was delayed until the mid-Cretaceous and thus cannot be linked simply to the presumed adaptive value of the drilling habit.

The apparent reinvention of the drilling habit in naticids, or other instances of parallel or iterative evolution within a single clade, fits well with some interpretations of the role of development in the evolution of morphological novelties. In a hierarchically organized developmental system, recurrence of certain morphologies is to be expected because (i) developmental constraints limit and channel the spectrum of available innovations, and (ii) major phenotypic effects can be the result of relatively few or small changes in regulatory genes (*22*). We are not claiming that enzymes, radula, and other features of Triassic drilling naticids were identical to those of Cretaceous drilling naticids, or that the drilling habit arose each time in a single evolutionary step, but that ancestors of both the Triassic and Cretaceous forms possessed similar anatomies [and thus similar preadaptations (*23*)] and similar developmental programs that would tend to respond to genetic modifications and subsequent selection in similar ways (*24*). With regard to the naticids, then, the more difficult problem may be why there is a gap of 120 million years between the two origination events (*25*).

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23. Or, more strictly, potential exaptations [S. J. Gould and E. S. Vrba, *Paleobiology* **8**, 4 (1982)].
24. A second, and no less spectacular, example of parallel evolution in carnivorous gastropods is the apparently independent derivation in two turrid subfamilies of the true toxoglossan, hypodermic radula. This hollow, barbed, dartlike tooth allows rapid capture and envenomation of active prey [R. L. Shimek and A. J. Kohn, *Malacologia* **20**, 423 (1981)].
25. C. R. Newton, *Geol. Soc. Am. Abstr. Programs* **15**, 652 (1983) reports possible naticid drillholes in Late Triassic mollusks in the Wrangellian Terrane of western North America. If these were also produced by naticids, they could indicate that the short-lived drilling lineage had reached a more widespread Tethyan distribution before terminating at the end of the Triassic. The Jurassic–Early Cretaceous gap remains.
26. We thank R. Zardini of Cortina d'Ampezzo, Italy, for the loan of the bulk of the investigated material and M. R. Carriker, S. M. Kidwell, J. A. Kitchell, N. F. Sohl, and two anonymous reviewers for comments. Supported in part by NSF grant EAR 81-21212 (D.J.).

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An Unusual Phycoerythrin from a Marine Cyanobacterium

Abstract. *Phycoerythrin conjugates are reagents for cell sorting and analyses in which the argon-ion laser line at 488 nanometers is used for excitation. Many marine* Synechococcus *strains contain phycoerythrins with absorption maxima at approximately 490 and 550 nanometers; these maxima indicate the presence of phycourobilin and phycoerythrobilin prosthetic groups in the protein. Phycoerythrins of red algae contain both groups, but those of freshwater and soil cyanobacteria contain only phycoerythrobilin. Phycoerythrin purified from* Synechococcus *WH8103 has molecular properties typical of red algal phycoerythrins, but its phycourobilin content is higher than that of other phycoerythrins. The protein has absorption maxima at 492 and 543 nanometers and corresponding molar extinction coefficients of 2.78 and 1.14 × 10^6; it fluoresces maximally at 565 nanometers with a quantum yield of 0.5. Conjugates of* Synechococcus *WH8103 phycoerythrin could increase the sensitivity of cell analysis techniques to almost twice that possible with other phycoerythrin conjugates.*

Phycoerythrins form a part of the photosynthetic light-harvesting antennae of cyanobacteria and red algae (*1*). In red algae, the visible absorption spectra of these proteins show peaks at about 566 nm and peaks or shoulders at about 540 and 500 nm with varying relative intensities (*2*). Red algal phycoerythrins carry two types of covalently attached tetrapyrrole prosthetic groups, phycoerythrobilin (PEB) and phycourobilin (PUB) (*3*). The PEB groups give rise to the 566- and 540-nm peaks, and the PUB's give rise to the 500-nm peak. In contrast, phycoerythrins purified from cyanobacteria isolated from soil or fresh water contain only PEB groups and do not exhibit the 500-nm peak. The cyanobacterium *Gloeobacter violaceus* does contain a phycoerythrin with both PEB and PUB chromophores (*4*), but this organism is atypical in other respects as well (*5*). The difference in the bilin composition of red algal and cyanobacterial phycoerythrins may be related to the changing nature of solar radiation as it penetrates seawater. Marine algae are exposed to maximum transmission of light at approximately 500 nm (*6*), and the presence in these organisms of a photosynthetic accessory pigment (PUB) that absorbs maximally at this wavelength appears to be more than coincidental.

