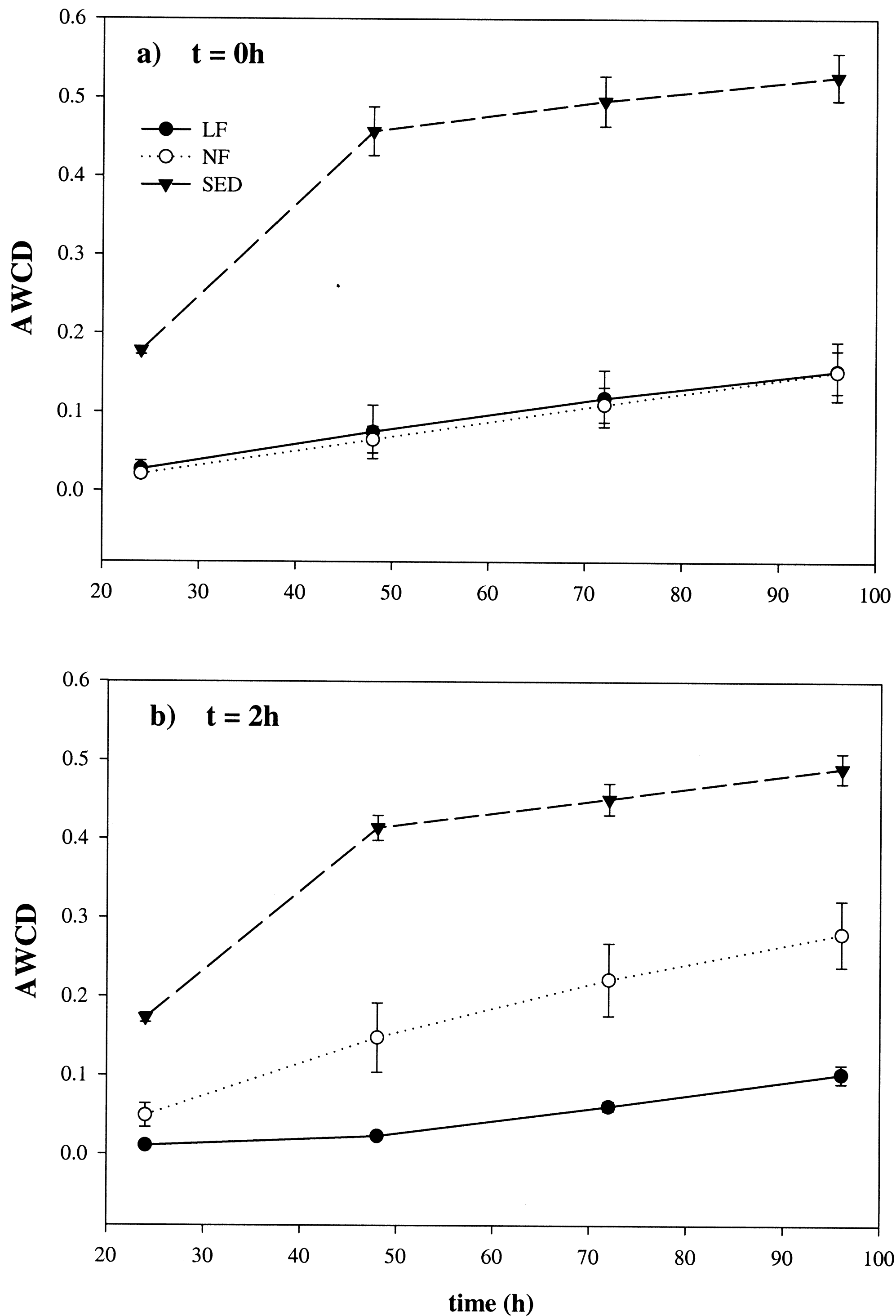

Fig. 6. Time course for AWCD (mean ± SEM) in Biolog plates inoculated with *B. aurantiacus* (a) fresh and (b) aged fecal coils and adjacent sediment.

h, significant differences between SED and either fecal sample type (usually both) were observed in the use of 24 substrates. Ten of these exhibited a more intense response in fecal samples (data not shown). The pattern persisted through 2 h, with 14 substrates used significantly more in LF versus SED samples (with 21 in the reverse direction). Amino acids in particular appeared to be more used by bacteria in *B. aurantiacus* egesta (six were used significantly more in LF, whereas two were used more in SED; data not shown). Especially given the significantly greater overall response in SED AWCD, it is clear that distinct bacterial assemblages are associated with *B. aurantiacus* egesta.

