ARTICLE

Human Milk Oligosaccharides And Site-Specific Glycosylation Heterogeneity Of Filipino Human Milk Glycoproteins Exhibit Dynamic Variations During Lactation As Influenced By Secretor Status And Coastal Environmental Condition – A Preliminary Study

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ABSTRACT

Human Milk Oligosaccharides (HMOs) have a role in the immune system development of infants. HMOs and the N-glycans of human milk glycoproteins, especially highly fucosylated ones, function as prebiotics to the bifidogenic gut microbiota. This study reports the first longitudinal profile of HMOs and human milk glycoproteins from coastal, noncoastal, secretor and nonsecretor Filipino mothers analyzed and annotated using Q-TOF LC/MS and QqQ LC/MS - MRM, respectively. A milk glycan profile from coastal mothers revealed higher ($P<0.05$) presence of fucosylated branched HMOs, such as DFLNHa, LDFT, LNDFH I, and LNFP II. Moreover, secretor mothers expressed the highest 2'FL from colostrum (Day 5) until the end of the study (Month 6). Interestingly, the predominance of secretory IgA (64%) on the total milk glycoproteins from Filipino mothers supported their biological adaptation in conferring immune protection to their breastfeeding infant. Glycoform variations on milk glycoproteins, sIgA, IgG, Lactoferrin, and α1-Antitrypsin, in coastal mothers were observed. Moreover, lactoferrin was found to be highly

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glycosylated with 20 fucosylated and sialylated glycoforms. These results suggest that the coastal mother's unique long-term seafood diet may have influenced their milk glycan profile and the potential incorporation of L-fucose derived from seafood in the biosynthesis of their milk glycans.

INTRODUCTION

The human milk is purposely designed by nature for optimum infant nutrition and protection. Thus, exclusive breastfeeding up to the first six months of life is strongly recommended (Eisenberg et al. 2015; Kimura et al. 2015; Qasem et al. 2015; WHO 1981). Even though the nutritional quality of infant formula approaches that of human milk, the incidence of infant mortality among non-breastfed remains 3- to 10-fold higher compared to breastfed ones and that, breastfeeding was associated with 50% decrease in mortality rate (Victora et al. 2016). It was believed that the protection provided by human milk was solely attributed to the presence of secretory antibodies. Various bioactive compounds present in human milk are known to modulate the developing microbiota within the infant's gastrointestinal tract, including immunoglobulins, lactoferrin, lysozyme, bioactive lipids, leucocytes, and various milk glycans such as glycoprotein, glycolipid, and free oligosaccharides (Bode 2012; Lis-Kuberka & Orczyk-Pawiłowicz 2019; Newburg 2013; Peterson et al. 2013).

Among the bioactive components present in human milk, the human milk oligosaccharides (HMOs) have been reported to be essential for its unique health benefits. These free oligosaccharides are distinct from the other milk components with respect to quantity and diversity. Human milk is noted for its high content (5-15 g/L) of HMOs (Zivkovic et al. 2011). It is this uniqueness and the lack of HMOs in bovine-based infant formula that strengthens breastfeeding practices and warrants further research into its significance and function to promote human health and its ability to prevent diseases. More than 200 HMOs have been identified in human milk (Smilowitz et al. 2014).

There are different factors that influence the diversity of HMOs and milk glycoproteins, and the variations are not fully understood. The most common factor and the one that is inherited is the secretor and Lewis blood group status of the mothers (Smilowitz et al. 2013). Recent reviews have indicated that not only does genetic factor influence the diversity of glycan structure, abundance, and changes in composition during lactation from different mothers, but also influenced by dietary, geographical, physiological determinants, and as well as the pathological state of both the mother and infant dyad (Golan & Assaraf, 2020; Hundshammer & Minge, 2020).

In a recent survey study, about 76.5% of resident respondents in Baybay City, Leyte indicate that fish serve as their most important source of protein (Mudge 2018). Seaweed salad is also a delicacy enjoyed by the locals living on the coastline of Baybay City, Leyte and often eaten as a vegetable substitute. Recent studies on the therapeutic effects of fucoidans due to its L-fucose residues derived from seaweed, sea cucumbers and, to a lesser extent, other marine invertebrates, have been extensively reviewed (Luthuli et al. 2019; Reyes et al. 2020). To date, there are no human milk oligosaccharide (HMOs) and glycoprotein profiles from Filipino mothers, especially from mothers living in coastal areas. Thus, these data would give information of unique glycan expression in the breastmilk of Filipino mothers living in coastal and noncoastal areas. These HMO and glycoprotein profiles could serve as a baseline for future studies on functional determination. The results would suggest on the influence of the coastal environment, as well as the possible incorporation of L-fucose derived from the long-term seafood diet of local mothers on HMO and human milk glycoprotein expression. With advances in technology, it is soon possible to fully elucidate the complexity of different HMOs and N-glycans of milk glycoproteins, along with their specific functions and uniqueness between ethnic groups.

MATERIALS AND METHODS

Materials and Reagents

Human milk oligosaccharides (HMOs) and glycoproteins were extracted from breast milk samples (n = 145) obtained from local mothers (n = 15) living in Baybay City, Leyte, Philippines. Autoclaved microcentrifuge tubes (1.5 mL) were used for sample storage. PGC 96-well carbon packed plates and C18 96-well cartridge plates for purification were purchased from Glygen (Columbia, MD). Both 0.2 and 0.8-mL 96-well polypropylene plates were purchased from Thermo Scientific. Nanopure water, absolute ethanol, sodium borohydride, acetonitrile, formic acid, and trifluoroacetic acid were of ACS quality or higher. Protein standards including LF, α-Lact, IgA, IgG, IgM, and A1AT were purchased from Sigma-Aldrich (St. Louis, MO), while LZ was purchased from Lee Biosolutions (St. Louis, MO). Peptide standards including α-, β-, κ-caseins, and osteopontin were purchased from Bionexus. Sequencing grade modified trypsin and dithiothreitol were purchased from Promega (Madison, WI), while iodoacetamide was purchased from Sigma-Aldrich (St. Louis, MO).

Human Milk Collection

A longitudinal breast milk collection was done from Filipino mothers living in the coastal (n = 9) and noncoastal (n = 6) areas of Baybay City, Leyte across the first six (6) months lactation period. Inclusion criteria of healthy mothers of legal age (18 or above) who delivered healthy full-term infants and exclusively breastfeeding were used. Mothers living directly at the coastline of Baybay City were binned as coastal mothers, while mothers living at least 2 kilometers away from the coast, situated at the mountain side were binned as noncoastal mothers. These mothers were randomly selected after delivery at the Western Leyte Provincial Hospital and have all given written consent for their participation in the study. Breast milk samples were collected according to the mother's preference, either by manual expression or with the aid of a breast pump, which lasted less than a minute. Each breastmilk samples were collected in sterile microcentrifuge tubes with a volume of 1.5 mL, sufficient for analyses. Maternal and infant data were also collected in the study such as maternal age, mode of delivery, BMI, diet, and the infant's growth measurements. Breast milk samples from Baybay City, Leyte were transported to UP Los Baños, kept frozen in an iced box with dry ice. Collected samples were properly stored in a -80°C biofreezer at the Institute of Biological Sciences, UP Los Baños. After the completed longitudinal breastmilk collection, samples were shipped to the Lebrilla Laboratory Group at UC Davis, CA, and stored at -80°C prior to milk glycan extraction and purification. The Eastern Visayas Health Research and Development Consortium Ethics Review Committee (EVHRDC-ERC) approved all aspects of the study with an assigned study protocol code 2018-005.

Rapid-Throughput Human Milk Oligosaccharide Analyses

HMOs were rapidly extracted from milk samples using previous reported methods (Totten et al. 2014; Totten et al. 2012; Wu et al. 2017). Briefly, milk samples were aliquoted into 96-well plates, diluted, and then defatted via centrifugation using a four-plate centrifuge [Eppendorf, model 5810R]. Milk proteins were precipitated out with ethanol, and the resulting glycan-rich

supernatant dried in a vacuum concentrator [Genevac™ miVac]. Dried samples were reconstituted and anomeric glycans reduced to their alditol form using $NaBH_4$. Desalting and HMO purification was done via Solid Phase Extraction (SPE) using PGC 96-well carbon packed plates [Glygen, Columbia, MD]. Eluted samples were dried, reconstituted, and diluted prior to Q-TOF LC/MS analysis.

HMOs were analyzed on a nano-HPLC-TOF-MS system with an optimized 45-minute complete run cycle. The Agilent 1200 series LC unit utilizes an autosampler fitted for 96-well plates (maintained at 4°C), a capillary pump for sample loading (4 µL/min), and a nano pump for analyte separation (0.3 µL/min), coupled to an Agilent 6530 TOF spectrometer. The pumps used a binary solvent system comprised of solvent A [3% ACN in 0.1% Formic Acid (v/v)] and solvent B [90% ACN in 0.1% Formic Acid (v/v)]. Loading and separation of a 10 µL sample injection volumes were respectively performed isocratically with solvent A1, and gradiently with solvents A1 and B1, as follows: 0-5 min, 3% B1; 5-7 min, 3-99% B1; 7-9 min, 99% B1; 9.01-16 min, 3% B1; 16-18 min, 3-99% B1; 18-20 min, 99% B1; 20.1-27 min, 3% B1; 27-29 min, 3-99% B1, 29-31 min, 99% B1; 31.01-32 min, 3% B1; and equilibration at 3% B1, 32-45 min. Detection was performed in the positive mode with electrospray as the ionization source. Calibration was achieved with a dual nebulizer electrospray source with calibrant ions of mass-to-charge (m/z) values ranging from 322.048 to 1521.971 (ESI-TOF Calibrant Mix, Agilent Technologies, Santa Clara, CA). A single calibrant ion, m/z 922.010, was used as reference. To ensure method reproducibility, a reference HMO pool was injected and analyzed on the first run and on every 10 sample injections to account any retention time shifts over long period of continuous instrument time.

HMO Structural Annotation

Acquisition data were collected using Agilent MassHunter Workstation TOF/QTOF Acquisition software (B.08.00) and deconvoluted using Agilent MassHunter Qualitative Analysis (B.08.00) and Agilent Profinder (B.08.00). Individual HMOs were annotated by accurate mass, retention time, and elution order using previously developed HMO libraries (Totten et al. 2014; Wu et al. 2011; Wu et al. 2010). Briefly, a reference HMO pool with fully annotated structures and retention times which were previously elucidated from exoglycosidase reactions and MS fragmentation patterns are matched with large HMO sample sets, making retention time reproducibility essential for rapid HMO identification. Agilent Profinder "Batch Target Feature Extraction" function made possible chromatographic spectra alignment across all sample data files, which allowed uniform peak assignment and accurate batch annotation of HMOs. Peak areas were also computed using Agilent Profinder to eliminate false positive and false negative results from Agilent MassHunter Qualitative Analysis "Find by Molecular Feature" function. HMOs were binned into classes according to sugar composition: fucosylated HMOs, sialylated HMOs, fuc-sialylated HMOs (containing both Fuc and Sia), and undecorated HMOs (containing neither Fuc nor Sia).

Secretor Status Determination

Rapid determination of secretor status without the need for serological testing was done following the published method (Totten et al. 2012). Briefly, secretor status was determined based on the abundance ratio cut-off of identified HMO secretor markers. The optimal cutoff for describing an individual as secretor have > 6.5 abundance ratio of 2'FL/3'FL, with a sensitivity and specificity of 95.5 and 100, respectively. Enrolled mothers with > 6.5 abundance ratio of 2'FL/3'FL were determined to be secretors, while those with < 6.5 abundance ratio of 2'FL/3'FL to be nonsecretors. In addition, secretor mothers were also identified to have a < 0.0235 6'SL/3'SL abundance ratio, although sensitivity is lower (93.2) with this reported criterion. As longitudinal collection of breast milk was conducted throughout the first six months of lactation, the secretor status was concluded based on the secretor status determination in the majority of the mother's samples.

Rapid-Throughput Human Milk Glycoprotein and Glycoform Analyses

Human milk glycoproteins were rapidly extracted using previous reported methods (Goonatilleke et al. 2019a; Huang et al. 2015; Huang et al. 2017). Briefly, protein standards including lactoferrin, α-lactalbumin, IgA, IgG, IgM, α-antitrypsin, and lysozyme were accurately weighed using a micro-balance [Mettler Toledo, XP26] and dissolved together in NH_4HCO_3 to make a standard protein mixture. Similarly, peptide standards including β-casein peptide 1 (SPTIPFFDPQIPK), β-casein peptide 2 (VLPIPQQVVPYPQR), κ-casein peptide 1 (RPAIAINNPYVPR), κ-casein peptide 2 (TYYANPAVVRPHAQIPQR), α-casein peptide 2 (CAEQFCR), α-casein peptide 3 (NNVMLQW), and osteopontin peptide (GDSVVYGLR) were dissolved together in NH_4HCO_3 to make a standard peptide mixture. Standard mixture of proteins, peptides, and milk samples were aliquoted into 96-well plates and reduced with dithiothreitol (DTT), followed by alkylation with iodoacetamide (IAA). Tryptic digestion was accomplished with the addition of trypsin and digests purified on C18 96-well cartridge plates [Glygen, Columbia, MD]. Eluted standards and samples were dried, reconstituted, and diluted prior to QqQ LC/MS – MRM analysis.