Unicellular cyanobacteria containing

phycoerythrin have been observed in abundance among marine phytoplankton (*7, 8*), and these organisms make a major contribution to the primary productivity in the ocean (*7*). Although the phycoerythrin of several of these organisms is of the ordinary cyanobacterial type, it is interesting that many strains contain phycoerythrins with both PEB and PUB chromophores (*9*). Phycoerythrin purified from a marine *Synechococcus* contains the largest number of PUB chromophores per molecule of any known phycoerythrin.

Synechococcus strain WH8103 was cultured in a natural seawater–based medium (*10*) at 23°C in a Fernbach flask; continuous warm white light (intensity of about 8×10^{-6} E/m^2·sec) was supplied, and the entire mixture was swirled once daily. Cells were harvested by centrifugation from approximately 3-week-old cultures that had reached an absorbance of about 0.15 at 750 nm. Phycobilisomes (*11*) were prepared as described by Yamanaka *et al.* (*12*) with the exception that our breakage buffer contained 0.01*M* EDTA and 0.001*M* phenylmethylsulfonyl fluoride. Preparation of phycoerythrin was as follows. Phycobilisomes were dialyzed against a 0.05*M* sodium phosphate–0.001*M* NaN_3 buffer (*p*H 7.0) for at least 3 hours to dissociate the particles and reduce the sucrose concentration. Ammonium sulfate was then added to 65 percent saturation, and the solution was allowed to stand overnight at 4°C. The precipitate was collected by centrifugation, resuspended in the *p*H 7.0 buffer, and dialyzed exhaustively against the same buffer at 4°C. The solution was then layered on density gradients of 0.2, 0.4, 0.6, 0.8, and 1.0*M* sucrose in the *p*H 7.0 buffer in 2.2-ml increments. Ultracentrifugation was performed in a Spinco SW41 rotor at 66,000g_{av} for 22.5 hours at 18°C, during which phycoerythrin sedimented down the gradient farther than the other biliproteins. The phycoerythrin zone was collected, and the protein was recovered by precipitation with ammonium sulfate as described above. The precipitate was dissolved in 0.001*M* sodium phosphate–0.1*M* NaCl (*p*H 7.0) at 4°C and dialyzed against the same buffer. The solution was applied to a column of hydroxylapatite (settled bed volume of 0.4 ml per milligram of protein), equilibrated with 0.001*M* sodium phosphate–0.1*M* NaCl (*p*H 7.0), and developed with buffers of increasing phosphate concentration. Pure phycoerythrin was eluted with 0.03 to 0.04*M* sodium phosphate–0.1*M* NaCl (*p*H 7.0).

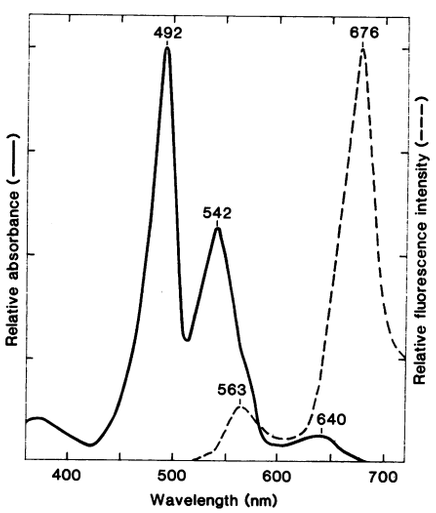

Fig. 1. Absorption and fluorescence emission spectra of *Synechococcus* WH8103 phycobilisomes in 0.75*M* sodium potassium phosphate buffer (*p*H 8.0). Excitation for the emission spectrum was at 490 nm, and the excitation and emission monochromator slits were set at 4-nm bandpass.