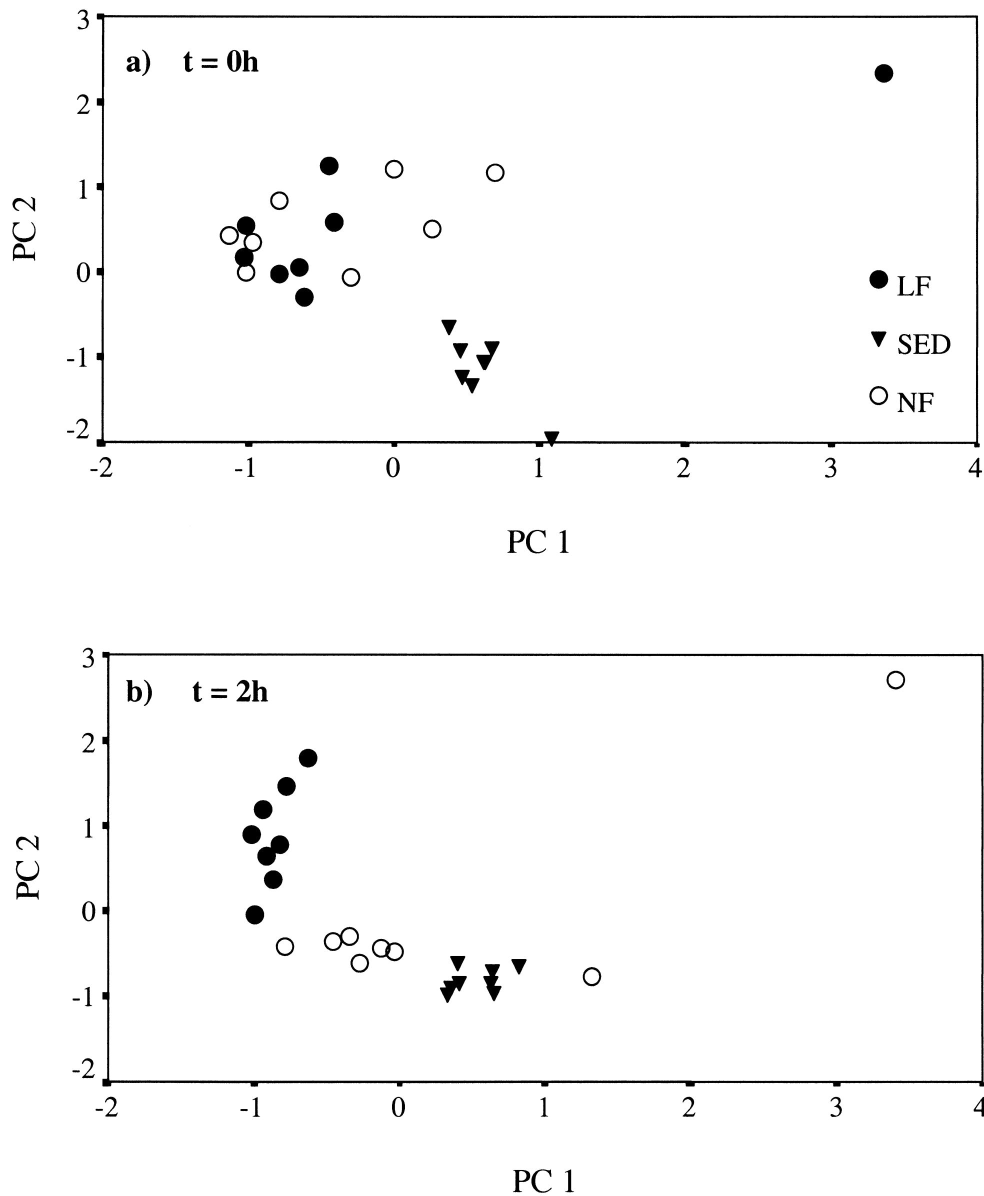

Fig. 7. Comparison of catabolic profiles from Breach Inlet sediment and fecal matter of *B. aurantiacus*; plot of first (PC 1) and second (PC 2) principal components derived from PCAs of 96-h Biolog readings.

Conclusions—The hypothesis that bacterial recolonization of egesta is by migration from underlying sediments was supported by the experiments conducted with the deposit feeders, *N. succinea* and *B. aurantiacus,* yet the patterns of digestive removal and recovery were both quite different. In *N. succinea,* clearly bacteria were removed because of gut passage, given that both numbers (total and active) and metabolic diversity (e.g., Biolog AWCD) were reduced in fresh egesta, compared with surrounding sediments. Although Biolog responses were reduced, no significant (net) numerical differences were noted between ambient sediment and fresh *B. aurantiacus* fecal material. The apparent depression of metabolic response may be more a result of compositional shifts, i.e., to strains unculturable on Biolog plates, than of numerical losses. These observations regarding *B. aurantiacus* are in agreement with those of Wilde and Plante (pers. comm.), who likewise saw no significant differences in epifluorescence counts of total or active bacteria between Breach Inlet sediments and *B. aurantiacus* egesta but did record significant losses in heterotrophic plate counts, AWCD, and metabolic diversity (Biolog substrate use). One possible explanation is that the relative proportion of anaerobes increased because of gut passage. Previous work has shown that the bulk of gut contents of large deposit feeders is anoxic (Plante and Jumars 1992). Although unquantified, it is clear that at least a fraction of fresh *B. aurantiacus* casts are reducing, as is indicated by their black or gray color (pers. obs.). More generally, Dobbs and Guckert (1988) con-