Glycoproteins and its site-specific glycosylation were analyzed on a UPLC-QqQ MS system with an optimized 16-minute complete run cycle. The Agilent 1290 series LC unit was equipped with a microwell-plate autosampler (maintained at 4°C), capillary sample loading pump, nano pump, Agilent Eclipse Plus C18 column (RRHD 1.8 µm, 2.1 mm x 100 mm), and coupled to an Agilent 6495 QqQ spectrometer. Analyte separation of a 1 µL injection volume was performed in gradient elution of binary solvent system comprising of solvent A [3% ACN in 0.1% Formic Acid (v/v)] and solvent B [90% ACN in 0.1% Formic Acid (v/v)], as follows: 1-1.5 min, 2% B1; 1.5-3 min, 15% B1; 3-4 min, 25% B1; 4-7 min, 30% B1, 7-10 min, 30% B1; 10-11 min, 35%; 11-12, 40% B1, 12-14 min, 100% B1; and equilibration at 2% B1, 14.1-16 min. The dynamic MRM (Multiple Reaction Monitoring) mode was used, whereby the transitions were monitored only when the target analyte was eluted, saving duty cycles for coeluting compounds. Individual glycoproteins were quantitated and their glycoforms monitored using the published MRM transition list (Huang et al. 2017). The MRM transition list consisted of the targeted peptide's precursor ion and two product ions, identified as the quantifier and qualifier. It is unlikely that an interference would share both quantifier and qualifier ions, which gave the MRM method high specificity and selectivity with the targeted peptides. Each targeted peptide transitions with precursor mass, product mass, retention time, and fragmentation voltage were optimized based on a reference milk pool injected and analyzed on the first run and on every 10 sample injections to ensure method reproducibility, account retention time shift, and minimize duty cycle (Hong et al. 2013).

Glycoprotein Quantitation and Glycoform Structural Annotation

The QqQ MS – MRM data were analyzed using Agilent MassHunter Quantitative Analysis (QQQ) B.08.00 software. The peak areas of the standards and samples were integrated by the software and used for quantitation. The limit of detection (LOD) and limit of quantitation (LOQ) were defined as S/N ≥ 3 and S/N ≥10, respectively. Limits of quantitation were determined as the concentrations of peptides with signal-to-

noise ratio ≥ 10. Absolute protein concentrations were obtained using standard calibration curves. The linear ranges spanned 3 orders of magnitude, which was sufficient to capture biological diversity, and with good linearity. Relative abundances of glycopeptides were calculated by normalizing the glycopeptide abundance to the quantifying peptide from the same glycoprotein in order to remove the contribution of protein concentration (Huang et al. 2017). The normalized abundance of the glycopeptides were transformed into natural log scale to determine the fold changes of the glycoprotein's predominant glycoforms at specific glycosites and allowed the determination of glycosylation changes at a protein-specific level (Goonatilleke et al. 2019a).

Statistical Analyses

All statistical analyses were performed using Graphpad Prism (version 8.4.2). To determine the odds of the mother's seafood diet and secretor status in coastal areas, Fischer's Exact Test was done on the odds ratio of the variables that defined these factors, with 95% Confidence Interval (CI) computed using Baptista-Pike Method. To determine the significant influence of the coastal environment and lactation period on HMO expression, two-way ANOVA with Repeated Measures was used. Two post hoc multiple comparisons tests were done after ANOVA: 1) The Tukey's test was used to find significant differences of fucosylated, fuc-sialylated, sialylated, undecorated, and total HMOs in each and between lactation points of coastal and noncoastal HMOs; while 2) the Sidak's test was used to determine significant difference of coastal and noncoastal HMOs in each lactation points. The same statistical analyses were done to assess the effect of secretor status and lactation period on HMO expression. Mixed Effects Model ANOVA was automatically fitted for missing values with repeated measures two-way ANOVA in Graphpad Prism, making it more robust to missing data. In the presence of missing values, the results of Mixed Effects ANOVA can be interpreted like repeated measures ANOVA.

For glycoprotein analyses, Two-way ANOVA with Repeated Measures was employed to determine significant changes of both protein concentration and fold change of site-specific glycoforms across lactation period between coastal and noncoastal environment. Similar to the HMO statistical analyses, two post hoc multiple comparisons tests were performed after ANOVA. Tukey's Multiple Comparisons test determined significant differences in each and between lactation points of coastal and noncoastal glycoproteins and glycoforms, separately. Sidak's Multiple Comparisons test determined the significant difference of coastal and noncoastal glycoproteins and glycoforms, separately, in each lactation points. To assess the effect of secretor status and lactation period on both glycoprotein concentration and glycoform fold changes, the same set of statistical analyses was performed. All statistical analyses were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The Filipino Locale of Baybay City, Leyte

The City of Baybay owes its name to having the longest coastline in Leyte and categorized as a fourth-class city according to the Philippines' six-stage income classification. It can be described as a typical shoreline rural town in the Philippines, with its traditional dietary habits being upheld due to its abundant agricultural lands and fresh seafood resources. The coastal residents of Baybay have a long-term diet that constitute primarily of seafoods due to its wide access of Camotes Sea. In the survey study of Mudge (2018), about 76.5% of resident respondents in Baybay indicate that fish serve as their most important source of protein. With its topography of mountains on the east and shoreline on the west, the residents of Baybay can be divided into coastal and noncoastal locals with distinct long-term diets. The spatial difference classification was also attributed to the local's sense of place and place attachment, especially in coastal and noncoastal contexts. These are defined by occupational roles such as farming or conservation, as well as home location (Brown et al. 2015; Gillespie et al. 2022). For noncoastal locals sloping uphill the mountains, their protein diet heavily relies on poultry and red meat, as compared to coastal protein diet comprising primarily of fish. In fact, the odds ratio of a seafood diet for coastal mothers was 3-times (3.151 exactly) the odds of non-seafood diet in the same area. Fisher's exact test revealed a highly significant result at $P<0.001$ and is shown in **Table 1**.

Table 1: Characteristics of the study population in Baybay City, Leyte.

	General (n=15)	Coastal (n=9)	Noncoastal (n=6)
Mother			
Age (y)	28.87 ± 5.74	28.11 ± 5.86	30 ± 5.90
BMI (kg/m^2)	20.73 ± 2.52	20.29 ± 1.82	21.38 ± 3.41
Secretor	47%	56%	33%
Secretor Mom Odds Ratio	n/a	2.50^{ns}	0.40^{ns}
Diet Composition			
Seafood	51%	63%	35%
Non-seafood	49%	37%	65%
Seafood Diet Odds Ratio	n/a	3.151***	0.3173***
Delivery of Birth			
Normal	40%	33%	50%
Caesarian	60%	67%	50%
Parity			
0	7%	11%	0%
1	47%	56%	33%
≥ 2	47%	33%	67%
Infant			
Male	40%	33%	50%
Female	60%	67%	50%

Values are Mean ± SD or %. Percentage reflect the proportion of representatives in respective study locations.
Diet composition reflect the mean diet proportion during the 6-month study duration.
^{ns} – not significant (P>0.05)
*** – highly significant (P<0.001)

Upon classifying the secretor status of breastfeeding mothers that participated in the study, 5 out of 9 coastal mothers were classified as secretors, while only 2 out of 6 were secretors for noncoastal mothers. Fisher's exact test was used to determine the significance of secretor mothers in the coastal areas of Baybay. These preliminary data suggest that the odds ratio of secretor mothers in coastal areas is 2.5. However, there is no evidence (P=0.608) that secretor mothers are significantly higher in coastal areas compared to noncoastal areas in Baybay City, Leyte.

Human Milk Glycoproteins

Glycoprotein analysis determines both the sites of N-glycosylation as well as the glycan structures associated with each site. The occupation of a potential N-glycosylation site with a consensus sequence NX(S/T), where X can be any amino acid except proline, however, is not obligatory. Thus, a glycoprotein may contain several potential N-glycosylated sites, each of which may or may not be glycosylated. Given that there is no template for glycosylation, nonhomogeneous structurally distinct glycans can lead to site heterogeneity of glycan structures for each glycosylation sites. Hence, glycan structural

heterogeneity and the presence of numerous glycosylation sites would result to different glycoforms of glycoproteins.

Human milk protein is the fourth most abundant following HMOs as the third most abundant component in human milk. The uniqueness of human milk also lies on the large amount of total glycosylation in its proteins and glycosylation of these proteins is currently being related to its immune function (Zhu & Dingess 2019). The underdeveloped immune system of the infant is supported by the acquired immune system brought by the mother's milk. In addition to milk proteins as a source of amino acids and for mineral absorption, the predominance of protein glycosylation in human milk implies a more structure-specific role. Combined with HMOs, there is now a high abundance of indigestible human milk glycans present to increase the survival of the breastfeeding infant (Newburg 2013).

The dynamic nature of these glycosylations may address the physiological needs of the infant and suggest the biological roles of these glycans. More likely, the digestion products of these glycoproteins may equally be important as the intact protein itself, as resulting glycopeptides could still carry the glycan structures with biological functions *in vitro* and *in vivo* (Peterson et al. 2013). As the infant's body, immune system, and gut microbiota rapidly develop during the first few days of life, profiling the changes in protein expression and glycosylation are critically important in understanding the activation of the immune system, its direct influence on beneficial gut microbiota colonization, and the overall growth and development of the infant. In the previous study of Froehlich et al. (2010), human milk proteins consist both caseins and whey proteins with a total concentration range of 10-20 μg/μL. Casein and whey proteins compose > 90% of the human milk proteins from healthy mothers during lactation (Goonatilleke et al. 2019a).

In this study, the three (3) main types of casein present in human milk: α-, β-, and κ-Casein; and as well as eight (8) abundant whey proteins: α-Lactalbumin, Lactoferrin, Immunoglobulins (IgA, IgM, and IgG), Osteopontin, Lysozyme, and α1-Antitrypsin, were quantitatively measured across the first six months of lactation from Filipino mothers living in Baybay City, Leyte. Multiple Reaction Monitoring (MRM) was used to examine quantitative changes in glycosylation at a site-specific level by normalizing the protein glycosylation to the absolute protein concentration. This strategy provided a comprehensive information on both the absolute amount of protein and the site-specific glycosylation profile, and thus determined if altered glycosylation profiles were solely due to the change in protein glycosylation or a consequence of the change in protein concentration. This dynamic method both determined the concentrations of individual proteins and as well as quantitatively monitored respective glycosylation at a site-specific level. Fragmentation patterns of peptides from the respective proteins had already been evaluated in the recent studies of Huang et al. (2017) and Goonatilleke et al. (2019a). A list of precursor masses, product masses, and optimized retention times of these targeted peptides from each respective protein monitored in this study were utilized (**SI Table 3**). Protein concentrations were calculated from the standard calibration curves derived from these targeted peptides using QqQ LC/MS MRM analysis.

The total glycoprotein content in human milk of lactating mothers was derived, with a concentration range of 5-15 μg/μL across the first six months of lactation (**Figure 1**). Coastal environment and secretor status have no significant influence (P>0.05) on the total human milk glycoprotein concentrations of Filipino mothers, which was also true in the longitudinal study done in Chinese mothers (Bai et al. 2018). Moreover, total glycoprotein concentrations for all milk samples were also observed to be significantly decreasing (P<0.05) throughout the first six months of lactation.

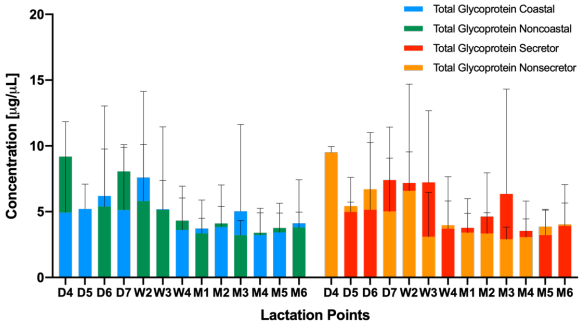


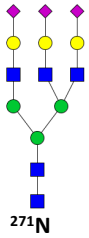
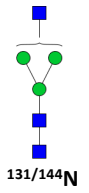
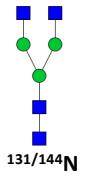
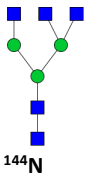
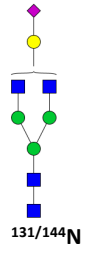
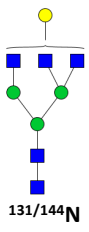
Figure 1: Total Glycoprotein Concentration (μg/μL) in Human Milk from Coastal, Noncoastal, Secetor and Nonsecretor Mothers living in Baybay City, Leyte.

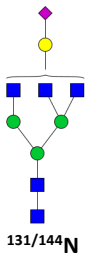
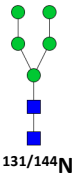
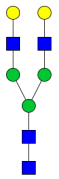
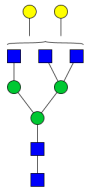
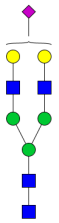
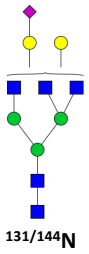
The percentage composition (%) of total glycoprotein in human milk differ for mothers. For all milk groups, secretory IgA had the highest percent composition (63-64%) of the total glycoprotein in human milk and in line with Peterson et al. (2013) being the most abundant antibody in human milk. This is necessarily due to its biological function as the first line of defense in protecting the intestinal epithelium from enteric pathogens and toxins (Mantis et al. 2011). The high abundance of IgA in human milk is followed by Lactoferrin and α-Lactalbumin, with percent composition range, 10-14% and 12-16%, respectively. The lowest glycoprotein components of human milk were IgM, IgG and Osteopontin, allocating only < 1% of the total glycoproteins, while α-Casein, β-Casein, κ-Casein, α1-Antitrypsin, and Lysozyme constitute the remaining 10-11% (**Figure 2**).

