A 25-y longitudinal dolphin cohort supports that long-lived individuals in same environment exhibit variation in aging rates

Stephanie Venn-Watson[a,b,1], Eric D. Jensen[c], and Nicholas J. Schork[b,d,e,f]

[a]Healthy Aging and Longevity Program, Epitracker, Inc., San Diego, CA 92106; [b]Seraphina Therapeutics, Inc., San Diego, CA 92106; [c]US Navy Marine Mammal Program, Naval Information Warfare Center Pacific, San Diego, CA 92016; [d]Quantitative Medicine and Systems Biology, The Translational Genomics Research Institute (TGen), Phoenix, AZ 85004; [e]Department of Population Science, The City of Hope National Medical Center, Duarte, CA 91010; and [f]Department of Molecular and Cellular Biology, The City of Hope National Medical Center, Duarte, CA 91010

While it is believed that humans age at different rates, a lack of robust longitudinal human studies using consensus biomarkers meant to capture aging rates has hindered an understanding of the degree to which individuals vary in their rates of aging. Because bottlenose dolphins are long-lived mammals that develop comorbidities of aging similar to humans, we analyzed data from a well-controlled, 25-y longitudinal cohort of 144 US Navy dolphins housed in the same oceanic environment. Our analysis focused on 44 clinically relevant hematologic and clinical chemistry measures recorded during routine blood draws throughout the dolphins' lifetimes. Using stepwise regression and general linear models that accommodate correlations between measures obtained on individual dolphins, we demonstrate that, in a manner similar to humans, dolphins exhibit independent and linear age-related declines in four of these measures: hemoglobin, alkaline phosphatase, platelets, and lymphocytes. Using linear regressions and analyses of covariance with post hoc Tukey–Kramer tests to compare slopes (i.e., linear age-related rates) of our four aging rate biomarkers among 34 individual dolphins aging from 10 y to up to 40 y old, we could identify slow and accelerated agers and differentiate subgroups that were more or less likely to develop anemia and lymphopenia. This study successfully documents aging rate differences over the lifetime of long-lived individuals in a controlled environment. Our study suggests that nonenvironmental factors influencing aging rate biomarkers, including declining hemoglobin and anemia, may be targeted to delay the effects of aging in a compelling model of human biology.

aging rates | longevity | bottlenose dolphins | anemia | immunosenescence

Understanding the basic biology of aging can enhance the discovery of strategies to live longer and healthier lives (1, 2). While how much longer humans can live beyond the current maximum lifespan of ~125 y has been highly debated (3–5), identifying mechanisms that effectively delay aging in long-lived mammals may lead to novel interventions that could slow the aging rate and thereby decrease chronic conditions associated with aging (6–8). Unfortunately, there have been key limitations to studying aging rates.

Aging research with nonhuman models has focused primarily on short-lived animals, such as worms, flies, and mice (9). Conserved aging-related pathways and genes, such as those associated with caloric restriction and use of the mechanistic target of rapamycin (mTOR), sirtuin-1, and insulin-like growth factor 1, have been identified in short-lived species and have shed light on the aging process in humans (10). However, the study of these short-lived species does not leverage unique evolutionary adaptations of long-lived mammals that have effectively allowed them to live for 50 to 100 y (1, 10, 11).

Owing to limitations of short-lived models, a growing number of longitudinal cohort studies in human populations are providing more direct and relevant insight into the biology of human

aging and aging rates (12–17). These population studies remain limited, however, due to the infrequency of routine sampling (e.g., three blood samples per individual over 12 to 13 y) and inherent differences in diets, socioeconomic status, and chronic medications that can impact interpretations of aging rate measures both within and among individuals (16, 18, 19). Thus, a clearer understanding of basic mechanisms contributing to aging rates in long-lived species requires the study of a long-lived, longitudinal cohort that is sampled frequently and living, to the degree possible, with a closely monitored diet, in a controlled environment, and without ongoing medications over their lifetime and, ideally, over generations.

Bottlenose dolphin (*Tursiops truncatus*) populations have been studied for their similarities with humans in terms of the diseases they experience (20–24). For example, it has been observed that aging dolphins, both in the wild and under human care, can develop chronic aging-associated conditions, including chronic inflammation, dyslipidemia, insulin resistance, hyperglycemia, and iron dysregulation, with biomarker reference ranges remarkably similar to those of humans (20, 24–27). Dolphins are one of the

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Significance

Aging is a degradative process that varies among individuals. Due to limitations in defining and differentiating aging rates in human populations, understanding why some people appear to age slower than others has proven difficult. We analyzed 44 blood-based indices of health as candidate aging rate biomarkers collected over a 25-y period on a relevant, long-lived population of dolphins. Evidence of subsets of dolphins exhibiting slow and accelerated aging rates were detected, despite sharing the same environment, diet, and health care. Furthermore, some dolphin subsets were more likely to develop clinically relevant conditions, including anemia and immunosenescence. Our results support the notion that aging rates in long-lived mammals may be defined and provide insight into novel interventions to delay aging.

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very few natural animal models for aspects of human pathophysiology relevant to highly prevalent chronic diseases in humans; for example, they can exhibit the spectrum of histological lesions consistent with nonalcoholic fatty liver disease, which is present in as many as 25% the population globally and will soon become the leading indication for liver transplantation (26–28). Dolphins can also naturally develop histological lesions mimicking Alzheimer's disease, the most common form of neurodegenerative disease in humans (29, 30). These similarities in aging and comorbidities may be due in part to shared traits between dolphins and humans that are not present in short-lived mammals, including rare and complete synteny of chromosome 1, slowed molecular rates, advanced cognition, and rapid glucose transport systems in red blood cells (RBCs) (31–38). Given the similarities between dolphins and humans with respect to age-related chronic conditions that develop over decades, studying dolphin aging rates and their impact on clinically relevant pathophysiological processes could shed light on human aging and its relationship to chronic diseases.

For more than 50 y, the US Navy has cared for a sustained population of approximately 100 bottlenose dolphins over three generations (22). Navy dolphins receive a high level of care from experienced veterinarians including, importantly, routine blood sampling to assess 44 clinical measures over their lifespan. Navy dolphins live in the same oceanic environment, are fed the same well-controlled and monitored fish diet, and do not typically receive chronic medications during their 30- to 50-y lifespan. This good care has resulted in Navy dolphins living longer than wild dolphins; whereas the average lifespan of wild dolphins is 20 y, Navy dolphins live an average of 32.5 y (39, 40). More than 30% of the current Navy dolphin population is between 30 and 55 y old, with an oldest recorded age of 57 y. Interestingly, not all aging dolphins at the Navy develop overt chronic aging-associated conditions despite sharing the same environment, health care, and diet (20, 41). As such, given the environmental control of this unique population, studying the Navy dolphin longitudinal cohort can provide valuable insight into inherent or genetically mediated factors influencing both aging and chronic, aging-associated conditions.