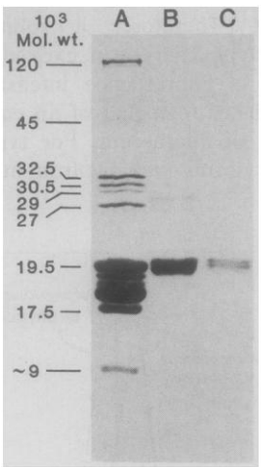

Fig. 2. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate of (lane A) *Anabaena variabilis* phycobilisome polypeptides as molecular weight markers; (lane B) WH8103 phycoerythrin; (lane C), a smaller amount of WH8103 phycoerythrin than applied in lane B to allow discrimination between the α and β subunits.

The absorption and fluorescence emission spectra of WH8103 phycobilisomes are shown in Fig. 1. The emission spectrum is similar to the spectra observed for cyanobacterial phycobilisomes of various biliprotein compositions (*11*). Energy absorbed by the PUB chromophores of WH8103 phycoerythrin is transferred efficiently to the terminal energy acceptors of the phycobilisome, which are responsible for the 676-nm emission peak (*11*). The small emission peak at 563 nm represents the direct emission of fluorescence from phycoerythrin.

Several properties of purified WH8103 phycoerythrin were characterized. The molecular weight of the native protein was determined by ultracentrifugation on linear sucrose density gradients by the method of Martin and Ames (*13*). WH8103 phycoerythrin settled as a single zone with a sedimentation velocity identical to that of *Porphyridium cruentum* B-phycoerythrin, for which a molecular weight of 240,000 has been determined (*14*).

On polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (Fig. 2), WH8103 phycoerythrin showed a polypeptide composition similar to that of red algal B- and R-phycoerythrins with α and β subunits of 19 to 20 kilodaltons and three red bands in the γ subunit region of about 29 kilodaltons (*14, 15*). Comparison of the amino acid composition of WH8103 phycoerythrin with that of *P. cruentum* B-phycoerythrin (Table 1) reveals a similarity between these two proteins. The bilin composition of WH8103 phycoerythrin was determined by the method of Glazer and Hixson (*14*) from spectra of solutions of known protein content in 8*M* urea (*p*H 1.9). Molar extinction coefficients (ε) used for PUB in this solvent were 104,000 (490 nm) and 0 (550 nm); those for PEB in the same solvent were 13,700 (490 nm) and 49,000 (550 nm) (*14, 16*). These measurements indicated the pres-

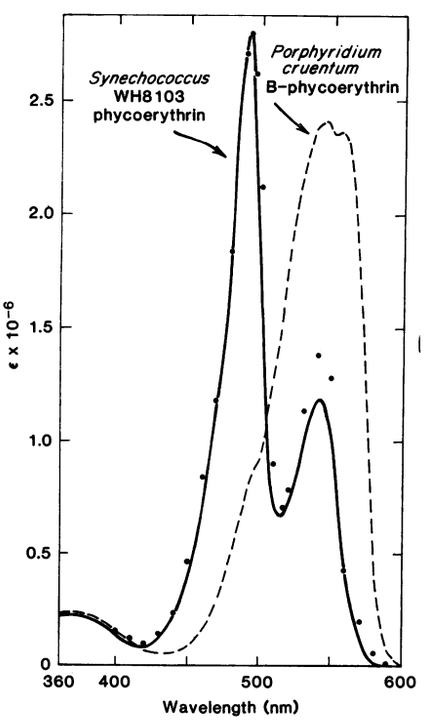

Fig. 3. Molar absorption spectra of WH8103 and *Porphyridium cruentum* phycoerythrins. Closed circles represent values from an excitation spectrum of WH8103 phycoerythrin determined for emission at 595 nm. The absorption and excitation spectra were normalized at 492 nm.

ence of 20.5 PUB and 13 PEB groups per 240,000 daltons of WH8103 phycoerythrin. Both red algal B- and R-phycoerythrins carry 34 bilins per 240,000 daltons (*15, 16*).