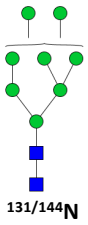
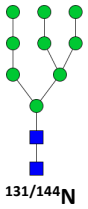
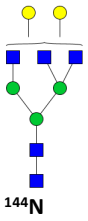
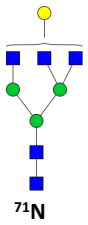
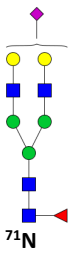
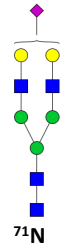
The mean concentrations of individual glycoproteins in human milk across the first six months lactation period from all mothers are presented on bar graphs in **Figure 3A-K**. The coastal environment had no significant influence (P>0.05) on the concentration of individual glycoproteins in human milk (**SI Table 4**). Interestingly, nonsecretor mothers have significantly higher lactoferrin percent composition (14%) in human milk compared to secretor mothers (10%), as shown in **Figure 2**. All glycoproteins except for lactoferrin did not significantly differ in concentrations between secretor and nonsecretor mothers, which agrees with the results of Goonatilleke et al. (2019a).

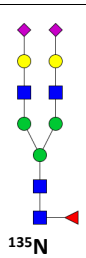
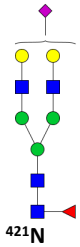
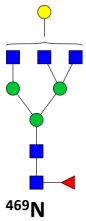
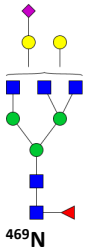
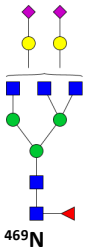
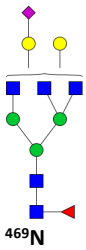
Individually, the concentrations of these glycoproteins significantly differ (P<0.05) throughout the first six months of lactation, with concentrations highest at the first week of lactation, followed by a general decreasing trend up to the end of the lactation period. This decreasing trend was observed in α-Casein, β-Casein, κ-Casein, IgA, IgM, α1-Antitrypsin, α-Lactalbumin, Lactoferrin, and Osteopontin. However, an increasing trend was observed for IgG and Lysozyme as lactation progressed, particularly with lysozyme reaching four times (4x) the concentration at Month 6 compared to Month 1 of lactation. These observed trend changes in glycoprotein abundances from Filipino mothers were in line with the observed changes from American mothers (Goonatilleke et al. 2019a).

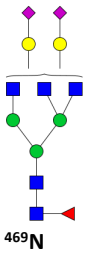
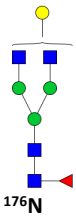
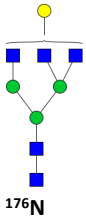
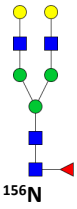
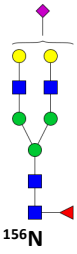
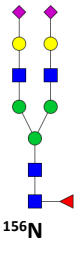
Table 2: Quantifiable Glycoforms of Human Milk Glycoproteins sIgA, IgG, Lactoferrin, and α1-Antitrypsin Across Six-month Lactation Period from Filipino Mothers living in Baybay City, Leyte.

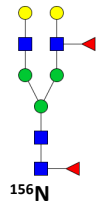
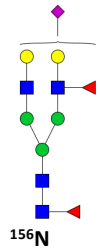
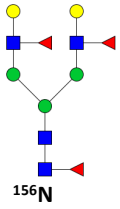
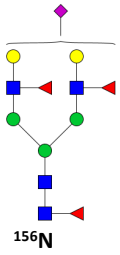
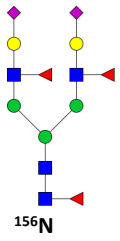
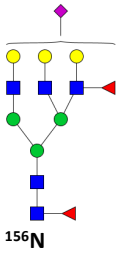
Glycoprotein	Glycoform	Precursor Ion	Product Ion	RT	D RT	Glycan Structure
α1-Antitrypsin	AT- 271 H6N5S3	1154.98	204.1	5.2	0.7	
sIgA	IGA-131/144 H3N3	1015.5	204.1	5.2	0.8	
sIgA	IGA-131/144 H3N4	1066.3	204.1	5.1	0.8	
sIgA	IGA-131/144 H3N5	1117.1	366.1	5.1	0.8	
sIgA	IGA-131/144 H4N4S1	1179.6	204.1	7.5	0.8	
sIgA	IGA-131/144 H4N5	1157.6	204.1	5.1	0.8	

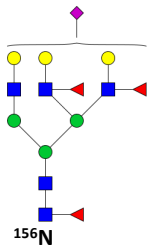
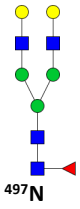
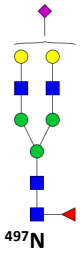
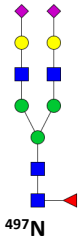
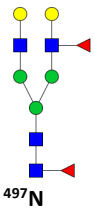
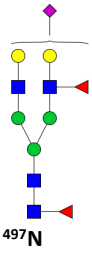
Glycoprotein	Glycoform	Precursor Ion	Product Ion	RT	D RT	Glycan Structure
sIgA	IGA-131/144 H4N5S1	984.5	204.1	5.7	0.8	
sIgA	IGA-131/144 H5N2	1045.8	204.1	5	0.8	
sIgA	IGA-131/144 H5N4	1147.3	204.1	4.9	0.8	
sIgA	IGA-131/144 H5N5	1198.1	366.1	6.2	0.8	
sIgA	IGA-131/144 H5N4S1	976.3	204.1	5.7	1	
sIgA	IGA-131/144 H5N5S1	1016.9	366.1	5.6	0.8	

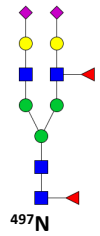
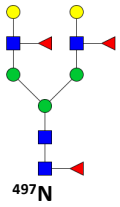
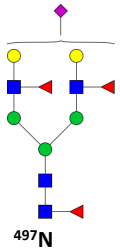
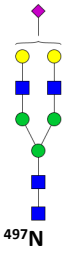
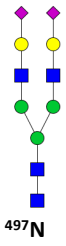
Glycoprotein	Glycoform	Precursor Ion	Product Ion	RT	D RT	Glycan Structure
sIgA	IGA-131/144 H8N2	934.1	204.1	5.1	1	
sIgA	IGA-131/144 H9N2	966.5	204.1	4.6	0.8	
sIgA	IGA-131/144 H5N5	1198.1	366.1	6.2	0.8	
sIgA	J-71 H4N5	1271.3	366.1	5.7	0.8	
sIgA	J-71 H5N4F1S1	1052.8	366.1	4.85	0.5	
sIgA	J-71 H5N4S1	1016.2	204.1	5.1	0.8	

Glycoprotein	Glycoform	Precursor Ion	Product Ion	RT	D RT	Glycan Structure
sIgA	SC-135 H5N4F1S2	906.2	204.1	5.1	0.8	
sIgA	SC-421 H5N4F1S1	1116.3	366.1	7.3	1	
sIgA	SC-469 H4N5F1	1069.8	366.1	6.8	0.8	
sIgA	SC-469 H5N4F1S1	1153.2	366.1	7.2	0.8	
sIgA	SC-469 H5N4F1S2	937.9	204.1	5.15	1	
sIgA	SC-469 H5N5F1S1	1060.2	366.1	4.25	0.5	

Glycoprotein	Glycoform	Precursor Ion	Product Ion	RT	D RT	Glycan Structure
sIgA	SC-469 H5N5F1S2	1132.9	366.1	4.4	0.5	
IgG	IGG H4N4F1_2	922.1	204.1	2	0.5	
IgG	IGG H4N5_2	941.1	204.1	2.1	0.5	
Lactoferrin	LF-156 H5N4F1	1250.6	204.1	5.8	0.8	
Lactoferrin	LF-156 H5N4F1S1	1058.9	204.1	6.8	0.8	
Lactoferrin	LF-156 H5N4F1S2	1117.1	204.1	7.4	0.8	

Glycoprotein	Glycoform	Precursor Ion	Product Ion	RT	D RT	Glycan Structure
Lactoferrin	LF-156 H5N4F2	1287.1	204.1	5.8	0.8	
Lactoferrin	LF-156 H5N4F2S1	1088.1	204.1	6.7	0.8	
Lactoferrin	LF-156 H5N4F3	1323.6	204.1	5.6	0.8	
Lactoferrin	LF-156 H5N4F3S1	1117.3	204.1	7.35	0.8	
Lactoferrin	LF-156 H5N4F3S2	1175.3	204.1	9	0.8	
Lactoferrin	LF-156 H6N5F2S1	1161.1	204.1	6.4	0.8	

Glycoprotein	Glycoform	Precursor Ion	Product Ion	RT	D RT	Glycan Structure
Lactoferrin	LF-156 H6N5F3S1	1190.3	366.2	6.3	0.8	
Lactoferrin	LF-497 H5N4F1	966.7	204.1	4.85	0.8	
Lactoferrin	LF-497 H5N4F1S1	1039.4	204.1	4.86	0.8	
Lactoferrin	LF-497 H5N4F1S2	1112.2	204.1	5.05	0.8	
Lactoferrin	LF-497 H5N4F2	1003.2	204.1	4.9	0.8	
Lactoferrin	LF-497 H5N4F2S1	1075.9	204.1	4.86	0.8	

Glycoprotein	Glycoform	Precursor Ion	Product Ion	RT	D RT	Glycan Structure
Lactoferrin	LF-497 H5N4F2S2	1148.7	204.1	5.1	0.8	
Lactoferrin	LF-497 H5N4F3	1039.7	204.1	4.8	0.8	
Lactoferrin	LF-497 H5N4F3S1	1112.4	204.1	5.1	0.8	
Lactoferrin	LF-497 H5N4S1	1002.9	204.1	4.9	0.5	
Lactoferrin	LF-497 H5N4S2	1075.7	204.1	4.8	0.8	

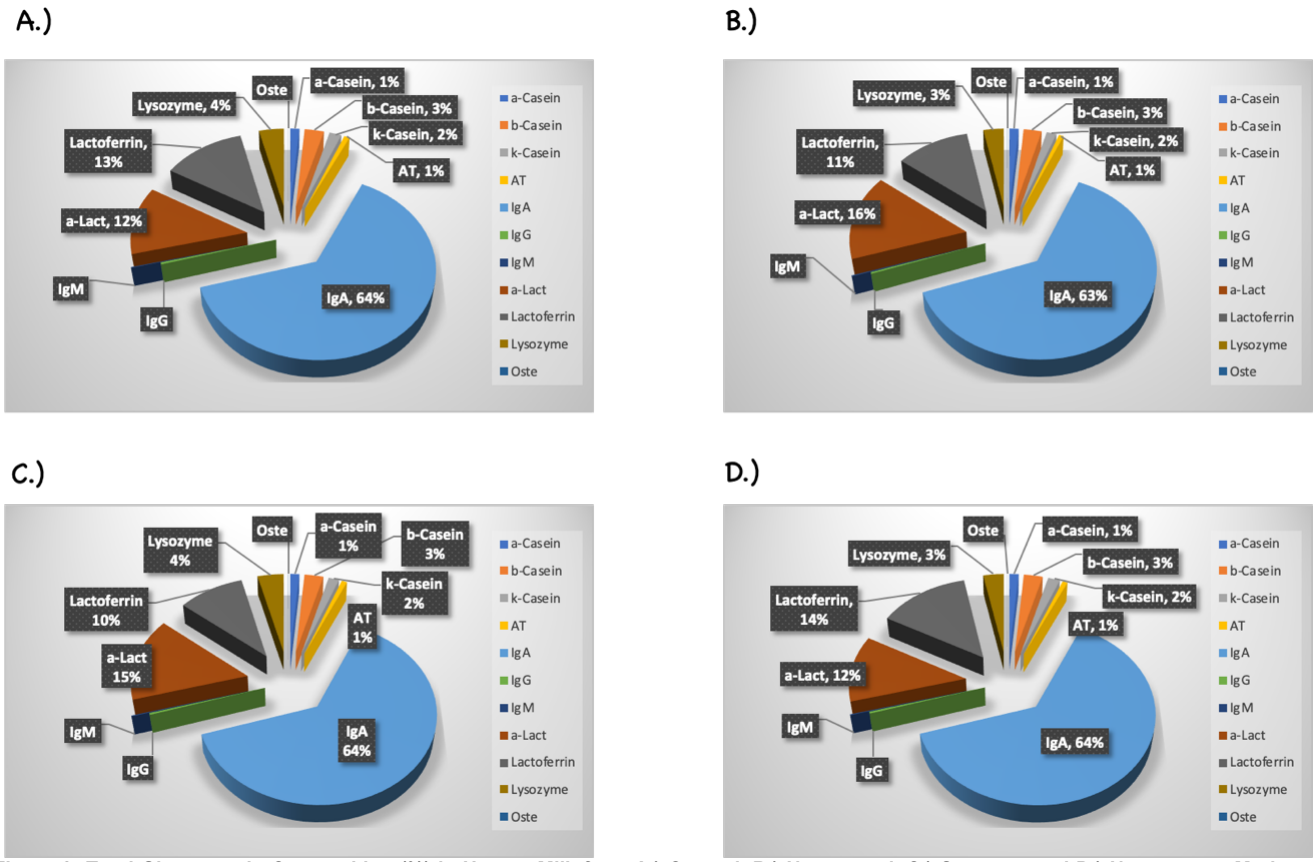
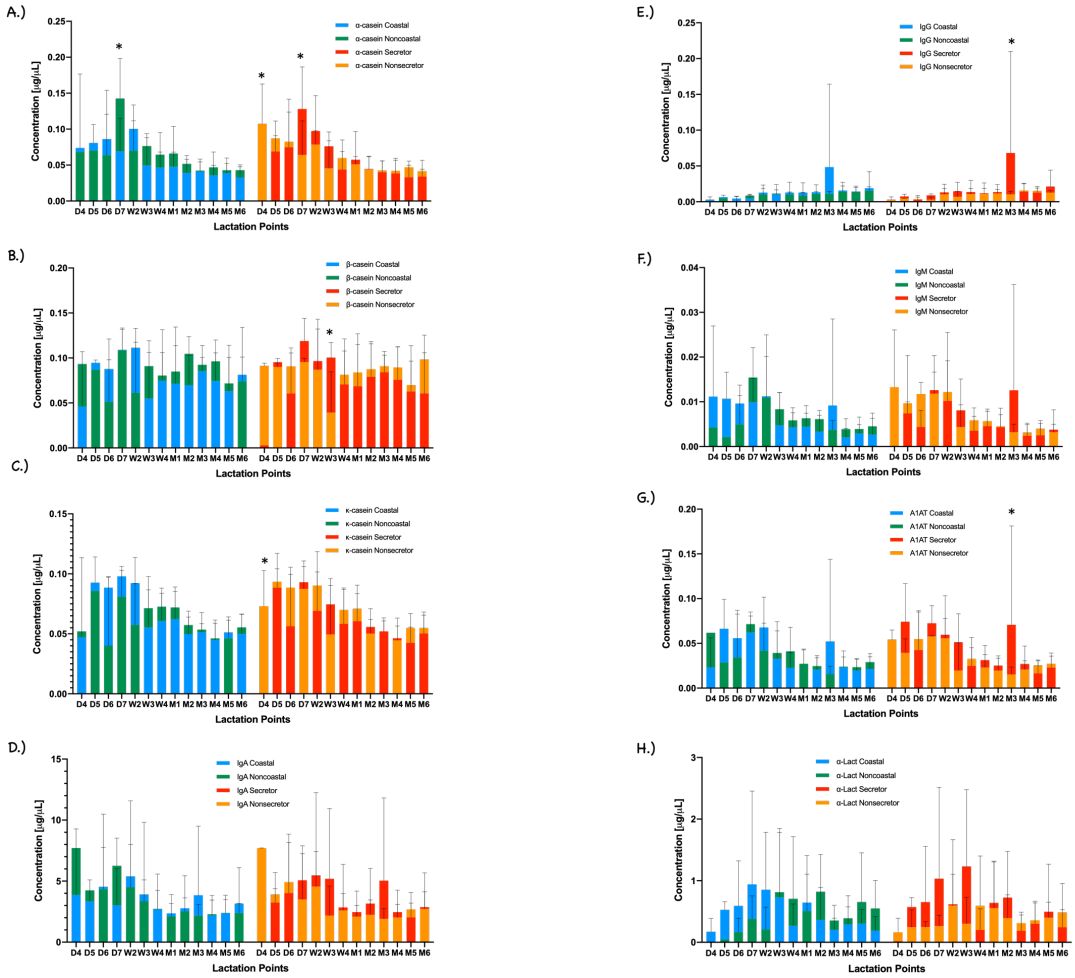