cluded that the confamilial *Ptychodera bahamensis* digests ~30% of total prokaryotic fatty acid biomass (a small proportion relative to deposit feeders in general; Kemp 1990) but that particular bacterial functional groups (e.g., those with the anaerobic desaturase pathway) increase with gut passage. In addition, bacteriolytic activity of the gut fluids of enteropneusts has previously been observed to be weak relative to deposit-feeding polychaetes and holothuroids (Plante and Mayer 1994; Plante and Shriver 1998).

Patterns of numerical recovery of bacterial assemblages in egesta also showed some differences between the two deposit feeders. When *N. succinea* egesta were isolated from underlying sediments, bacterial numbers continued to decline, whereas Biolog responses were unchanged, between 0 and 2 h. This continued lysis is somewhat surprising, given that bacteriolytic agents (Plante and Mayer 1994) and digestive enzymes (Mayer et al. 1997) of polychaetes are found in low levels in the posterior gut and, presumably, the feces. Some cells observed in fresh egesta may have been dead or damaged but not yet lysed; Plante and Mayer (1994) showed that the digestive fluids of the polychaete, *Arenicola marina,* kills bacteria well before it lyses them. Alternatively, oxygenation over the 2 h of air exposure could have killed strict anaerobes in fecal casts. This could result in reduced direct counts, yet Biolog results would not be affected, because these anaerobes would be essentially invisible at 0 or 2 h whether active or dead. In contrast, in egesta incubated on underlying sediments, active and total cell numbers increased. Metabolic responses increased and began to approach those seen for ambient sediments, although recovery was incomplete over the 2-h incubation. Likely, the delayed loss of cells was in this case simply overwhelmed by the immigration of bacteria from adjacent sediments.