The molar absorption spectrum of WH8103 phycoerythrin is compared to that of *P. cruentum* B-phycoerythrin in Fig. 3. The numerous PUB chromophores in WH8103 phycoerythrin give rise to the sharp peak at 492 nm. *Porphyridium cruentum* B-phycoerythrin contains only two PUB groups (*14*). The fluorescence emission spectra for 490 nm excitation of WH8103 and *P. cruentum* phycoerythrins are compared in Fig. 4. Quantum yield determinations were performed on these two proteins with a Perkin-Elmer MPF44B spectrofluorimeter equipped with a DCSU-2 corrected spectra unit, a 500-nm blazed emission grating, and a Hamamatsu R926 phototube. Rhodamine-101 in methanol was used as a primary standard with a quantum yield (Q) of 1.0 as in the method of Karstens and Kobs (*17*). Measurements were performed at a sample absorbance of 0.05 cm^{-1} at the excitation wavelength of 490 nm. For WH8103 phycoerythrin, $Q = 0.50$; for *P. cruentum* B-phycoerythrin, $Q = 0.57$. Upon 532-nm excitation with a mode-locked Nd^{3+}:YAG laser, the fluorescence of WH8103 phycoerythrin exhibited single-component exponential decay with a lifetime of 1.85 ± 0.08 nsec. This indicates that the procedure used leads to a preparation of phycoerythrin of uniform size. A lifetime of 2.1 ± 0.1 nsec has been determined for *P. cruentum* B-phycoerythrin under similar conditions (*18*).

All of these observations indicate that WH8103 phycoerythrin is similar in its molecular and energy transfer properties to phycoerythrins obtained from red algae. However, the distinctive feature of this protein—its high content of PUB—makes this phycoerythrin potentially valuable as a fluorescent tag for analytical use.

The value of biliprotein conjugates as reagents for fluorescence-activated cell sorting and analysis, fluorescence microscopy, and fluorescence immunoassay has been shown (*19, 20*). Phycoerythrin conjugates emit in the orange region of the spectrum, where background autofluorescence of cells is much lower than at shorter wavelengths. The fluorescence intensity of such conjugates is also higher than that of fluorescein conjugates. For example, for excitation at the argon-ion laser line (488 nm), $\epsilon = 2.78 \times 10^6$ and $Q = 0.5$ for WH8103 phycoerythrin; the corresponding values for fluorescein are $\epsilon = 8 \times 10^4$ and $Q = 0.9$. Therefore, a solution of WH8103 phycoerythrin excited at 488 nm has a fluorescence intensity 19.3 times higher than that of an equimolar solution of fluorescein. For typical R-phycoerythrins available from numerous red algae (*2*), $\epsilon = 1.28 \times 10^6$ (*19*). Assuming that these proteins have a fluorescence quantum yield similar to that of B-phycoerythrin ($Q = 0.57$), the corresponding fluorescence intensity ratio for these proteins relative to fluorescein is 10.1. A phycoerythrin-fluorescein intensity ratio of 10 was measured when equimolar solutions of *Gastroclonium coulteri* R-phycoerythrin and fluorescein were fluxed through a cell sorter (*19*). Hence, WH8103 phycoerythrin conjugates would nearly double the sensitivity of assays employing this class of biliproteins.

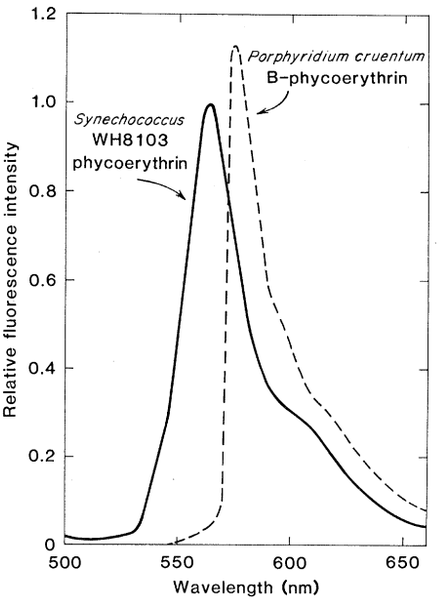

Fig. 4. Fluorescence emission spectra of WH8103 and *Porphyridium cruentum* phycoerythrins. Excitation was at 490 nm and sample absorbance at the excitation wavelength was 0.05 cm^{-1}.