Figure 2: Total Glycoprotein Composition (%) in Human Milk from A.) Coastal, B.) Noncoastal, C.) Secretor, and D.) Nonsecretor Mothers living in Baybay City, Leyte throughout the First Six Months of Lactation.



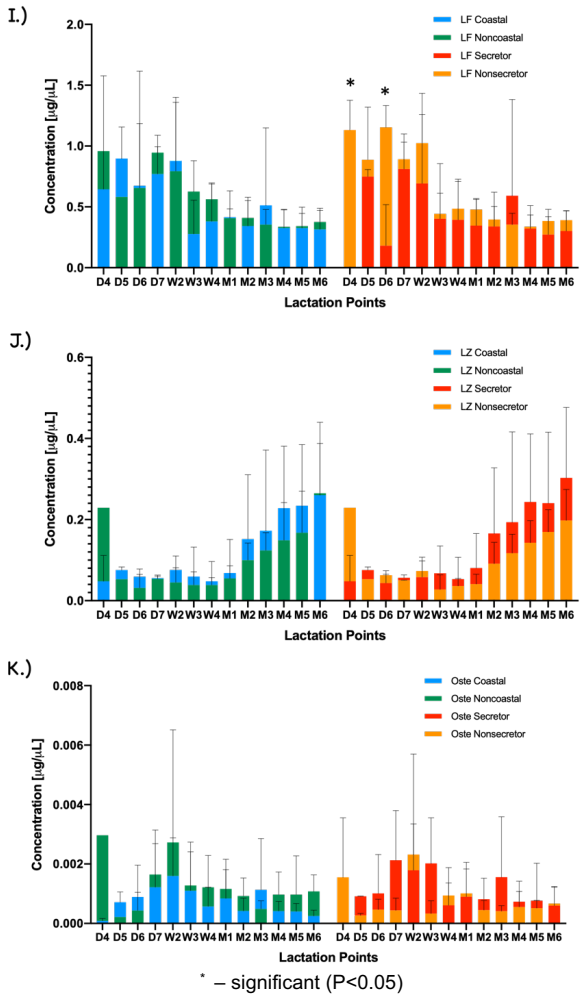


Figure 3: Human Milk Glycoprotein Concentrations (µg/µL): A.) α-Casein, B.) β-Casein, C.) κ-Casein, D.) IgA, E.) IgG, F.) IgM, G.) α1-Antitrypsin, H.) α-Lactalbumin, I.) Lactoferrin, J.) Lysozyme, and K.) Osteopontin from Coastal, Noncoastal, Secretor, and Nonsecretor Mothers living in Baybay City, Leyte.

Filipino Mothers have High Milk IgA Content

Filipino mothers have higher immunoglobulin A concentration (3.16 g/L, **Figure 3D**) compared to American mothers (~0.5 g/L), while American mothers have higher α-Lactalbumin (~6 g/L) compared to Filipino mothers (0.522 g/L, **Figure 3H**). The highest glycoprotein component in Filipino breastmilk in this study was IgA, in contrast to American breastmilk that was α-Lactalbumin (Goonatilleke et al. 2019a). In the earlier study of Klein et al. (2018), urban Filipino mothers living in Cebu City had a lower immunoglobulin A (0.145 g/L) and a similar α-Lactalbumin (0.534 g/L) concentrations to Filipino mothers living in Baybay City. It is worth noting that the authors employed the same protocol with our study and that of Goonatilleke et al. (2019a).

Since protein synthesis is a gene translation product, difference in protein expression is expected between different ethnicities. Klein et al. (2018) indicated that α-Lactalbumin is an innate immune milk protein which should not greatly differ within the same race, while secretory IgA is an acquired immune milk protein in which its abundance reflect the mother's past and recent pathogen exposures. It is reasoned that the similar abundance of α-Lactalbumin between Cebu Filipino mothers (0.534 g/L) and Baybay Filipino mothers (0.522 g/L) was due to these groups belonging to the same ethnicity, while the higher sIgA concentration from Baybay Filipino mothers (3.16 g/L) compared to Cebu Filipino mothers (0.145 g/L) was in line with the difference in their socioeconomic status. According to Ruiz et al. (2017), mothers with lower socioeconomic status tend to have higher sIgA concentration due to higher pathogen exposure brought by different environmental settings (rural and urban). American mothers also have low sIgA concentration (~0.5 g/L) which reflects the lower maternal exposure to pathogens associated with Western lifestyle practices (Goonatilleke et al. 2019a). This complements well with the observations that lactating mothers from countries with high infant mortality rate due to infection (Bolivia, Nepal, and Philippines) tend to have higher concentrations of acquired immune milk proteins (Klein et al. 2018).

These results illustrate the biological adaptation of Filipino mothers prioritizing intestinal protection of their breastfeeding infant, as secretory IgA is the predominant antibody in human milk that mediates the immune protection of the gut mucosa (Baumann et al. 2010). The predominance of α-lactalbumin in the breastmilk of American mothers also signifies the prioritization of nutrient transfer in the breastfeeding American infants, with α-Lactalbumin as a nutrient transporter in addition to its well-known role in lactose biosynthesis (Barbana et al. 2011; Layman et al. 2018). The difference in human milk glycoprotein predominance could also give insight on growth rate discrepancy between interracial babies, as American babies are known to be bigger in size compared to their Filipino counterparts. Although a smaller number of Filipino mothers were enrolled in this study, the trend in glycoprotein abundances (specifically sIgA, IgM, and IgG) show the changes occurring in general over the course of lactation (Gao et al. 2012; Zhang et al. 2013). This suggests that the abundance and temporal progression of major immune milk glycoproteins is a well-conserved evolutionary trait.

The higher abundance of secretory IgA (**Figure 3D**) and IgM (**Figure 3F**) at early stages of lactation and the increasing IgG content (**Figure 3E**) as breastmilk matures were particularly observed in this study. This was consistent with the proteomic study of Zhu et al. (2021a) that immunoglobulins (IgA, IgM, and IgD) were decreasing in abundance throughout lactation, while IgG increased over time. The high level of sIgA at the early stages of lactation would provide greater protection against increasing pathogenic exposure during the early postnatal life as the infant's immune system is still developing (Zhang et al. 2013). The increasing IgG abundance in mature milk also complements the diminishing maternal IgG of the infant derived from transplacental transport due to the newborn's immune system naivety. In addition, sIgA and IgM are more abundant in transitional milk, while IgG in mature milk. This reflects the transformation of human milk function from direct pathogen neutralization conferred by secretory IgA to adaptive immune learning via antigen intake facilitated by IgG, as lactation progresses (Gao et al. 2012). Follow-up research with a larger group of Filipino mothers would nonetheless be needed to confirm these trends in Filipino mothers. It is our immediate objective to help illuminate the predominant difference in breastmilk composition which could significantly impact the growth rate of breastfeeding infants.

Other immunoglobulins like IgG and IgM, compose < 1% of the total glycoproteins in human milk due to the difference in their mechanism in providing immunological defense as these antibodies mainly confer systemic immunity. Specifically, IgG harnesses the innate immune system by coordinating with Fc-mediated antibody functions of neutrophiles, macrophages, etc., which also implies that IgG levels are regulated to avoid overshooting immune responses (Epp et al. 2016). Moreover, increase in lysozyme concentration (**Figure 2J**) as lactation progresses inhibits growth of pathogens by hydrolyzing bacterial cell walls, which leads to instability and bacterial cell death (Callewaert & Michiels 2010). Despite the increasing

lysozyme concentration, the presence of the protease inhibitor, α1-antitrypsin (**Figure 2G**), in human milk protects immunoglobulins from degradation and direct insult from pathogens in the digestive tract. With the upregulation of lysozyme and the association of α1-antitrypsin on both innate and adaptive/acquired immune-related glycoproteins, it is proposed by Zhang et al. (2016) that there is a possible synergistic interaction in human milk glycoproteins, promoting infant immunity and growth-related development.

Coastal Environment has No Influence on Protein Expression in Human Milk

Comparison of the milk glycoprotein profile from coastal and noncoastal mothers presented here is not meant to differentiate between the samples, but rather to show a preliminary data in the glycoprotein diversity. Individual glycoproteins, α-Casein, β-Casein, κ-Casein, IgA, IgG IgM, α1-Antitrypsin, α-Lactalbumin, Lactoferrin, Lysozyme and Osteopontin, in human milk showed no significant difference ($P>0.05$) in concentration between coastal and noncoastal Filipino mothers living in Baybay City, Leyte. These results suggest that the coastal environment has no significant influence on protein expression in human milk. This was not surprising since protein biosynthesis is a gene translation product and was already anticipated that environmental factors hold no influence in protein expression. Immunoglobulin A (IgA) had the highest percent composition (63-64%) of the total glycoprotein in human milk for coastal and noncoastal mothers throughout lactation. The other glycoproteins that constitute the total glycoprotein content in human milk were also comparable for coastal and noncoastal mothers. α-Casein, β-Casein, κ-Casein, α1-Antitrypsin (AT) and Lysozyme constitute 10-11% of the total glycoprotein content in human milk, while < 1% is comprised of IgM, IgG and Osteopontin (Oste). Human milk proteins drive infant growth not only as a source of essential amino acids but also for enhanced nutrient absorption and digestion (Zhu and Dingess 2019). Not only immunological protection, but these milk glycoproteins also confer immunomodulatory function as lactoferrin and osteopontin can regulate TLR4 signaling (Ando et al. 2010) and promote the production of anti-inflammatory cytokines (Lönnerdal et al. 2016), respectively.

In general, these glycoproteins were significantly decreasing ($P<0.01$) in concentration after the first week of lactation except for β-Casein, IgG, α-Lactalbumin, and Osteopontin. Lysozyme, on the other hand, was significantly increasing in concentration for both coastal and noncoastal mothers starting at Month 2 until Month 6 of lactation (**Figure 3I**). The increase in lysozyme concentration is attributed to its antimicrobial and anti-inflammatory function (Callewaert & Michiels 2010), which is coordinative to the maturation of the infant's innate and adaptive immune system. Milk colostrum had the highest total glycoprotein concentration, which means higher levels of antibodies to compensate for the infant's undeveloped immune system. The abundance of milk glycoproteins later decreased in concentrations as lactation progresses and as the immune system matures. This agrees with the general composition that defines mature milk, from high protein content to a higher fatty acid content (Ballard & Morrow 2013). This observed shift is necessary to support the increasing caloric demand of the rapidly growing infant.