We analyzed data obtained from the US Navy's well-controlled longitudinal dolphin cohort, focusing on the 44 blood-based biomarkers since 1) these biomarkers are also routinely studied in human clinical and epidemiologic studies, 2) they can be used as proxies to assess variation in individual dolphin aging rates, and 3) they can be evaluated for their associations with clinically relevant pathologies that are similar in orientation to others that have been studied in humans and other longer-lived species (42, 43).

Methods and Materials

Study Population and Animal Welfare. This study was limited to archived, retrospective health data and biospecimen analyses that were collected on Navy bottlenose dolphins (*Tursiops truncatus*) as part of their routine health care. The US Navy Marine Mammal Program is an Association for Assessment and Accreditation of Laboratory Animal Care International-certified program that adheres to animal care and welfare requirements outlined by the Department of Defense and US Navy Bureau of Medicine.

Sample and Data Collection. Archived data for this study were limited to measures obtained from routine blood samples collected from Navy bottlenose dolphins in the morning following an overnight fast between January 1994 and December 2018 (*n* = 5,889 samples from 144 dolphins). Blood samples collected either as an initial response or follow-up to acute clinical health concerns were excluded. Methods of routine blood sampling and the measurements obtained from the Navy dolphins have been described previously (20, 41).

Data on the following 44 measures were available for analysis: red blood cell (RBC) indices (RBC count, hemoglobin, hematocrit, mean corpuscular

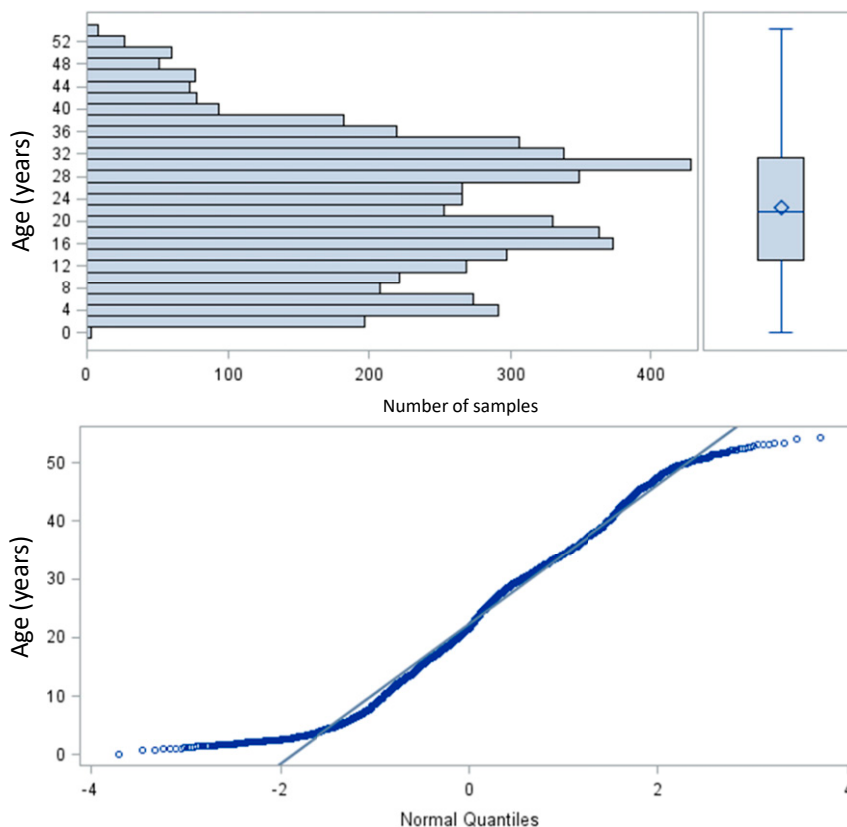**Fig. 1.** Age distribution of routine blood samples collected from 144 US Navy bottlenose dolphins (*T. truncatus*), 1994 to 2018. (*Upper*) Distribution of total number of routine blood samples collected from individual dolphins by age (y) at time of sample collection. (*Lower*) Distributed normal quartiles of routine blood samples collected (*x* axis), based on age at time of sample collection (*y* axis).

volume, mean corpuscular hemoglobin concentration, RBC distribution width, and nucleated RBCs); platelets and mean platelet volume; white blood cell count; eosinophils, lymphocytes, monocytes, and neutrophils (percent and absolute counts); glucose, blood urea nitrogen, creatinine, uric acid, sodium, potassium, chloride, carbon dioxide, total protein, albumin, calcium, inorganic phosphate, alkaline phosphatase, lactate dehydrogenase, aspartate amino-transferase, alanine aminotransferase, gamma-glutamyl transpeptidase, bili-rubin, total cholesterol, triglycerides, iron, creatine kinase, erythrocyte sedimentation rate, magnesium, and estimated glomerular filtration rate.

Independent Biomarkers Associated with Age. A single stepwise generalized linear regression model including all 44 blood-based biomarkers and dummy variables to control for sex (female = 0, male = 1), subspecies (*Tursiops truncatus truncatus* = 0, *Tursiops truncatus gilli* = 1), and facilities conduct-ing the blood analyses (simple 0,1 dummy variables were created for each of four laboratories) was applied to identify the most significant independent linear correlates of advancing age (defined as biomarkers with regression coefficients with $P < 0.0001$ and partial $R^2 \geq 0.01$). To remove biomarkers that had threshold effects or whose differences over time were driven primarily by single age categories, biomarkers that were independent predictors of age in our stepwise regression model were further assessed for consistent trends across percentile-defined age groups using a generalized linear model (GLM) with a random effect accounting for the correlated observations within dolphins and post hoc Scheffé between-group comparisons.