Table 1. Comparison of the amino acid compositions of WH8103 phycoerythrin and *Porphyridium cruentum* B-phycoerythrin. Lyophilized protein samples (~ 0.25 mg) were dissolved in 6*N* HCl containing 0.15 (weight to volume) percent phenol and hydrolyzed in vacuo at 110°C for 24, 48, and 72 hours. A value of 14 residues of His and 6 residues of Trp and a molecular weight of 240,000 were assumed for WH8103 phycoerythrin. Data on *P. cruentum* B-phycoerythrin are from (*4*). S.E.M., standard error of the mean.

Amino acid	Value (mean ± S.E.M.)*	
	WH8103	*P. cruentum*
Lys	85.0 ± 0.8	84.2 ± 2.1
His	(14.0)	13.8 ± 0.7
Arg	115.0 ± 0.6	117.4 ± 2.6
Asp	221.5 ± 0.8	228.3 ± 1.6
Thr†	104.7	75.1
Ser†	171.4	172.8
Glu	157.7 ± 0.6	152.9 ± 2.1
Pro	97.9 ± 1.9	63.6 ± 2.4
Gly	184.8 ± 0.5	151.3 ± 2.8
Ala	309.0 ± 0.9	320.3 ± 6.9
1/2 Cys	34.4 ± 0.1	24.5
Val	142.1 ± 1.4	167.7 ± 3.1
Met	46.5 ± 0.1	53.4 ± 0.3
Ile	98.8 ± 0.5	98.4
Leu	170.0 ± 0.4	154.1 ± 3.1
Tyr	83.2 ± 0.7	96.0 ± 1.6
Phe	46.5 ± 0.2	45.1 ± 1.2
Trp	(6.0)	6.0

*Values obtained from three analyses. †Values obtained by linear extrapolation to time zero.

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10. Culture medium contained (milligrams per liter) $NaNO_3$, 750.0; K_2HPO_4, 15.3; Na_2CO_3, 10.0; EDTA-Na_2, 5.0; (milliliters per liter) trace metal mix, 1; seawater, 877; deionized water, 120; vitamin mix, 2. Trace metal mix contained (grams per liter) $ZnSO_4 \cdot 7H_2O$, 0.222; $MnCl_2 \cdot 4H_2O$, 1.40; $Co(NO_3)_2 \cdot 6H_2O$, 0.025; $Na_2MoO_4 \cdot 2H_2O$, 0.39; citric acid monohydrate, 6.25; ferric ammonium citrate, 6.00. Seawater was diluted with deionized water to 30 parts per thousand salinity. Vitamin mix contained (grams per liter) thiamine, 2.0; B_{12}, 0.001; biotin, 0.001. The stock solution was sterilized by passage through a 0.2-μm Nalgene Nuclepore filter. The medium was autoclaved and allowed to cool to room temperature before addition of the vitamin mix.
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Olfactory-Based Orientation in Artificially Imprinted Sea Turtles

Abstract. *Sea turtles (*Lepidochelys kempi*) are being artificially imprinted to Padre Island, Texas, in an effort to establish a new nesting population. These turtles spent more time per exposure in solutions made of Padre Island sand and seawater than in control solutions in a multiple-choice test. This is evidence that sea turtles may detect differences in natural water samples and remember olfactory cues to which they were exposed neonatally and that these differences may affect their orientation behavior. This suggests that imprinting could be used as a conservation technique for establishing new breeding populations of endangered sea turtles.*

Kemp's ridley, *Lepidochelys kempi*, is a rare and endangered sea turtle species (*1*). Almost the entire species nests on a 15-km section of beach near Rancho Nuevo, Mexico. In 1978, the United States joined Mexico in an intense conservation project attempting to establish a second nesting population of Kemp's ridleys on Padre Island, Texas, 400 km north of Rancho Nuevo.

Typically, eggs are collected at Rancho Nuevo during oviposition, placed in containers of Padre Island sand, and transported to Padre Island National Seashore where they are hatched (*2*). Hatchlings are released on the beach and allowed to enter the surf. This experimental conservation strategy, known as artificial "imprinting," is based on olfactory imprinting (*1, 3*), an unproven hypothesis suggesting a learning of the olfactory nature of the nesting beach or the adjacent waters, or both, by the hatchlings as they leave the nest and migrate out to sea. The turtles would store this olfactory information without reexposure until many years later when they return as adults to nest. At that time, the stored olfactory information would facilitate the animals' navigation as they approach the nesting beach.