Nonsecretor Mothers have Higher Milk Lactoferrin compared to Secretor Mothers

Not all glycoproteins were comparable in concentrations for secretor and nonsecretor mothers. Lactoferrin was significantly higher ($P<0.05$) with a percent composition of 14% on the total milk glycoproteins from nonsecretor mothers, while only 10% on the total milk glycoproteins for secretor mothers (**Figure 2**). However, this higher lactoferrin content (14%) in nonsecretor milk was offset by the relatively lower α-Lactalbumin content (12%) in nonsecretor milk compared to (15%) in secretor milk. The higher lactoferrin expression in nonsecretor mothers could result in increased antimicrobial property of nonsecretor milk, as lactoferrin functions as an iron (Fe^{3+}) chelator and would inhibit microbial growth (Georgi et al. 2013; Liu & Newburg 2013). Our results suggest that nonsecretor mothers may have other protective mechanisms that ensure the healthy growth of their breastfeeding infant. For example, low FUCT2 activity in nonsecretor mothers and the subsequent low fucosylation of milk glycans in nonsecretor milk protects breastfeeding infant from *E. hystolytica* infection, the 3rd leading cause of death by parasitic diseases after malaria and schistosomiasis (Pritt & Clark 2008). According to Bode and Jantscher-Krenn (2012), a free terminal Gal is essential in the lectin-ligand interaction of *E. hystolytica* and human milk glycans. Thus, α1-2 fucosylated milk glycans highly expressed in secretor milk would potentially be less effective in its soluble decoy function with *E. hystolytica*.

Generally, all identified glycoproteins were not significantly influenced by secretor status. Immunoglobulin A (IgA), the predominant glycoprotein which constitute 64% of the total glycoprotein content in human milk, was not significantly influenced by secretor status and coastal environment. Our results were in line with the earlier findings of Bai et al. (2018) that concentrations of immune milk proteins, namely IgA and IgG, were not significantly different between secretor and nonsecretor mothers. Since whey proteins are much more heavily glycosylated, α-lactalbumin, lactoferrin, and immunoglobulins are also more resistant to proteolytic cleavage, which remain intact and are not hydrolyzed into endogenous milk peptides (Zhu & Dingess 2019).

The evolutionary rationale of HMO predominance in human milk is to serve as decoy receptors for these pathogens as well as prebiotics for the growth of beneficial gut microbiota. The same is true with human milk glycoproteins conferring protective function, as well as immunomodulation of the innate immune system of the growing infant. Perhaps the multivalent extensively branched glycans of these glycoproteins provide a superior mechanism for pathogen capture compared to the relatively simple structures of HMOs (Bernardi et al. 2013). More likely, human milk glycoproteins and HMOs work synergistically to confer optimum protection evolution could offer to breastfeeding infants. Nonetheless, glycoproteins in human milk have warranted investigation in its function as competitive inhibitors of pathogen binding, immunomodulators, and prebiotics for commensal gut microbiota. Recent studies of Karav et al. (2019) and Goonatilleke et al. (2019b) have led to a better understanding on the importance of milk glycoproteins in shaping the gut microbiota and immune system of the growing infant. Considering the effects of glycosylation in modulating the severity of allergic reactions via antibody glycan-mediated immune response, long-term effects of human milk glycoproteins relating to prevention of autoimmunity should be spearheaded in future research.

Specific glycoproteins in human milk exhibiting antimicrobial activities including IgA, IgG, and Lactoferrin, are the most studied antimicrobial proteins. The glycoprotein secretory IgA is the dominant antibody fraction in human milk as depicted previously in **Figure 2**. Immunoglobulin A (IgA) has glycan structures with Neu5Ac, GlcNAc and Man terminus that are known as pathogen binding epitopes (Peterson et al. 2013). The diversity of glycan epitopes used by pathogens in binding to epithelial cell surfaces is impressively countered by the equally diverse glycan moieties on IgA and other glycoproteins in

human milk that act as decoy receptors to pathogen binding. The importance of glycoform abundances and its dynamic changes in human milk glycoproteins as lactation progress could illustrate a scheme of how evolution has directed biological function of glycoproteins that are efficiently utilized by the growing infant, either for immunity and/or optimal colonization of beneficial gut microbiota.

Dynamic Changes of Glycoforms in Human Milk Glycoproteins

Secretory IgA is characterized with its four protein sub-units, IgA1, IgA2, Joint Chain (J Chain), and Secretory Component (SC), having N-glycosylation varying for each sub-unit. The glycopeptides for IgA1 and IgA2 with respective glycosylation sites, ^{144}N and ^{131}N, share the same tryptic peptide backbone, making site-specific glycan structures indistinguishable for the two sub-units (Huang et al. 2017). Site heterogeneity quantification of site-specific glycosylation for sIgA reveal fold changes of glycoforms at specific glycosites on its protein sub-units with tryptic digestion. Quantification of the site-specific glycosylation of other human milk glycoproteins, including lactoferrin, α1-Antitrypsin, IgG, and IgM throughout the six months lactation period were also performed.

Lactoferrin was observed to be the second most abundant milk glycoprotein next to immunoglobulin A (IgA) from Filipino mothers. It has three N-glycosylation sites, two of which are highly occupied (^{156}N and ^{497}N). This study presents the first detailed site-specific glycosylation profile of human milk glycoproteins from Filipino mothers as influenced by coastal environment and secretor status. This study offers novel insight on the dynamic changes in glycosylation at a site-specific level, as well as the variation of glycoforms in human milk glycoproteins. The dynamic expression of these N-glycans is to address the needs of the breastfed infant, in terms of gut health, immunity, and overall growth and development. **Table 3** shows the glycoforms of human milk glycoproteins sIgA, IgG, lactoferrin, and α1-Antitrypsin that had abundance suitable for quantification (S/N>10) throughout the first six months of lactation. Unfortunately, IgM had no glycans that could be quantified (S/N>10) out of the 11 glycoforms monitored for IgM in the MRM assay, while only 2 from the total of 9 glycans on the ^{176}N site of IgG2 were quantitatively observed. There is no well characterized role for the IgM glycosylation in mediating effector function, unlike for IgA and IgG, which could explain its low glycosylation level (Keyt et al. 2020). Moreover, the immunological redundancy of IgM in individuals with high sIgA concentration, which we observed in the breastmilk of Filipino mothers (Michaud et al. 2020). For the sIgA components, 13 glycans structures with high-mannose and a certain degree of sialylation were quantified out of the 18 complex glycans monitored for IgA1/2. Three out of eight glycoforms were observed at the single ^{71}N glycosylation site of the J Chain, while seven Fuc-sialylated glycans from the fifteen complex glycans had abundance suitable for quantification on the secretory component (SC) of sIgA. It was remarkable that all 20 highly sialylated glycan structures monitored on the ^{156}N and ^{497}N glycosylation sites of lactoferrin were quantitatively observed in Filipino mothers while only ≤12 quantifiable lactoferrin glycoforms from American mothers (Goonatilleke et al. 2019a; Huang et al. 2017). For α1-Antitrypsin, however, only a single tri-antennary sialylated glycan could be quantified on the ^{271}N glycosylation site out of the 10 highly sialylated glycoforms monitored on two of its N-glycosites, ^{107}N and ^{271}N. Due to either resistance of many glycoproteins to tryptic digestion adding up to the glycoprotein's relatively large size or the complete lack of glycoform expression on glycosylation sites monitored, glycans on several N-sites were not quantitatively observed. It is recommended that optimization of tryptic digestion be conducted in future studies to allow complete quantification of glycosylation sites.

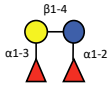
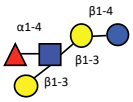
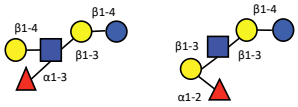
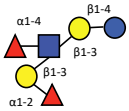
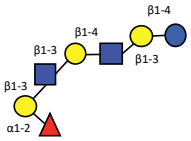
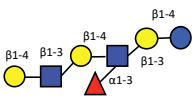
The role and underlying mechanism of the dynamic changes in human milk protein glycosylation is poorly understood. Glycosylation in proteins have several biological functions. It protects the peptide backbone from proteolysis which meant that availability of bioactive peptide in human milk may be influenced by the degree of glycosylation of human milk glycoproteins (Goettig 2016). Glycans also activate the infant's naïve immune system through direct and indirect interactions in the immune regulatory pathways (Baum & Cobb 2017). In the previous study on human milk lactoferrin, enzymatic removal of terminal fucose residues resulted in a significant increase in *Salmonella enterica typhimurium* adhesion, implicating the role of the fucose residue in lactoferrin's binding inhibition to this bacterial strain (Barboza et al. 2012). For the Heidelberg strain, however, the authors noted that binding inhibition was attributed to lactoferrin's terminal galactose residues, which demonstrated that different glycoforms interact with specific bacteria more effectively. To the best of our knowledge, this is the first study that provided insight on how these glycans are biologically directed and actively involved in the optimal growth and development of the infant through mapping the dynamic changes of glycoforms in human milk glycoprotein throughout the first six months of lactation (**Figure 4)**.

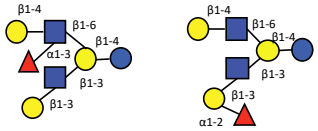
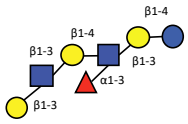
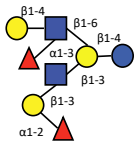
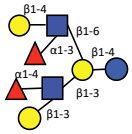
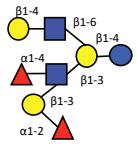
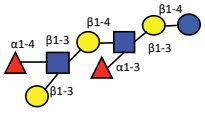
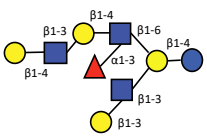
Coastal Environment has a Degree of Influence on Glycoform Expression

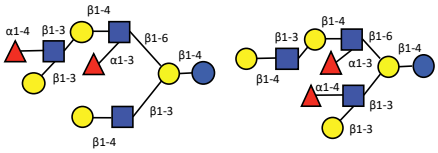
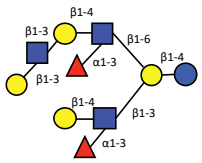
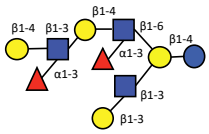
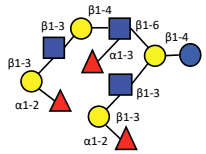
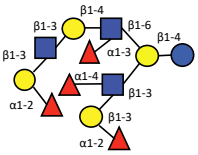
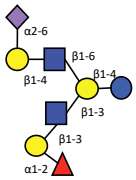
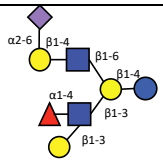
N-glycosylation on the four sub-units of secretory IgA, the most predominant immune glycoprotein in human milk, were monitored in this study. The sub-units, IgA1, IgA2, Joint Chain (J Chain), and Secretory component (SC), have heterogenous glycan structures known as glycoforms on their respective N-glycosylation sites and vary in degree of expression. IgA1 and IgA2 glycosites, ^{144}N and ^{131}N, share the same tryptic peptide backbone and thus, glycoforms on these glycosites could not be differentiated. Instead, glycoforms on both IgA1 and IgA2 glycosites were normalized to a common tryptic peptide and monitored as glycoforms of IgA1/2. **Figure 4A** shows the glycoforms of IgA1/2 quantitatively monitored throughout the first six months of lactation from coastal and noncoastal mothers. Glycoforms, H3N4, H4N5S1, H5N4S1, H8N2, and H9N2, were significantly influenced ($P<0.01$) by the coastal environment and that expression of these sialylated glycoforms were significantly higher in coastal milk compared to noncoastal ones. The other glycoforms, H3N3, H4N4S1, and H5N5S1, did not significantly differ ($P>0.05$) in expression between coastal and noncoastal milk and throughout the first six months of lactation. Although the exact role of these glycoforms is not fully understood, this study gives insight that the expression of specific glycoforms is regulated and targeted to address the immediate needs of the growing infant notwithstanding the personal energy toll for the mother.

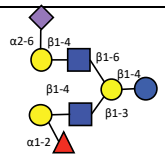
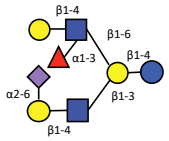
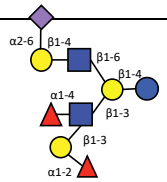
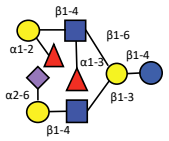
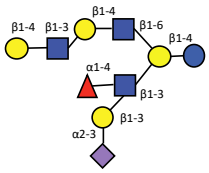
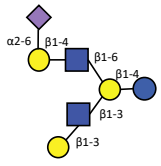
The secretory component (SC) glycoforms on glycosylation sites, ^{135}N, ^{421}N, and ^{469}N, were not significantly influenced ($P>0.05$) by the coastal environment. This means that expressions of quantifiable core-fucosylated, heavy sialylated glycoforms shown in **Figure 4B** were not significantly different in coastal and noncoastal milk. However, significant decrease ($P<0.01$) of expression was observed for the core-fucosylated glycoform H4N5F1 (^{469}N), serving as putative precursor to other sialylated glycoforms H4N5F1S1 (^{469}N) and H4N5F1S2 (^{469}N), across the first six months of lactation. As the general trend of glycoproteins in human milk decreased throughout lactation (**Figure 3**), it gives a higher chance for glycosyltransferases in decorating precursor glycoforms resulting to the decorated glycan structures and could explain the observed significant

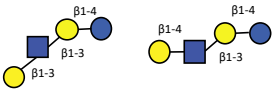
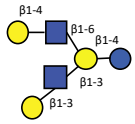
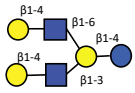
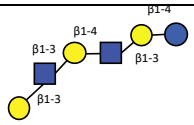
Table 3: Annotated HMOs from Filipino Mothers living in Baybay City, Leyte.