The five age categories used in the GLM were based on the 0 to 10th (group 1), >10th to 25th (group 2), >25th to 75th (group 3), >75th to 90th (group 4), and >90th (group 5) age percentiles identified from the main dataset. Candidate aging rate biomarkers were further assessed using a mixed GLM with the animal identifier as a random effect. Thus, blood-based biomarkers that were independent, linear predictors of age ($P \leq 0.0001$ and partial $R^2 \geq 0.01$), exhibited consistent and significant trends with increasing age ($P \leq 0.05$), and remained significant when including animal identifiers as a random effect ($P \leq 0.05$) were defined as independent, linear correlates of advancing aging (i.e., candidate aging rate biomarkers). The slopes obtained for each biomarker's linear association with age for each dolphin—based on individual linear regressions using each dolphin's longitudinal data and the GLMs using all the data—provide a proxy for those dolphins' aging rates.

Comparisons of Aging Rates among Individual Dolphins. Given the homoge-nous environment of the Navy's dolphin population, our null hypothesis was that there would be no meaningful differences in aging rates among individual animals if environmental factors were the primary contributors to variation in our aging rate biomarkers. To make use of data that were as complete as possible for this analysis without being affected by early developmental bio-logical processes and overt late age-related morbidity and disease phenotypes, we limited our analyses to 34 individual dolphins that aged from young adults to elder dolphins (i.e., from as young as 10 y and up to 40 y) during the 25-y study period. This age range was selected because it had the largest repre-sentation of individuals in the study population, and chronic subclinical path-ological processes related to aging and our aging rate biomarkers would be expected to arise and progress as the dolphins aged from 10 y to 40 y.

To test for accelerated vs. slow aging individuals in this study population, we used two overarching and complementary strategies. We first used standard linear regression analysis applied to each dolphin's data indepen-dent of the other dolphins to assess the significance of the relationship between each biomarker and age (in the form of the slope). We also used a GLM-based regression analysis that considered data from all dolphins to-gether but included a random effect for slope difference across the dol-phins. We performed these analyses for each biomarker selected from the stepwise regression analyses (hemoglobin, alkaline phosphatase, platelet, and lymphocytes). Dolphins that had statistically significant ($P \leq 0.01$) linear declines in biomarker values based on the standard linear regression anal-yses with age were defined as "accelerated" agers, whereas dolphins with nonsignificant ($P > 0.01$) linear declines in biomarker values with age or significant increases with age were defined as "slow" agers.

For the GLM analysis, we also assessed the differences in aging rates by fitting all data into a single model and including a term reflecting the in-teraction between age and individual dolphins, thus allowing assessments of deviations of aging-driven slopes for individual dolphins from the average dolphin slope. Post hoc between-animals Tukey–Kramer adjustment tests were applied with each of the four targeted biomarkers of aging. Using this method, dolphins with significant, relatively negative least squares means interaction effect estimates (i.e., more rapid declines in a given biomarker with age) were defined as accelerated agers, and dolphins with significant relatively positive least squares means interaction effect estimates (i.e., less rapid declines in a given biomarker with age) were defined as slow agers. Statistical significance was defined as a P value ≤ 0.05. As is well known, individual curve fits for each biomarker on age would not necessarily result in the same slopes as those obtained from a GLM analysis with individual taken as a random effect, since the GLM uses all the observations and can fit an average curve with greater power than individual curves. We chose to use both individual curve fits and GLM fits to contrast results and explore the behavior of the biomarkers for each individual dolphin, including these biomarkers' relevance to clinically meaningful endpoints, such as anemia and lymphopenia. Potential differences in sex distribution and origin (e.g., born under human care vs. acquired from the wild) between aging rate groups were further evaluated using Fisher's exact test and two-sided P values.

Clinical Relevance of Accelerated Aging Rates. It was hypothesized that different aging rate subgroups would have different risks of developing clinically relevant concentrations of biomarkers as they aged from 10 y to up to 40 y. Definitions of low hemoglobin (i.e., anemia), low alkaline phosphatase, low platelets (i.e., thrombocytopenia), and low lymphocytes (i.e., lymphopenia) were determined using the lowest 10th percentile values among dolphins from the main dataset ($n = 144$ dolphins) that were at least 10 y old at the time of blood sampling.

In most cases, clinically relevant concentrations of our aging rate bio-markers appeared when dolphins were between 20 and 40 y old. As such, individuals that had at least 10% of routine samples collected between age 20 and 40 y with aging rate biomarkers that met our criteria for clinically rel-evant concentrations were categorized as having the blood-based abnor-mality, while dolphins with <10% of routine samples that met our criteria for abnormal values were categorized as not having the given abnormality. Fisher's exact test and two-sided P values (≤ 0.05) were used to evaluate whether animals with accelerated aging for a given aging rate biomarker were more likely to have clinically relevant concentrations of biomarkers compared with animals with relatively positive slope estimates.

Data Availability. To ensure transparency, and knowing that there are many approaches to analyzing data of the type we discuss, the data and data subsets we used have been made available in Datasets S1–S4.

Table 1. Independent blood-based predictors of advancing age in a 25-y longitudinal cohort of bottlenose dolphins (*T. truncatus*) ($n = 144$ dolphins, 5,888 samples)