Recovery patterns differed for the egesta of *B. aurantiacus* in that total bacterial numbers continued to decrease in both isolated egesta and those incubated on the sediment surface. It would appear that either the rate of migration into the feces of *B. aurantiacus* is lower or the rate of continued bacteriolysis is higher, compared with that of *N. succinea.* Although hemichordates are well known to produce halogenated aromatics, concentrations of these compounds in fecal materials is low and is unlikely to inhibit bacteria (King 1986). Moreover, changes in metabolic profiles of these aged feces clearly suggest that immigration does occur, albeit not as rapidly as in *N. succinea* casts. The prospect that bacteriolytic rates in egesta might be relatively high is supported by observations that digestive agents in other hemichordates are not confined to midgut regions but rather can be found at fairly uniform levels throughout the alimentary canal (and potentially into the feces; Plante and Mayer 1994). It is also possible that redox changes could cause these declines, as hypothesized above for *N. succinea.* Although we know of no published studies with regard to redox changes in fecal casts, temporal changes are obvious as those initially black casts of (some) *B. aurantiacus* quickly turn to the brown color of surrounding sediments (pers. obs.).

Given that recovery was absent when egesta were isolated on latrines, yet rapid (but incomplete) in fecal materials incubated on sediments, the simplest interpretation is that recolonization is primarily by immigration from adjacent sediments. Our initial assumption was that elevated digestive reactants or products in egesta could result in rapid bacterial growth, thus accounting for recovery of microbial communities in these disturbed sediments. Both treatments should have been similar with respect to these potential nutritive sources. The latrines, however, isolated egesta not only from bacterial migration but also from chemical exchange with sediments (as well as potential protozoan and metazoan predators). If we assume *B. aurantiacus* fecal coils to be cylinders of radius on the order of 0.3 cm, we can calculate that a minimum of 80% of solute gradients between egesta and sediments would disappear in 2 h (see Plante and Jumars 1992). Thus, naturally incubated feces could have become enriched in some growth-stimulating solutes as they aged, relative to feces on latrines. It is highly unlikely that a rapid equilibration of solute concentration could have led to the numerical responses observed within our 2-h incubations, however, given that doubling times in porewaters of sandy intertidal sediments generally are on the order of days (e.g., Fallon et al. 1983; Karl and Novitsky 1988; Deming and Baross 1993 and references within). Outward diffusion of metabolic byproducts would also be inhibited on latrines. These metabolites do not appear to limit bacterial growth, however, because similar conditions prevail in hindguts (Plante et al. 1990), yet growth is stimulated (Deming and Colwell 1982; Plante et al. 1989).

Because the present study examined bacterial recovery in only two sympatric species of deposit feeder at one intertidal site, there remains the question of generalizability. That the study was restricted to the intertidal should not preclude the ability to make inferences about subtidal settings. Although colonization from overlying waters could occur in the latter and was avoided in our study, this would no doubt be trivial in comparison to colonization from sediments. Although fecal materials are likely to disrupt boundary layer flow, potentially enhancing the flux of both nutrients and microorganisms, these effects are unlikely to be important in the early stages of recolonization and succession (although subsequent effects on growth may not be trivial), because bacterial numbers in waters are typically only 0.1%–1% of those in sediments (Schmidt et al. 1998). Variation in the physical nature of egesta, however, might indeed limit the extrapolation of our findings. Although many suspension- and deposit-feeding detritivores produce fecal pellets packaged within mucus coatings, both animals studied by us egest feces with no such covering. We know of no studies that have examined the permeability of the pellet membrane in deposit feeders, but those studies of terrestrial (e.g., Peters 1976) and marine pelagic (Gowing and Silver 1983) invertebrates suggest that the membrane is an effective barrier to bacterial migration, at least until it begins to degrade. In those deposit feeders producing such pellets, the importance of in situ growth of ingested or enteric bacteria relative to immigration likely is greater, as has been argued for pelagic pellet-producing invertebrates (Gowing and Silver 1983; Nagasawa 1992).