Hex_HexNAc_Fuc_NeuAc	Mass	RT	Class	Annotation/Structure
2_0_1_0				
(C18 H34 O15)	490.189	12.83	Fuc	2'FL
2_0_1_0				
(C18 H34 O15)	490.189	1.55	Fuc	3'FL
2_0_2_0				
(C24 H44 O19)	636.246	14.83	Fuc	LDFT
3_1_1_0				
(C32 H57 N O25)	855.323	12.44	Fuc	LNFP II
3_1_1_0				
(C32 H57 N O25)	855.323	14.67	Fuc	LNFP III/LNFP I
3_1_2_0				
(C38 H67 N O29)	1001.381	11.92	Fuc	LNDFH I
4_2_1_0				
(C46 H80 N2 O35)	1220.453	19.21	Fuc	IFLNH I
4_2_1_0				
(C46 H80 N2 O35)	1220.452	17.37	Fuc	IFLNH III

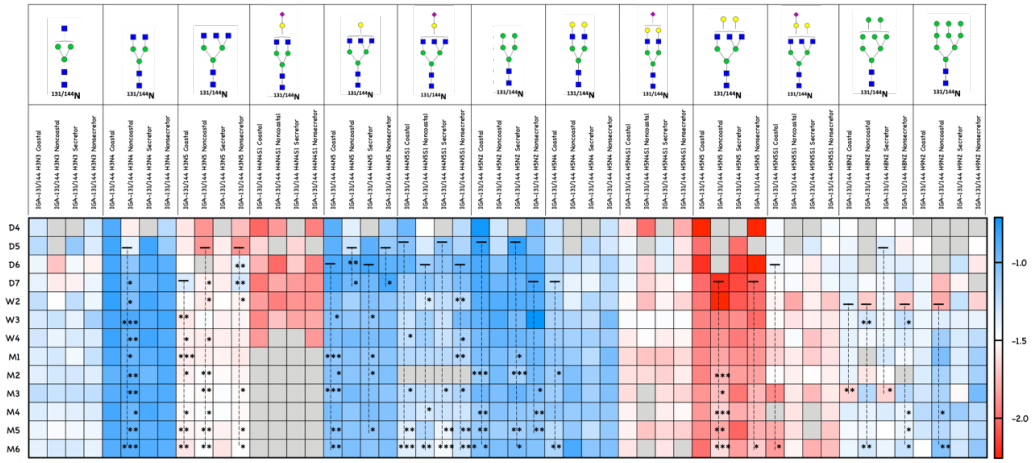
Hex_HexNAc_Fuc_NeuAc	Mass	RT	Class	Annotation/Structure
4_2_1_0				
(C46 H80 N2 O35)	1220.454	17.12	Fuc	MFLNH III/ MFLNH I
4_2_1_0				
(C46 H80 N2 O35)	1220.455	16.43	Fuc	MFpLNH IV
4_2_2_0				
(C52 H90 N2 O39)	1366.513	15.72	Fuc	DFLNHa
4_2_2_0				
(C52 H90 N2 O39)	1366.513	14.61	Fuc	DFLNHb
4_2_2_0				
(C52 H90 N2 O39)	1366.511	18.28	Fuc	DFLNHc
4_2_2_0				
(C52 H90 N2 O39)	1366.513	14.00	Fuc	DFpLNH II
5_3_1_0				
(C60 H103 N3 O45)	1585.587	19.69	Fuc	FLNO

Hex_HexNAc_Fuc_NeuAc	Mass	RT	Class	Annotation/Structure
5_3_2_0				
(C66 H113 N3 O49)	1731.646	19.20	Fuc	DFLNnO I / DFLNO II
5_3_2_0				
(C66 H113 N3 O49)	1731.647	18.25	Fuc	DFLNnO II
5_3_2_0				
(C66 H113 N3 O49)	1731.646	18.10	Fuc	DFLNO I
5_3_3_0				
(C72 H123 N3 O53)	1877.706	17.95	Fuc	TFiLNO
5_3_4_0				
(C78 H133 N3 O57)	2023.761	17.05	Fuc	Tetra-iso-LNO
4_2_1_1				
(C57 H97 N3 O43)	1511.551	20.98	Fuc Sia	FS-LNH I
4_2_1_1				
(C57 H97 N3 O43)	1511.550	19.07	Fuc Sia	FS-LNH III

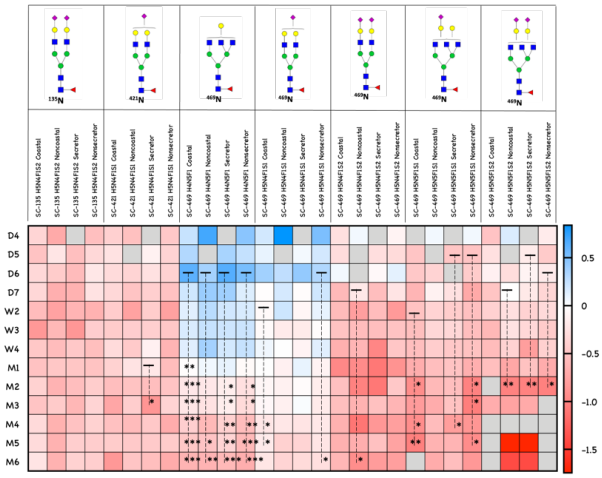
Hex_HexNAc_Fuc_NeuAc	Mass	RT	Class	Annotation/Structure
4_2_1_1				
(C57 H97 N3 O43)	1511.551	19.88	Fuc Sia	FS-LNH
4_2_1_1				
(C57 H97 N3 O43)	1511.552	21.92	Fuc Sia	FS-LNnH I
4_2_2_1				
(C63 H107 N3 O47)	1657.611	21.67	Fuc Sia	DFS-LNH
4_2_2_1				
(C63 H107 N3 O47)	1657.592	23.20	Fuc Sia	DFS-LNnH
5_3_1_1				
(C71 H120 N4 O53)	1876.687	21.12	Fuc Sia	FS-LNO
2_0_0_1				
(C23 H41 N O19)	635.226	18.45	Sia	3'SL
2_0_0_1				
(C23 H41 N O19)	635.227	12.78	Sia	6'SL
4_2_0_1				
(C51 H87 N3 O39)	1365.492	20.69	Sia	S-LNH

Hex_HexNAc_Fuc_NeuAc	Mass	RT	Class	Annotation/Structure
3_1_0_0				
(C26 H47 N O21)	709.265	15.02	Undec	LNT/LNnT
4_2_0_0				
(C40 H70 N2 O31)	1074.397	17.41	Undec	LNH
4_2_0_0				
(C40 H70 N2 O31)	1074.397	17.87	Undec	LNnH
4_2_0_0				
(C40 H70 N2 O31)	1074.394	20.00	Undec	pLNH

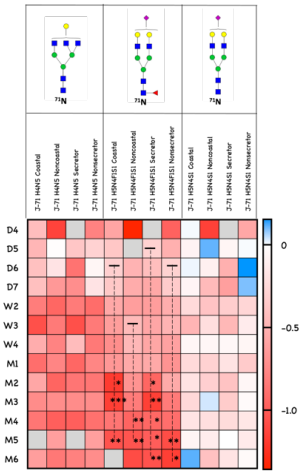
A.) IgA1/2 Glycoforms



B.) Secretory Component (SC) Glycoforms



C.) Joint (J) Chain Glycoforms



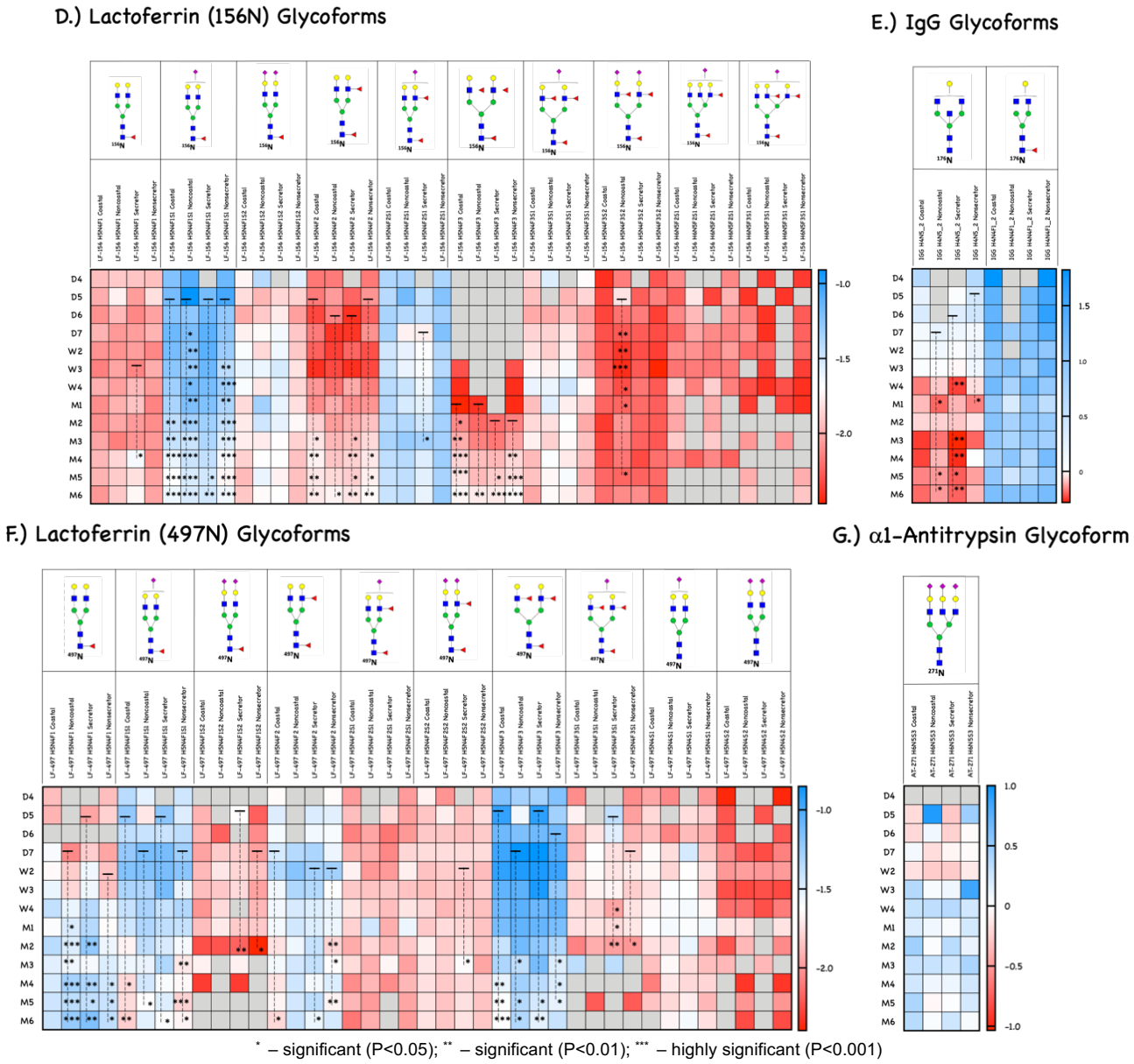


Figure 4: Dynamic Changes of Glycoforms in Human Milk Glycoproteins sIGA, IgG, Lactoferrin, and α1-Antitrypsin throughout the First Six Months Lactation Period of Coastal, Noncoastal, Secretor, and Nonsecretor Mothers living in Baybay City, Leyte. sIgA components: A.) IgA1/2, B.) SC, and C.) J Chain Glycoforms. E.) IgG2 Glycoforms, D.) Lactoferrin N156 and F.) Lactoferrin N497 Glycoforms. G.) α1-Antitrypsin Glycoform. Data reported as log value (mean).

decrease of precursor glycans as lactation progresses. We hypothesize that as the immune system of the breastfeeding infant matures through the course of lactation, the expression of some of these glycoforms would taper down as they served to prime only the naïve immune system of the breastfeeding infant.

For the Joint chain (J Chain) glycoforms, the expression of the core-fucosylated sialylated glycoform, H5N4F1S1 (^{71}N), was significantly influenced (P<0.05) by the coastal environment while the other two quantifiable glycoforms, H4N5 (^{71}N) and H5N4S1 (^{71}N), were not. However, it can be seen in **Figure 4C** that core fucosylation significantly decreases (P<0.001) as lactation progresses, evident on the decrease of glycoform H5N4F1S1 (^{71}N) expression. The role of core fucose as molecular switches is known to control biological activities including immune response, signal transduction, and cell adhesion. In addition, core fucose N-glycans are endogenous ligands to Dectin-1, a pattern recognition receptor expressed on many immune cells, including dendritic cells, monocytes, and macrophages, triggering superoxide production, phagocytosis enhancement, and cytokines induction (Manabe et al. 2019). Not only that, core fucosylation of N-glycans also plays a role in shaping the gut microbiota of the breastfeeding infant. Moreover, core-fucosylated N-glycans selectively promote the colonization of *Bifidobacterium* spp. and *Lactobacillus* spp. in the infant's gut and the resulting L-fucose metabolites evoke B cell activation via the B cell receptor (BCR) mediated signaling pathway. Hence, glycoproteins contribute in shaping the gut microbiome by releasing N-glycans as free oligosaccharides and would complement the existing abundance of HMOs as prebiotics in optimal colonization of beneficial gut microbiota.

Only two quantifiable glycoforms of IgG were observed in subclass, IgG2 on glycosite ^{176}N. These two glycoforms, H4N5 (^{176}N) and H4N4F1 (^{176}N), have a bisecting GlcNAc and a core fucose residue, respectively. The expression of these two glycoforms are not significantly influenced (P>0.05) by coastal environmental condition. The core fucosylated glycoform, H4N4F1 (^{176}N), did not significantly decrease (P>0.05) in expression through the first six months of lactation (**Figure 4E**). However, the expression of the bisecting GlcNAc glycoform, H4N5 (^{176}N), significantly decreased (P<0.05) in noncoastal

milk. Bisecting GlcNAc has been described to induce antibody-dependent cell-mediated cytotoxicity (ADCC) and the increased levels of this glycan type in IgG has been linked to autoimmunity (Maverakis et al. 2015). Bisecting GlcNAc are also no longer substrates of other glycosyltransferases and do not undergo further elongation (Nakano et al. 2019). The authors concluded that bisecting GlcNac suppresses terminal modification of N-glycans, which could explain other glycoforms of IgG2 not quantitatively observed in this study.