| Biomarker	Age group 1, 0 to 5.7 y ($n = 60$)	Age group 2, 5.8 to 13.1 y ($n = 1,308$)	Age group 3, 13.2 to 31.2 y ($n = 1,628$)	Age group 4, 31.3 to 37.5 y ($n = 1,623$)	Age group 5, >37.5 y ($n = 1,210$)	Significant post hoc group comparisons ($P < 0.05$)
Hemoglobin, g/dL	14.4 ± 0.9	14.3 ± 1.3	14.6 ± 1.4	13.7 ± 1.5	13.9 ± 1.3	1, 2 > 3 > 4 > 5
Alkaline phosphatase, U/L	1,008 ± 134	579 ± 290	354 ± 139	280 ± 113	280 ± 98	1 > 2 > 3 > 4 > 5
Platelets, × 10³ cells/μL	134 ± 24	113 ± 30	99 ± 31	86 ± 33	82 ± 35	1 > 2 > 3 > 4, 5
Lymphocytes, %	25 ± 10	18 ± 8	16 ± 7	13 ± 8	12.5 ± 6.7	1 > 2 > 3 > 4, 5
Creatinine, mg/dL	1.2 ± 0.3	1.2 ± 0.2	1.4 ± 0.3	1.4 ± 0.3	1.5 ± 0.4	1, 2 < 3 < 4 < 5
Protein, g/dL	6.4 ± 0.5	6.6 ± 0.4	7.0 ± 1.0	7.2 ± 0.5	7.2 ± 0.6	1, 2 < 3 < 4, 5
Albumin, g/dL	4.6 ± 0.4	4.6 ± 0.6	4.5 ± 0.6	4.4 ± 1.1	4.0 ± 1.4	1, 2, 3, 4 > 5
Bilirubin, mg/dL	0.11 ± 0.11	0.14 ± 0.12	0.21 ± 0.13	0.18 ± 0.26	0.13 ± 0.13	3, 4 > 1, 2, 5
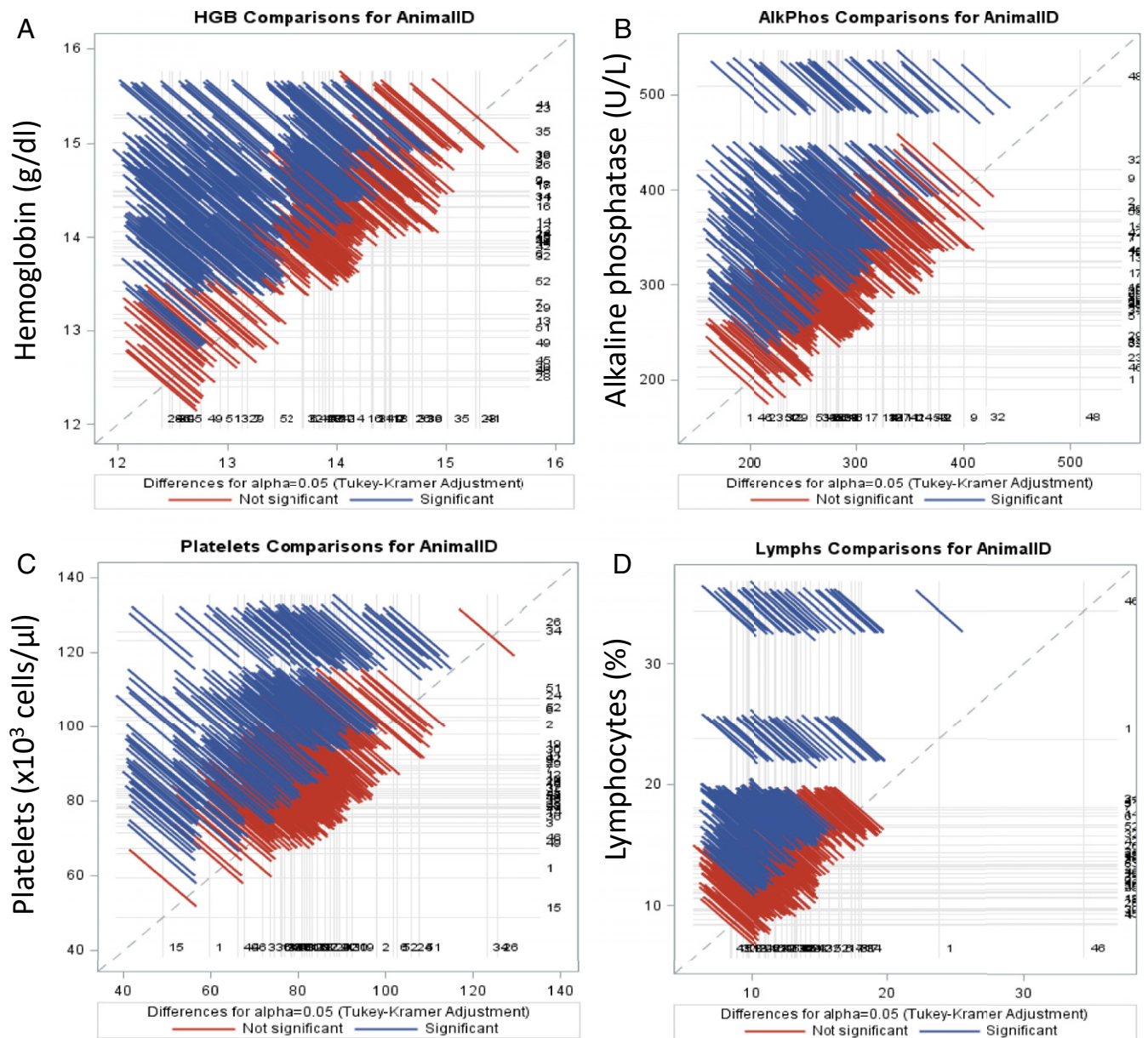
Fig. 2. Tukey–Kramer post hoc comparisons obtained from the generalized linear regression analysis with slopes considered as a random effect in capturing aging rates between individual dolphins for four blood-based biomarkers: hemoglobin (*A*), alkaline phosphatase (*B*), platelets (*C*), and lymphocytes (*D*).

Results

Study Population. Data were available from 5,889 routine and overnight fasted blood samples collected between January 1994 and December 2018 from 144 bottlenose dolphins between 0 and 54 y old (mean ± SD, 22.3 ± 12.0 y; median, 21.7 y) at the time of the blood collections (Fig. 1). Age categories were defined as follows: group 1, 0 to 5.7 y (*n* = 62 samples; 1%); group 2, >5.7 to 13.1 y (*n* = 1,326 samples; 23%); group 3, >13.1 to 31.2 y (*n* = 1,645 samples; 28%); group 4, >31.2 to 37.5 y (*n* = 1,636 samples; 28%); and group 5, >37.5 y (*n* = 1,219 samples; 21%).

The mean number of samples collected per dolphin was 41 ± 36. A total of 2,373 (40.3%) and 3,516 (59.7%) samples were collected from female and male dolphins, respectively. Almost all (98.5%) samples were collected from *T. truncatus truncatus* (Atlantic bottlenose dolphins); 1.5% of samples were collected from *T. truncatus gilli*. The samples were analyzed primarily at

either Quest Diagnostics (2,817; 47.8%) or the Naval Medical Center San Diego (2,357; 40%).