Previous studies have shown that bacterial growth (Deming and Colwell 1982; Plante et al. 1989) and activity (Juniper 1981; Plante and Jumars 1992) can be accelerated in the guts and fecal casts of deposit feeders, compared with sediments. Our latrine samples allow us to isolate the effects

of in situ growth within casts from numerical increases resulting from immigration. In casts of neither animal were substantial numerical or metabolic recoveries observed via growth. These results would seem to call into question the assumptions of numerous previous studies (e.g., Juniper 1981; Levinton and Bianchi 1981; Grossmann and Reichardt 1991; Wilde and Plante pers. comm.) regarding the cause of higher numbers or activities in fecal casts. Our findings do not preclude growth within the gut or feces, however, but rather suggest that any such growth was, in our study, overwhelmed by immigration. Indeed, increased metabolic response in latrine feces from 0 to 2 h for a few Biolog wells (substrates) suggest that select (symbiotic and/or digestion-resistant) bacterial groups are growing within the fecal casts. Moreover, our study examined only two deposit-feeding species and may not represent all or even most other taxa.

To summarize, we observed rapid colonization of fecal casts via migration from underlying sediments. One significant effect is that the sedimentary resource for deposit-feeding invertebrates should be renewed more rapidly than what would be expected through repopulation by survivors of ingestion. Bacteria can swim much faster than they can grow and divide, and in the latter case of in situ growth, grazer-resistant taxa could come to dominate fecal casts and sediments. A second consequence is that it is unlikely that deposit feeding leads to important changes in microbial community structure. We found little evidence that guts and egesta serve as spatio-temporal refugia for "fugitive" bacterial strains, although numerous lines of evidence suggest that aerobically incubated Biolog plates may be a poor method to detect these taxa (Konopka et al. 1998).

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Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters

Abstract—Bottle incubations, during which the activity and growth of prokaryotes is monitored during several days, are frequently carried out to study functional aspects of marine prokaryotic assemblages. These experiments will relate directly to in situ activities if all populations grow harmonically during the incubation. We tested whether this was the case by analyzing the composition of bacterial assemblages at the beginning and at the end of the incubation by denaturing gradient gel electrophoresis. Five experiments were done in different Antarctic regions. Bacterial assemblages north and south of the Polar Front were very different. In all cases, the final assemblages were very different from the initial ones, and these changes were often accompanied by a significant decrease of diversity indices. Our experiments included treatments with different temperature and organic matter amendments. Whereas the increase in temperature tested had a minor effect on prokaryotic growth rate and specific composition, the addition of organic matter strongly stimulated growth rate and selected a particular bacterial assemblage in some experiments but not in others. A significant component of bacterial assemblages from waters south of the Polar Front appeared to be *Polaribacter franzmannii,* a gas vacuolated bacterium of the Cytophaga-Flavobacterium-Bacteroides group that was originally isolated from Antarctic sea ice. This phylotype was enriched and dominated in almost all final assemblages. Our results indicate that long-term bottle incubations mostly measure the activity of a few opportunistic bacteria and not that of the original assemblage. This should be taken into account if data obtained in these experiments are used for balancing whole ecosystem carbon budgets and to derive biogeochemical conclusions.

Controlled incubations are often used to determine the activities of marine prokaryotic assemblages. In some cases, incubations last only a few hours, during which the composition of the assemblage is probably stable. Examples of such short-term incubations are experiments to estimate prokaryotic production (Kirchman et al. 1985) or the proportion of actively respiring cells (Sherr et al. 1999*a*). Other experiments, however, require much longer incubation times. In such experiments, grazing by protists is minimized by dilution or filtration, and the response of the prokaryotic assemblage is measured by cell growth. Examples of this approach are incubations used to determine empirical conversion factors of leucine or thymidine incorporation to bacterial production (Kirchman and Ducklow 1993; Ducklow et al. 1999) or to estimate prokaryotic growth efficiency or rates of dissolved organic carbon degradation (Carlson and Ducklow 1996; del Giorgio and Cole 1998). Other examples are bioassays to study potential limiting factors, such as temperature or organic and inorganic nutrients (Kirchman