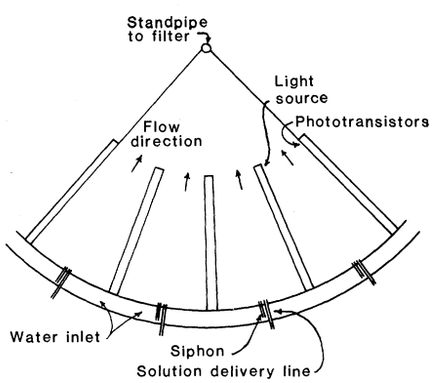

Fig. 1. Schematic diagram of automated system for monitoring marine turtle behavior (*4*). Solutions made of seawater that was used to wash beach sand were pumped into compartments, and the turtles' responses to these solutions were recorded.

As part of the effort to save the Kemp's ridley, juveniles are raised for 9 to 12 months at the National Marine Fisheries Laboratory, Galveston, Texas. We reasoned that if these juvenile ridleys are in fact imprinted to Padre Island, they might show responses (other than homing) to solutions derived from Padre Island as part of their normal juvenile behavior. We report that 4-month-old Padre Island imprinted ridleys showed a preference for solutions made from Padre Island sand and seawater in a multiple-choice situation. The turtles can apparently detect differences in naturally occurring waters, and this sensory information affects the animal's orientation behavior. This observation suggests that imprinting to new beaches can occur and that artificial imprinting could be developed as a strategy for saving these endangered sea turtles.

Beach sand and seawater samples were collected at the imprinting site and at a site 293 km to the northeast on Galveston Island (4.2 km west of Jamaica Beach) in the summer of 1980 and refrigerated for later use. Each test involved placing a single turtle in an automated monitoring tank (*4*) (Fig. 1). After a 30-minute acclimation period in the tank, each turtle could choose among four compartments; these contained solutions from either Padre or Galveston Island (*5*) and two untreated solutions. Seawater was continuously siphoned from a peripheral moat into the back of each compartment at 45 liters per hour, and treatment solutions were pumped into the back of compartments at 1 liter per hour. The order in which solutions were pumped into compartments was systematically varied between tests to eliminate compartment bias. Galveston solution served as one control with two compartments being untreated controls. Total entries, total time spent, and time spent per entry (*6*) into compartments were recorded during 4-hour monitoring periods for 12 4-month-old ridley turtles hatched in 1981.

Turtles spent significantly more time per entry (*7*) ($P \leq 0.05$) in water treated with Padre solution than in either that treated with Galveston solution or the untreated solutions, in which they spent approximately equal time per entry (Fig. 2). We interpret this as a preference by Padre Island imprinted Kemp's ridleys for the solution from there. This suggests that turtles may orient to solutions through a behavior not directly attributable to feeding (*8*).

The turtles also distinguished Galveston solution from both the Padre solution

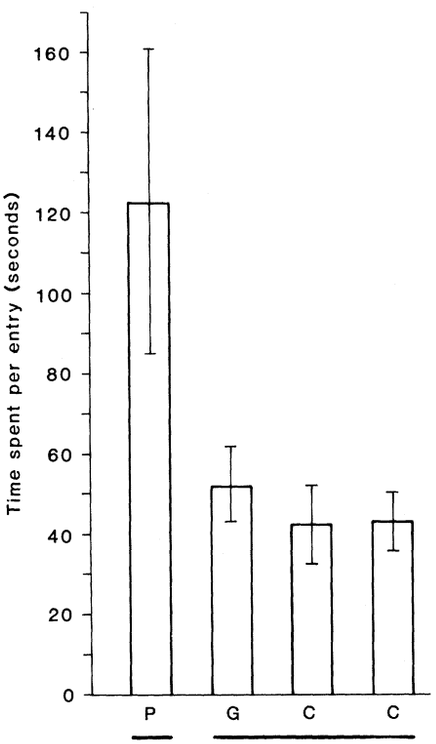

Fig. 2. Mean (± standard error of the mean) time spent per entry in compartments treated with Padre solution (*P*) ($N = 131$), Galveston solution (*G*) ($N = 254$), and untreated (*C*) ($N = 207$ and 218) compartments. Compartments underlined with the same line are not significantly different (Tukey's test, $P \leq 0.01$).