Independent Biomarker Associations with Age. Of the 44 blood-based biomarkers assessed within a single stepwise regression model that controlled for sex, subspecies, and laboratory, the following eight (18%) were the strongest independent correlates of age (*P* < 0.0001): alkaline phosphatase (partial R^2 = 0.34), platelets (partial R^2 = 0.06), protein (partial R^2 = 0.04), hemoglobin (partial R^2 = 0.04), albumin (partial R^2 = 0.03), creatinine (partial R^2 = 0.02), lymphocytes (%) (partial R^2 = 0.01), and bilirubin (partial R^2 = 0.01). Of these, four biomarkers—hemoglobin, alkaline phosphatase, platelets, and lymphocytes—had consistent, significant trends of decreasing values across increasing age categories (Table 1). In addition, protein and creatinine increased with advancing age. Aging trends with albumin and bilirubin were driven primarily by a single age category or grouped younger vs. older categories, respectively; as

Table 2. Normal reference intervals for blood-based aging rate biomarkers in bottlenose dolphins (*T. truncatus*) compared with humans

| Blood-based biomarker | Dolphin normal reference interval | | | Human normal reference range* |
	10th percentile	90th percentile	n	
Hemoglobin, g/dL	12	16	4,785	12 to 17
Alkaline phosphatase, U/L	163	470	4,749	36 to 150
Platelets, ×10³ cells/μL	49	130	4,740	150 to 350
Lymphocytes, %	6	23	4,810	20 to 40
Creatinine, mg/dL	1.1	1.8	4,747	0.7 to 1.3
Protein, g/dL	6.4	7.8	4,756	6.0 to 7.8

Data to determine references were limited to routine samples collected from US Navy dolphins age ≥10 y.
*Merck Manuals: https://www.merckmanuals.com/professional/resources/normal-laboratory-values/blood-tests-normal-values.

such, these biomarkers were excluded from further assessment as aging rate biomarkers. Of the remaining six aging rate biomarker candidates, the four biomarkers with declining concentrations with age remained significant ($P < 0.001$) in a generalized linear mixed model in which the animal identifier was included as a random effect to control for the repeated measures collected on each dolphin.

Comparisons of Aging Rate Biomarkers among Individuals. Individual dolphins ($n = 34$) with longitudinal measures routinely obtained between age 10 y up to 40 y during the 25-y study period had a mean of 81 ± 28 routine samples collected per dolphin (a total of 2,747 samples, with a range of measures from 26 to 148 samples per dolphin). Individual dolphins were defined as an accelerated ager or a slow ager based on either the presence or absence of significant linear associations with age or significant deviations in least squares mean estimates from the average dolphin based on the GLM that included an interaction term between age and individual dolphin. There was significant variation in slopes based on both the individual standard linear regression values (Dataset S3) and the generalized mixed model analyses (Fig. 2) for all four selected aging rate biomarkers. There were no differences in sex distribution between accelerated and slow agers in all aging rate biomarkers except hemoglobin. All 15 females (100%) were accelerated agers, defined by significant declines in hemoglobin with age, compared with only 11 of 19 males (57%) ($P = 0.005$). There were no significant differences (percent female vs. males) in accelerated agers for the other three aging rate biomarkers: declining alkaline phosphatase (13 of 15 females [87%] vs. 15 of 19 males [79%]; $P = 0.67$), declining platelets (11 of 15 females [73%] vs. 14 of 19 males [74%]; $P = 1.0$), and decreasing lymphocytes (11 of 15 females [73%] vs. 12 of 19 males [63%]; $P = 0.72$).

There were also no significant differences based on animal origin (born under human care vs. acquired from the wild) between accelerated agers and slow agers for the all four aging rate biomarkers: declining hemoglobin (4 of 6 born under human care [67%] vs. 22 of 28 wild-acquired [79%]; $P = 0.61$), declining alkaline phosphatase (4 of 6 born under human care [67%] vs. 24 of 28 wild-acquired [86%]; $P = 0.28$), declining platelets (4 of 6 born under human care [67%,] vs. 21 of 28 wild-acquired [75%]; $P = 0.65$), and decreasing lymphocytes (3 of 6 born under human care [50%] vs. 20 of 28 wild-acquired [71%]; $P = 0.36$).

Clinical Relevance of Accelerated Aging Rates. We assessed the degree to which aging rate subgroups were more or less likely to

have clinically relevant concentrations of the blood-based aging rate biomarkers. To do so, we determined proper reference values for the measures used to quantify clinically relevant values. Using the lower 10th percentile values from normally distributed age groups, definitions for each biomarker contributing to clinically relevant low concentrations of hemoglobin (i.e., anemia), low alkaline phosphatase, low platelets (i.e., thrombocytopenia), and low lymphocytes (i.e., lymphopenia) were determined (Table 2). Definitions for anemia in dolphins matched those used for humans (Table 2). Note that the defined low alkaline phosphatase, platelets, and lymphocyte levels in dolphins differed from the reference values used for humans.

There were no differences in the presence or absence of clinically relevant conditions between accelerated agers and slow agers based on standard linear regression fits to each individual dolphin. These nonsignificant comparisons included (percent of animals with a given condition) between accelerated agers and slow agers were as follows: 10 of 26 (38%) accelerated agers (defined by significantly declining hemoglobin with age) had anemia, compared with 2 of 8 (25%) slow agers ($P = 0.68$); 10 of 28 (36%) accelerated agers (defined by significantly declining alkaline phosphatase with age) had clinically low alkaline phosphatase, compared with 2 of 6 (33%) slow agers ($P = 1.0$); 13 of 25 (52%) accelerated agers (defined by significantly declining platelets with age) had thrombocytopenia, compared with 3 of 9 (33%) slow agers ($P = 0.45$); and 10 of 23 (43%) accelerated agers (defined by significantly declining lymphocytes) with age had lymphopenia, compared with 4 of 11 (36%) slow agers ($P = 1.0$).

However, the GLM including a random effect for slope and an interaction term for age x dolphin effect, suggested that dolphins exhibiting steeper negative slopes (i.e., accelerated aging rates) with hemoglobin and lymphocytes were more likely to progress to clinically relevant low hemoglobin and lymphocyte concentrations, respectively, by age 20 to 40 y compared with dolphins with flatter, less negative or even positive slopes (slower aging rates; Figs. 3 and 4). Here, 9 of 10 (90%) dolphins with steeper negative slopes (based on hemoglobin by age slopes) developed anemia, compared with none of the 14 dolphins with flat, less negative or positive slopes ($P < 0.0001$). All nine (100%) dolphins with steeper slopes based on lymphocyte percentage by age developed lymphopenia, compared with only 1 of 13 (7.7%) dolphins with flat or positive slopes ($P < 0.0001$) (Fig. 5).