Among the innate immune proteins of human milk, there has been renewed interest in lactoferrin bioactivity for its inhibitory effect on SARS-CoV-2 (Chang et al. 2020; Habib et al. 2021). Remarkably for lactoferrin in this study, all twenty (20) monitored highly fucosylated and/or sialylated glycoforms were quantitatively observed on glycosites, ^{156}N and ^{497}N. For the glycosite ^{156}N, the glycoform H5N4F1S1 (^{156}N) was observed to be expressed significantly higher (P<0.05) in noncoastal milk compared to coastal ones, while no significant differences (P>0.05) in expression were observed for the rest of the monitored glycoforms on glycosite ^{156}N. Few of the monitored ^{156}N glycoforms shown in **Figure 4D**, specifically H5N4F1 and H5N4F1S2, did not significantly change (P>0.05) in expression throughout lactation. On the other hand, three glycoforms namely H5N4F3 (^{156}N), H6N5F2S1(^{156}N), and H6N5F3S1 (^{156}N), were only expressed at certain lactation points. Glycoform H6N5F2S1(^{156}N) expression tapers out at Month 5 for coastal mothers while earlier at Month 1 of lactation for noncoastal mothers. To our knowledge, this is the first preliminary study on a small number of cohorts that has reported environmental influence on the expression of these highly fucosylated N-glycan structures. It is also important to point out that difference in the degree of terminal fucosylation of these glycoforms represent a structural homology of the different fucosylated HMO structures. This is of particular interest because similar glycan structures share almost the same biological functionality, which is not well characterized for terminal fucosylation on human milk glycoproteins. Moreover, results from this study also suggested the degree of influence the coastal environment had in glycoform expression of these milk glycoproteins, which could be associated to the environmental condition or the dietary intake of coastal mothers as these glycoforms are highly fucosylated. The incorporation of dietary fucose from the long-term seafood diet of coastal mothers into their milk glycans is a strong possibility since exogenous sources of fucose can be salvaged into GDP-fucose via the salvage pathway (Becker & Lowe 2003). Furthermore, there are about 109 nonhuman peptides mostly originating from bovine milk proteins (bovine caseins and β-lactalbumin) that were previously detected in human milk (Zhu et al. 2018) and supports our claim that maternal diet is indeed translated into breastmilk composition.

Similar to the monitored glycoforms at glycosite ^{156}N of lactoferrin, the glycoforms on glycosite ^{497}N also showed no significant difference (P>0.05) in glycoform expression between coastal and noncoastal mothers. As observed previously on ^{156}N glycoforms, about two of the ^{497}N glycoforms (**Figure 4F**) showed quantifiable expression only at early points of lactation. In contrast, sialylated glycoform H5N4S2 (^{497}N) expression persist longer for noncoastal mothers. Few ^{497}N glycoforms of lactoferrin, namely H5N4F1S2 (^{497}N) and H5N4F2S2 (^{497}N), did not significantly differ (P>0.05) in expression for both coastal and noncoastal mothers, while the remaining four glycoforms significantly decreased (P<0.05) throughout the first six months of lactation. In contrast to the remarkable glycoform expression of lactoferrin observed in this study, only one quantifiable glycoform was observed on the glycosylation site, ^{271}N, of α1-Antitrypsin (**Figure 4G**). The expression of this tri-antennary sialylated glycoform, H6N5S3 (^{271}N), however was not significantly influenced (P>0.05) by coastal environment and did not significantly differ (P>0.05) in expression throughout the first six months of lactation.

Secretor Status Generally has No Influence on Glycoform Expression

Secretor status has no significant influence (P>0.05) in the glycosylation of most IgA1/2 glycoforms at glycosites ^{144}N/^{131}N, and that any difference in glycoform expression were the same for secretor and nonsecretor milk. This came with the exception on high-mannose glycoforms, Man8 (H8N2) and Man9 (H9N2) as shown in **Figure 4A**, in which these glycans are expressed significantly higher (P<0.05) in nonsecretor milk. Fewer glycoforms were also observed significantly decreasing (P<0.001) in expression as influenced by secretor status as compared to coastal environment. This gives insight that environmental factor has a degree of influence on the glycosylation of secretory antibodies in human milk early in lactation. Prenatal vaccination stimulates production of maternal antibodies and confer protection to infants via acquired passive immunity *in utero* or through breastmilk (Cerini & Aldrovandi 2013). Moreover, vaccination triggers specific antibody glycosylation profile, it can be hypothesized that the glycosylation of antibodies giving it antigen-specificity can be reflected on both maternal immunologic memory and environmental antigenic stimulation (Mahan et al. 2016). This would imply that long-term environmental exposure would have a stronger interplay on glycosylation specificity of antibodies in human milk compared to secretor status.

The expression of core fucosylated-heavy sialylated glycoforms of sIgA secretory component (SC) shown in **Figure 4B** at glycosites, ^{135}N, ^{421}N, and ^{469}N, were also not significantly influenced (P>0.05) by secretor status. The glycoforms, H5N4F1S2 (^{135}N) and H5N4F1S1(^{421}N), did not significantly differ (P>0.05) in expression throughout lactation. A general decreasing trend in expression was monitored for the other quantifiable glycoforms, H4N5F1 (^{469}N), H4N5F1S1 (^{469}N) and H4N5F1S2 (^{469}N). ST6GAL1 (α-2,6-sialyltransferase 1) was observed to be downregulated starting at Month 2 and throughout the later course of lactation, which could potentially support our result on the decreasing trend of these sialylated glycoforms, regardless of secretor status (Zhu et al. 2021a).

The same absence of secretor status influence was observed for the quantifiable glycoforms of the sIgA Joint chain (J Chain) sub-unit shown in **Figure 4C**. The J chain glycoforms, H4N5 (^{71}N), H5N4F1S1 (^{71}N) and H5N4S1 (^{71}N), did not significantly differ (P>0.05) in expression between secretor and nonsecretor mothers. It was also observed that only the core-fucosylated glycoform H5N4F1S1 (^{71}N) was significantly decreasing (P<0.05) throughout lactation. Based on quantifiable glycoform expression of other milk glycoproteins monitored in this study, there is no association observed between secretor status and core-fucosylation of glycans. There is a high possibility that expression of core-fucosylated glycoforms vary between milk glycoproteins throughout lactation depending on its biological function. This is evident on the high expression of the quantifiable core-fucosylated glycoform, H4N4F1 (^{176}N), on immunoglobulin G (IgG2), a minor immunoglobulin (**Figure 4E**). Expression of this core-fucosylated glycoform also did not significantly differ (P>0.05) between secretor and nonsecretor mothers, which also did not significantly decrease (P>0.05) in expression throughout the first six months of lactation. This is not surprising since, to our knowledge, FUT2 (α-1,2-fucosyltransferase) is highly expressed in secretor mothers and not FUT8 mediated core-fucosylation. In addition, sialylated glycoform of α1-Antitrypsin, H6N5S3 (^{271}N), monitored in

Figure 4G was also not influenced by the secretor status, and remained at constant level of expression throughout lactation. This should bode well with nonsecretor mothers since core-fucosylated N-glycans selectively promotes B cell activation and effective colonization of *Bifidobacterium* and *Lactobacillus* spp. (Li et al. 2019).

Lactoferrin from Filipino Mothers is Highly Glycosylated

Perhaps the glycoforms of lactoferrin present the most interesting observation of all the milk glycoproteins monitored as twenty (20) highly fucosylated and sialylated glycoforms were all quantitatively observed on glycosites, ^{156}N and ^{497}N. Each glycoforms on glycosite ^{156}N were not significantly influenced (P>0.05) by secretor status. Half of the ^{156}N glycoforms monitored, including H5N4F1, H5N4F1S2, H5N4F3S1, H6N5F2S1, and H6N5F3S1, did not significantly decrease (P>0.05) in expression throughout lactation (**Figure 4D**). As discussed previously, few highly fucosylated glycoforms specifically H5N4F3 (^{156}N), H6N5F2S1(^{156}N), and H6N5F3S1 (^{156}N), were observed to be only expressed at certain windows of lactation. The fucosylated glycoform, H5N4F3 (^{156}N), only reached quantifiable abundance at Month 2 for secretor mothers, while earlier at Week 3 for nonsecretor mothers. Fucosylation levels of immune milk proteins, including lactoferrin, were reported to be significantly increasing starting at Month 3 of lactation, regardless of secretor status (Bai et al. 2018). Surprisingly, increased fucosylation in the expression of glycoform H6N5F3S1 (^{156}N) persist more apparently in nonsecretor milk in this study throughout lactation compared to secretor ones. This might be a biological adaption for nonsecretor mothers with an evolutionary pressure to fortify their breastfeeding infant with highly fucosylated glycans even for a short period of time. Considering that proteins compose a smaller fraction (6%) in human milk compared to HMOs (11%), this seemingly high fucosylated glycoform persisting in lactoferrin would somehow offer very little advantage for nonsecretor mothers (Zivkovic et al. 2011). Nonetheless, these fucosylated glycoforms can still be utilized by the breastfeeding infant as prebiotics.

In the study of Karav et al. (2016), N-glycans released from these milk glycoproteins, namely lactoferrin and immunoglobulins, via endoglycosidase (EndoBI-1) activity of infant-borne *B. infantis* would serve as prebiotics for the selective growth of infant-associated taxa. Other infant-associated strains of *Bifidobacterium*, specifically *B. longum* and *B. breve*, also exhibited this activity (Garrido et al. 2012). These complex N-glycans released from lactoferrin and immunoglobulins comprise of sialylated and/or fucosylated bi- and triantennary glycan structures, which *B. infantis* showed an ability to consume as the sole carbon source and mimicking its strong growth on complex HMOs. *B. infantis* consumes about 73% of neutral N-glycan and a higher 92% of sialylated N-glycans (Karav et al. 2016), which showed an evolutionary preference on the sialylated glycoforms highly expressed in milk glycoproteins (**Figure 4**) observed in this study.

Similarly, two fucosylated glycoforms at glycosite ^{497}N of lactoferrin, specifically H5N4F1S2 (^{497}N) and H5N4F3S1 (^{497}N), were expressed significantly higher (P<0.05) in nonsecretor mothers compared to secretor ones. Only few glycoforms, namely H5N4F2S1 (^{497}N), H5N4F2S2 (^{497}N), H5N4S1 (^{497}N), and H5N4S2 (^{497}N), did not significantly decrease (P>0.05) in expression across lactation (**Figure 4F**). Dynamic glycosylation was indeed observed from the N-glycans on the monitored milk glycoproteins. For example, the difference in the degree of sialylation of the same precursor glycan H5N4F3 to the sialylated glycan structure H5N4F3S1 was observed at glycosite ^{156}N and ^{497}N of lactoferrin. The glycoform H5N4F3 (^{156}N) shown in **Figure 4D** was easily sialylated and a precursor of glycoform H5N4F3S1 (^{156}N). This was evident on the change of expression levels of these two glycoforms, in which H5N4F3 (^{156}N) expression levels inversely complement H5N4F3S1 (^{156}N). In contrast, the dynamic sialylation of glycoform H5N4F3 (^{497}N) resulting in the sialylated glycoform H5N4F3S1 (^{497}N) of lactoferrin. At this glycosylation site, the putative sialylation of glycoform H5N4F3 (^{497}N) to the sialylated glycoform H5N4F3S1 (^{497}N) was not evident (**Figure 4F**), with H5N4F3S1 (^{497}N) expression low in lactoferrin.

The emerging picture of the human milk glycoproteome is complex and dynamic, likely reflecting the immediate biological function of these glycoproteins at certain time points of lactation. A clearer understanding on the variation in human milk proteome and the microheterogeneity of its glycoforms would allow us to better understand their biological significance and guide future efforts in improving human health and wellness in early life. Certainly, glycosylation in human milk proteins play critical roles in the development and protection of the infant, and its complexity suggest that human milk is more than just liquid food for babies.

Human Milk Oligosaccharides

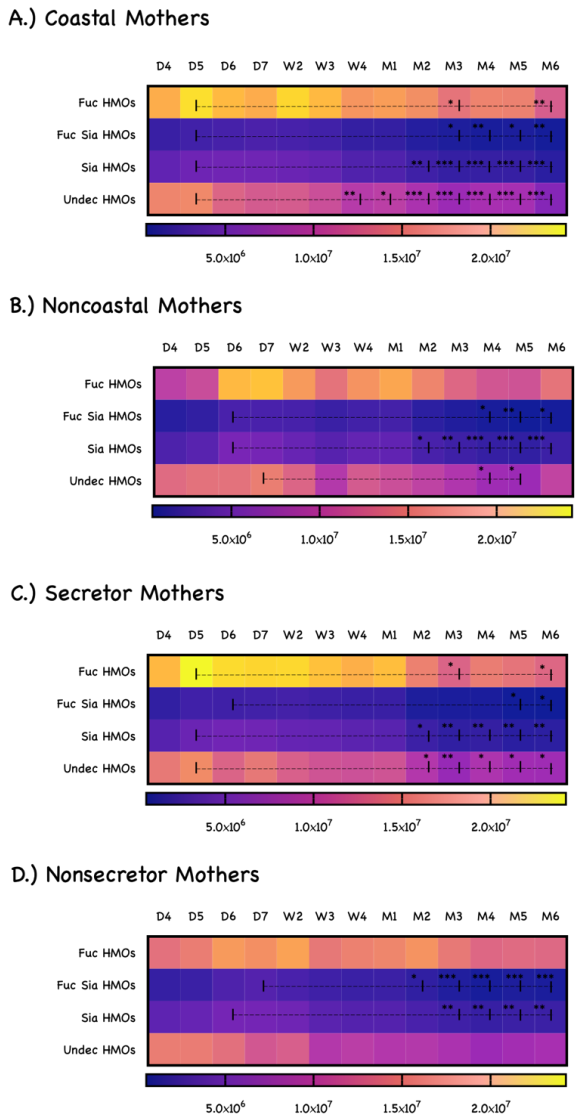
HMOs from breastmilk of mothers living in Baybay City were extracted and analyzed in this study throughout the first six months lactation period. A profile of 172 human milk oligosaccharides from the Filipino mothers of Baybay was elucidated (**SI Table 11**), with exact masses, retention times, and monosaccharide compositions. These HMOs were classified according to their sugar composition: fucosylated, sialylated, fuc-sialylated, and undecorated HMOs, with HMO isomers identified and characterized HMOs annotated (Totten et al. 2014; Wu et al. 2011; Wu et al. 2010).