We emphasized that some of these outcomes can possibly be attributed to variation in *y*-intercepts associated with individual dolphin regressions involving each biomarker on age, in that some dolphins with relatively negative slope estimates had initial (or values at younger ages) that were closer to pathological ranges. More analyses of this phenomenon and its biological relevance are being pursued. Clearly, the values of a biomarker with which a dolphin was born could be as much a genetically mediated phenomenon as the rate at which it ages.

Discussion

While it has long been assumed that long-lived individuals naturally age at different rates, few studies have verified this assumption using data that are free of the confounding effects of varying environmental exposures. Here, our study assessed differences in measures of the aging rate and determined clinically relevant correlates among long-lived dolphins, all raised in the same environment.

We found that, independent of biomarkers for chronic inflammation and iron status, declining hemoglobin emerged as an independent, strong linear correlate of age throughout the lifetimes of dolphins. Based on multiple analyses, we could identify accelerated-aging dolphins with significant, linear declines of hemoglobin from age 10 y to 40 y, as well as slow-aging dolphins with stable or less precipitous declines in hemoglobin levels during the same aging period. Furthermore, dolphins that were found to have more rapid declines in hemoglobin levels with age also had
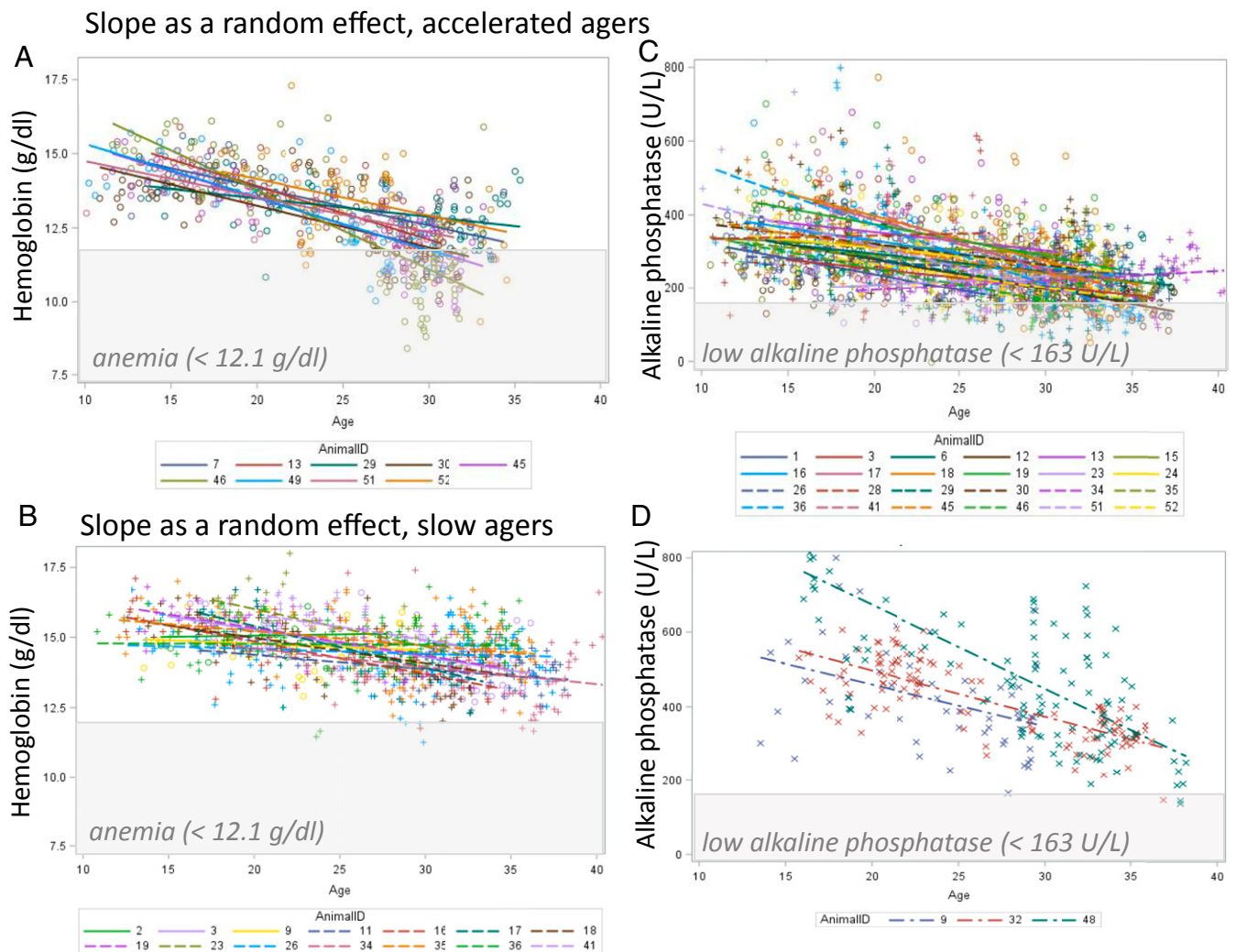
Fig. 3. Scatterplots of hemoglobin values (*A* and *B*, *Left*) and alkaline phosphatase values (*C* and *D*, *Right*) as a function of age with superimposed individual regression lines for each dolphin, for accelerated agers (*A* and *C*, *Upper*) and slow agers (*B* and *D*, *Lower*), among individual dolphins with longitudinal measures obtained between age 10 y and 40 y. Accelerated and slow agers we identified from the GLM analysis. Gray-shaded boxes represent clinically relevant, abnormal biomarker values using data from ~4,750 routine dolphin blood samples.

declines in hemoglobin that were more likely to progress to anemia (hemoglobin <12 g/dL) between 20 and 40 y compared with dolphins with relatively positive least squares means estimates, though many had hemoglobin levels that were low early in life as well.

Similarly to dolphins, as humans age, they exhibit declining hemoglobin levels and a higher prevalence of anemia (also defined as hemoglobin <12 g/dL) (44). Anemia with aging has been attributed to greater RBC fragility and is a risk factor for increased morbidity and mortality (45–48). The estimated prevalence of older individuals with anemia globally ranges from 20% to 76% (48, 49). While one-third of cases of anemia among the elderly are attributed to nutritional deficiencies and another third are characterized as anemia of chronic disease, one-third of cases are classified as "unexplained" anemia or "anemia of old age" (47, 49). Along with our present findings, these data support the notion that anemia of old age may be associated with, or the product of, an accelerated aging rate in a subset of individuals. Because increasing RBC fragility with aging can serve as a window to increased cellular senescence throughout the body (50–52), monitoring hemoglobin trends over time may help

identify people with faster aging rates and evaluate the near-term efficacy of novel approaches aimed at slowing the aging rate.

In addition to declining hemoglobin, platelet counts decreased linearly with age in dolphins, some of which progressed to thrombocytopenia. Like our findings in dolphins, it has been well established that advancing age and declining platelets are correlated in humans; older people have a higher risk of developing thrombocytopenia, and this trend is observed from birth to old age (53–58). While proposed etiologies for declines in platelet counts with age in humans have included decreased thrombopoietin levels and reduced hematopoietic stem cell availability, the actual underlying drivers for this aging rate biomarker have not yet been confirmed (59). Similarities in declining platelet counts with age between dolphins and humans support the relevance of dolphins as a model for human aging.

An overall decrease in lymphocytes was also observed as a function of age in dolphins, providing another measure of the aging rate. Our analyses identified accelerated-aging dolphins with significant, linear declines in lymphocytes while aging from 10 y to 40 y, as well as slow-aging dolphins that maintained stable lymphocyte levels during the same aging period. Furthermore, dolphins that had biomarker-related age-associated slopes with
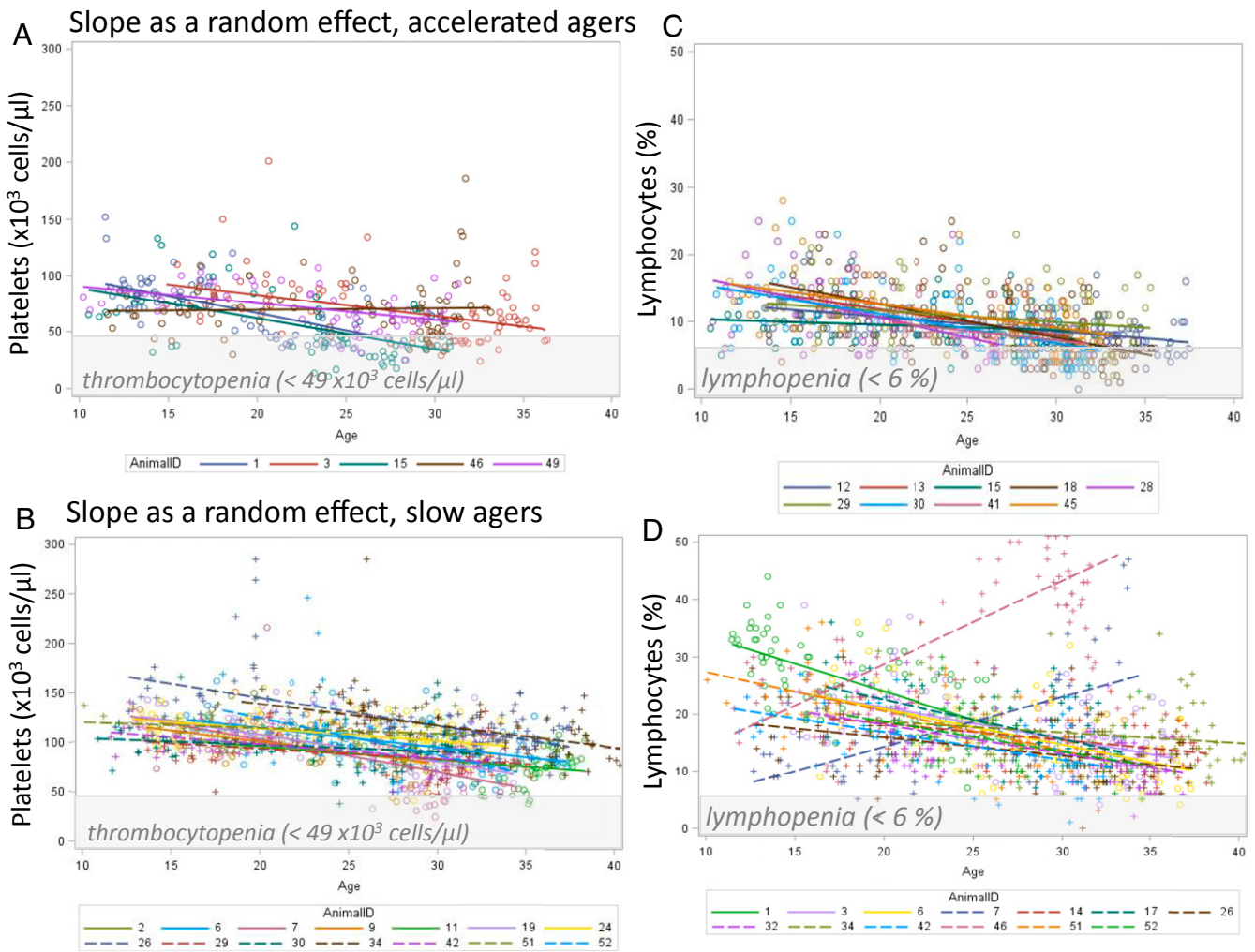
Fig. 4. Scatterplots of platelet values (*A* and *B*, *Left*) and lymphocyte values (*C* and *D*, *Right*) as a function of age with superimposed individual regression lines for each dolphin, for accelerated agers (*A* and *C*, *Upper*) and slow agers (*B* and *D*, *Lower*), among individual dolphins with longitudinal measures obtained between 10 y and 40 y. Accelerated and slow agers we identified from the GLM analysis. Gray-shaded boxes represent clinically relevant, abnormal biomarker values using data from ∼4,750 routine dolphin blood samples.

significantly and relatively negative least squares means estimates demonstrated not only declining lymphocytes with increasing age, but also declines in lymphocytes that were more likely to progress to lymphopenia (lymphocytes <6%) between 20 y and 40 y compared with dolphins with relatively positive least squares means estimates. Declining numbers of lymphocytes can occur in older humans as well, and an impaired immune response with age, or immunosenescence, has been previously postulated as a measurement of aging rate in humans (60). Lymphopenia has been reported in 25% of hospitalized elderly patients and is a predictor of longer hospital stays, in-hospital mortality, and 1-y mortality (60). Thus, the discovery of novel interventions to restore higher levels of functioning lymphocytes in dolphins may help delay aging and lengthen the lifespan in humans (61, 62).

While our findings demonstrate associations between accelerated aging rates and morbidity, including higher risks of developing anemia and lymphopenia compared with dolphins with slower aging rates, we did not include an analysis of mortality in our study, for two primary reasons. First, the annual mortality rate of dolphins at the Navy Marine Mammal Program is low (2.7%), and many dolphins in this study were still alive when we initiated it (40). Second, in general, evaluating associations between aging rate and mortality in natural, long-lived populations that are relatively small has

significant limitations due to mortalities not associated with aging-related processes (e.g., acute infections with primary pathogens). In addition, while the current study did not evaluate specific genes that may drive aging rates—including across generations—we are currently pursuing such studies.

Although sex differences in human populations can explain a large fraction of interindividual variation in disease susceptibility and aging phenotypes, aging rate biomarkers in our study remained independent and linear predictors of aging, controlling for sex. This is despite the fact that female dolphins are known to live longer than male dolphins (39, 40). Potential reasons why male dolphins have shorter lives compared with females in the wild may include higher risks of trauma due to combat with other males; greater vulnerability to predation from being in smaller groups; larger territorial ranges, which takes them through riskier habitats; and accumulation of higher concentrations of persistent organic pollutants. Most of these reasons for the shorter lifespan of male dolphins are due to acute events and would not be expected to be reflected in linear aging rate indices. As with humans, further investigations are warranted to evaluate potential nontraumatic chronic factors that may contribute to shorter lifespans in males.

In conclusion, despite having a homogenous ocean environment, fish-based diet, and routine healthcare, individual dolphins
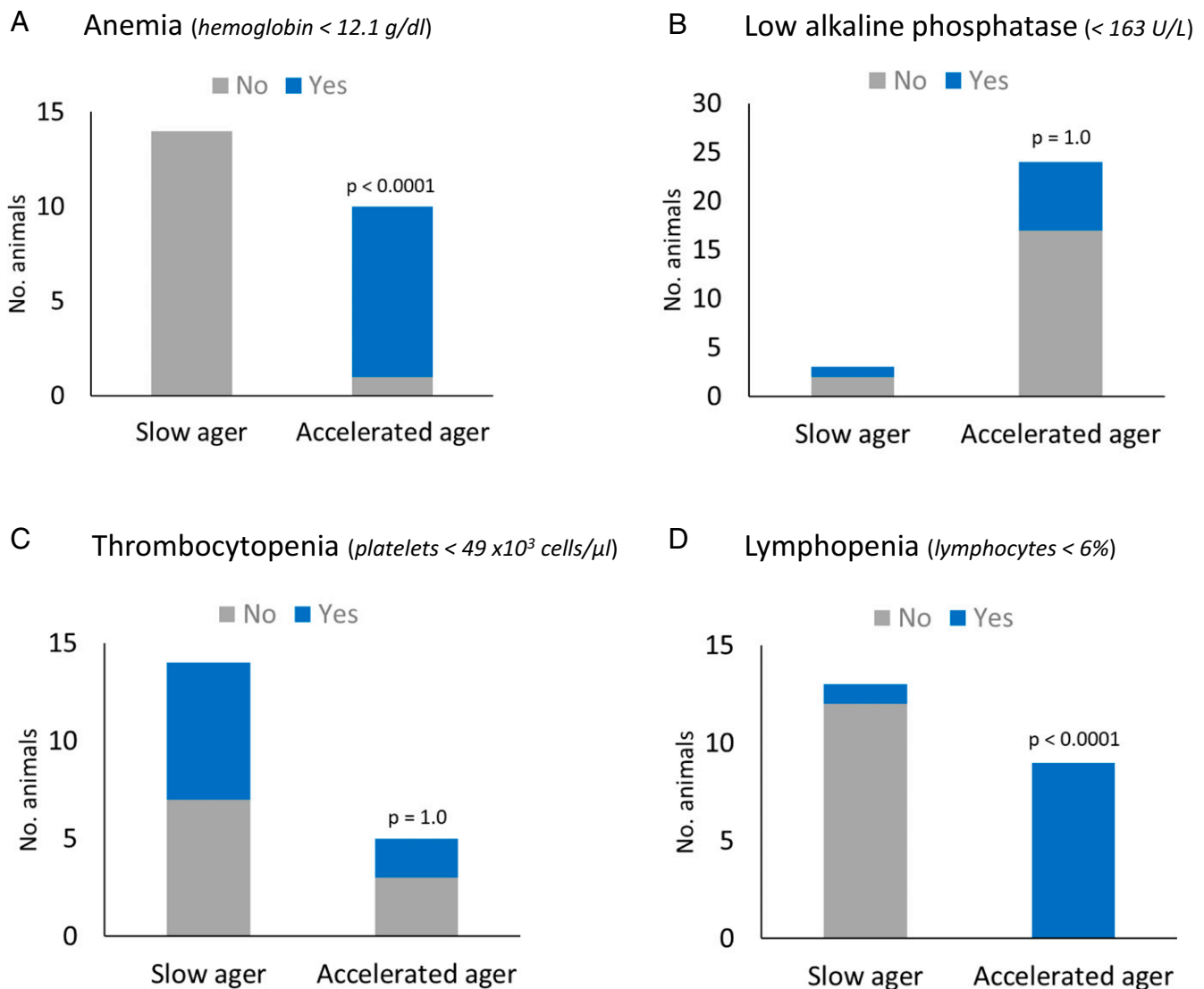
Fig. 5. Comparisons of the numbers of dolphins with and without clinically relevant biomarker values between dolphins aging from 10 y to 40 y with accelerated and slow aging rates based on the GLM analysis, including anemia (hemoglobin <12.1 g/dL) (*A*), low alkaline phosphatase (<163 U/L) (*B*), thrombocytopenia (platelets <49 × 10³ cells/μL) (*C*), and lymphopenia (lymphocytes <6%) (*D*).

exhibited variation in the rate of aging based on commonly used blood-based clinical biomarkers. Our data also support the notion that individual dolphins with accelerated aging are more susceptible to clinically relevant and aging-associated conditions, including anemia and lymphopenia. Thus, our unique dolphin longitudinal cohort may be ideal for prioritizing nonenvironmental, genetic factors as targets for longevity-enhancing or geroprotective agents that may slow aging rates and improve the health span of long-lived mammals. Thus, identifying the genetic determinants of the four blood-based aging rate indices in dolphins would be

of great value, as these indices reflect hematologic and immune changes with age that could in turn reflect organismal or system-wide cellular senescence processes associated with aging.

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