The mean absolute abundance of HMOs derived from the breastmilk of coastal, noncoastal, secretor, and nonsecretor mothers are grouped in **Figure 5** according to its sugar type composition, with mean values shown in **SI Figure 3**. Mixed Effects ANOVA revealed significant changes (P<0.001) in the abundance of fucosylated, fuc-sialylated, sialylated, and undecorated HMOs from all the mothers throughout the first six months of lactation (**SI Table 12**). A general trend of highest HMO abundances can be observed as early as Day 5 of lactation, which started only to significantly decrease at Month 2 of lactation with continuous decrease up to Month 6 as seen in **Figure 5**.

Coastal Environment has a Degree of Influence in Fucosylation of HMOs.

Comparison of the HMO profile from coastal and noncoastal mothers presented here is not meant to differentiate between the samples, but rather to show a preliminary data in the oligosaccharide diversity. Coastal mothers exhibited the highest abundance of fucosylated HMOs (2.25E+07) in colostrum (Day 5 of lactation). Higher concentration of fucosylated HMOs is generally observed in colostrum (Gabrielli et al. 2011) and through the first month of lactation from previous studies (Albaladejo et al. 2021; Xu et al. 2016) Other types of HMOs, such as fuc-sialylated, sialylated, and undecorated HMOs also exhibited high abundances in colostrum (Day 5), which only decreased significantly at Month 3 (P<0.05). These observations were followed by continuous decrease (P<0.001) throughout the end of the study. The observed decreasing HMO abundances over the course of lactation is in agreement with previous results (Thurl et al. 2010; Xu et al. 2016). HMO function as a prebiotic which stimulates the growth of bifidogenic gut microbiota and as well as, soluble decoys that protect the infants from pathogenic infection (Bode 2012). As the infant's gut microbiota and immune system mature over the course of lactation, the need

for these protective benefits, as well as high abundance of HMOs decreases over time.



* – significant (P<0.05); ** – significant (P<0.01); *** – highly significant (P<0.001)

Figure 5: Absolute Abundances of Fucosylated, Fuc-Sialylated, Sialylated, and Undecorated HMOs from A.) Coastal, B.) Noncoastal, C.) Secretor, and D.) Nonsecretor Mothers living in Baybay City, Leyte.

On the other hand, noncoastal mothers did not exhibit significant changes in fucosylated HMOs throughout the six-month lactation period (P>0.05). To be accurate, fucosylated HMOs showed no significant increase in abundance and maintained low concentration at Day 5 throughout Month 6 of lactation. This result suggests that aside from secretor status, the coastal environment may also influence fucosylation of HMOs. This could be attributed to the regular food intake of seaweed, which is known for its high fucoidan content (Vo & Kim 2013), that is eventually catabolized and the L-fucose incorporated in the biosynthesis of fucosylated glycans via the salvage pathway (Becker & Lowe 2003). However, this was not the case for fuc-sialylated, sialylated and undecorated HMOs. Their abundance levels did not significantly differ between coastal and noncoastal mothers (**SI Table 12**). It was already reported that the expression of these acidic HMOs is not related to secretor status nor Lewis blood group status (Kunz et al. 2017). Thus, our results indicate that environmental condition also may not influence acidic HMO abundances.

Similar to coastal mothers, fuc-sialylated, sialylated and undecorated HMOs from noncoastal ones had the highest concentrations on the first week of lactation. Fuc-sialylated and sialylated HMOs reached peak abundance levels at Day 6 of lactation and started decreasing significantly for fuc-sialylated HMOs (Month 4) and sialylated HMOs (Month 2). On the other hand, undecorated HMOs from noncoastal mothers peaked at Day 7 and only significantly decreased at Month 4 of lactation, which differ on the early decrease of undecorated HMOs at Week 4 from coastal ones. The early decrease of undecorated HMOs in coastal mothers can be attributed to the possible incorporation of L-fucose, derived from their seafood diet, into the biosynthesis of fucosylated HMOs via the GDP-fucose salvage pathway, in which fucose liberated from catabolism becomes available to the catalytic domains of fucosyltransferases in the secretory pathway (Faijes et al. 2019). The free fucose would serve as substrate in the synthesis of fucosylated glycans and glycoconjugates, in which this salvage pathway ultimately incorporates dietary fucose (Becker & Lowe 2003). Thus, it can be implied that dietary fucose is incorporated in the biosynthesis of HMOs from lactating mothers.

Secretor Mothers have Higher Total HMO Abundance

Mothers were classified as secretors without serological testing based on the optimal cut-off ratio value of selected HMO secretor markers identified by Totten et al. (2012). These mothers exhibited the highest fucosylated HMO abundance at Day 5 of lactation. At Month 3, a significant decrease was observed for fucosylated HMOs. Similarly, sialylated, fuc-sialylated, and undecorated HMOs reached highest abundance at the first week of lactation. Both sialylated and undecorated HMOs significantly decreased in abundance at Month 2 of lactation, while later at Month 5 for fuc-sialylated HMOs.

Mixed Effects ANOVA revealed significantly lower (P<0.001) fucosylated HMO abundances in nonsecretor milk compared to secretor milk. In fact, fucosylated HMOs from nonsecretor milk showed low abundance with no significant increase from Day 5 throughout Month 6 of lactation (**Figure 5**). This observation was consistent with the other findings of Thurl et al. (2010), Totten et al. (2012), and Kunz et al. (2017). Moreover, Zhu et al. (2021a) gave insight that the increasing concentration of FUCA1 (α-L-fucosidase), complementing the decreasing FUT3 concentration during lactation, may contribute to the overall decline in fucosylated HMO abundance. Low abundance at Day 5 with constant levels throughout lactation was also observed for undecorated HMOs from nonsecretor milk, while secretor undecorated HMOs started to significantly decrease at Month 2 of lactation.

The discrepancy of undecorated HMOs from secretor and nonsecretor can be attributed to the major HMO composition between milks. Our results indicate that secretor status does not give any significant influence on sialylated HMO abundance, and that difference between secretor and nonsecretor milk were identical at all time points (**SI Table 12**). This is similar to the previous study of Kunz et al. (2017), that sialylated HMOs were neither associated to secretor status nor Lewis blood groups. Secretor status also does not significantly influence fuc-sialylated HMO formation. Abundance level peaked at Day 6 and Day 7 for sialylated and fuc-sialylated HMOs, respectively, from nonsecretors. These abundance levels shown in **Figure 5** later decreased significantly at Month 2 and Month 3 for fuc-sialylated and sialylated HMOs, respectively.

In general, the mean absolute abundances of total HMOs from coastal mothers were higher compared to their noncoastal counterparts as shown in **Figure 6**. In addition, the same higher abundance trend was observed from secretor mothers compared to their nonsecretor counterparts. The total HMO abundance levels peaked at Day 5 of lactation for coastal and secretor mothers. Higher levels of total HMOs from secretor milk has always been attributed to high levels of fucosylated and undecorated HMOs expressed by secretor mothers (Kunz et al. 2017; Thurl et al. 2010). Although our results showed the high expression of total HMO abundance from coastal mothers compared to noncoastal ones, there was no strong influence from the coastal environment in this study. Moreover, Mixed Effects ANOVA shows no significant difference (P>0.05) on the coastal environment and secretor status, and difference were identical at all time points. Results in this statistical analysis can be attributed to the low number of enrolled participants. Current efforts are being done to recruit more participants in our future studies.

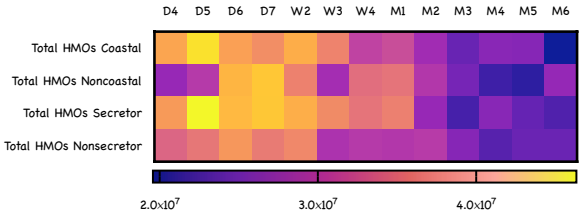


Figure 6: Total HMO Abundances from Coastal, Noncoastal, Secretor, and Nonsecretor Mothers throughout the Six-Month Lactation Period Living in Baybay City, Leyte.

Annotated HMOs from Filipino Mothers living in Baybay City, Leyte

Each annotated human milk oligosaccharide was classified according to sugar type composition: fucosylated, fuc-sialylated, sialylated and undecorated HMOs. We have identified thirty-eight (38) HMOs from Filipino mothers living in Baybay City, Leyte. Absolute abundances of annotated HMOs derived from coastal, noncoastal, secretor and nonsecretor mothers living in Baybay City, Leyte throughout the first six-month lactation period, are drawn in heatmaps depicted together in **Figure 7**.

Higher Fucosylated HMO Abundance in Coastal and Secretor Mothers

Mixed Effects ANOVA on the 20 annotated fucosylated HMOs (**SI Table 15**) revealed that the coastal environment had no significant influence (P>0.05) on the abundance of fucosylated HMOs, except for DFLNHa, LDFT, LNDFH I, and LNFP II, which were found in higher concentration in coastal mothers. These preliminary data show that these HMOs were the only ones that differed in abundance between coastal and noncoastal mothers, while the rest of the annotated fuc-sialylated, sialylated and undecorated HMOs did not. Moreover, LDFT, LNDFH I, and LNFP II remained abundantly high in coastal milk throughout the six months lactation period. Meanwhile, 3'FL was observed to be significantly increasing (P<0.001) from Day 6 to Month 4, while its isomer 2'FL, and two other fucosylated HMOs, DFpLNH II and MFLNH III/I, remained constantly high in abundance across the six months lactation period. The rest of the fucosylated HMOs, however, significantly decreased (P<0.001) throughout the end of the study.

Unlike for secretor status, the coastal environment did not show significant influence on the abundance of 2'FL in human milk, as evident on the comparable abundance of 2'FL in coastal and noncoastal milk. This observed no significant difference in 2'FL between coastal and noncoastal mothers can be due to the limited cohort sample size. Nonetheless, results showed that coastal mothers express higher total abundance of fucosylated HMOs compared to noncoastal mothers. The 2'FL is a major source of H-2 epitope in human milk and competes with the H-2 epitopes of the host cell surface receptors for binding of *C. jejuni*, thus preventing infection. It was demonstrated that 2'FL quenches *C. jejuni*-induced inflammation in human epithelial cells, thus protecting infants from severe diarrhea – one of the most prominent causes of infant mortality (Yu et al. 2016).

A similar trend was observed on the fuc-sialylated HMOs. Although not significantly different (P>0.05), the fuc-sialylated HMOs from coastal mothers were slightly higher in abundance at the first week of lactation compared to noncoastal mothers. Majority of the fuc-sialylated HMOs, namely FS-LNH I, FS-LNH III, FS-LNnH I, and DFS-LNH, were slightly higher at the first week of lactation in coastal milk compared to noncoastal milk, while only a few fuc-sialylated HMOs, such as FS-LNO and DFS-LNnH, were slightly higher in abundance in noncoastal milk at the first week of lactation.

The remaining types of HMOs, such as the sialylated and undecorated HMOs, also did not show any significant difference on its abundance between coastal and noncoastal milk. The results of this study suggest that sialylated HMOs may not be influenced by environmental factors. Although not significant at all time points, it was interesting to observe that the sialylated HMOs from noncoastal mothers were slightly higher (0.85-fold) in abundance compared to their coastal counterpart. Only at colostrum (Day 5) did 6'SL reached significantly higher level of abundance (P<0.05) in noncoastal mothers compared to coastal ones, and then were comparable in abundance throughout the end of the lactation period. The sialylated HMOs, 6'SL and S-LNH, were about 10-fold higher in abundance compared to 3'SL, in both coastal and noncoastal mothers. In general, 6'SL decreased in abundance after Week 2 for both coastal and noncoastal mothers. In contrast to these decrease in abundance, the levels of 3'SL remained constant throughout the six months lactation period for both coastal and noncoastal mothers.

The undecorated HMOs, LNT/LNnT, were the most predominant HMOs observed in noncoastal mothers. These HMOs remained at constant high levels (P>0.05) in noncoastal milk from Day 5 to Month 6 of lactation, while decreased significantly for coastal milk at Week 4. LNT was also reported by Totten et al. (2012) to be the most abundantly present in all milk types. For LNT/LNnT, however, its abundance levels were taken as the sum of the two undecorated HMOs, LNT and LNnT, which were not baseline separated. Unsuccessful baseline separation of LNT and LNnT also occurred in the previous work of Albaladejo et al. (2021) and will be addressed in future studies. A similar trend was observed for LNnH which only decreased significantly in coastal milk. The remaining undecorated HMOs, LNH and pLNH, were significantly decreasing in both coastal and noncoastal milk. A plausible explanation for the significant decrease of undecorated HMOs in coastal milk is that they serve as putative precursors for fucosylated ones, as evident on the significantly higher abundance of fucosylated HMOs in coastal milk. Overall, this preliminary data makes undecorated HMOs the predominant HMO type in noncoastal milk, fucosylated HMOs the predominant HMO type in coastal milk throughout the first six months of lactation.

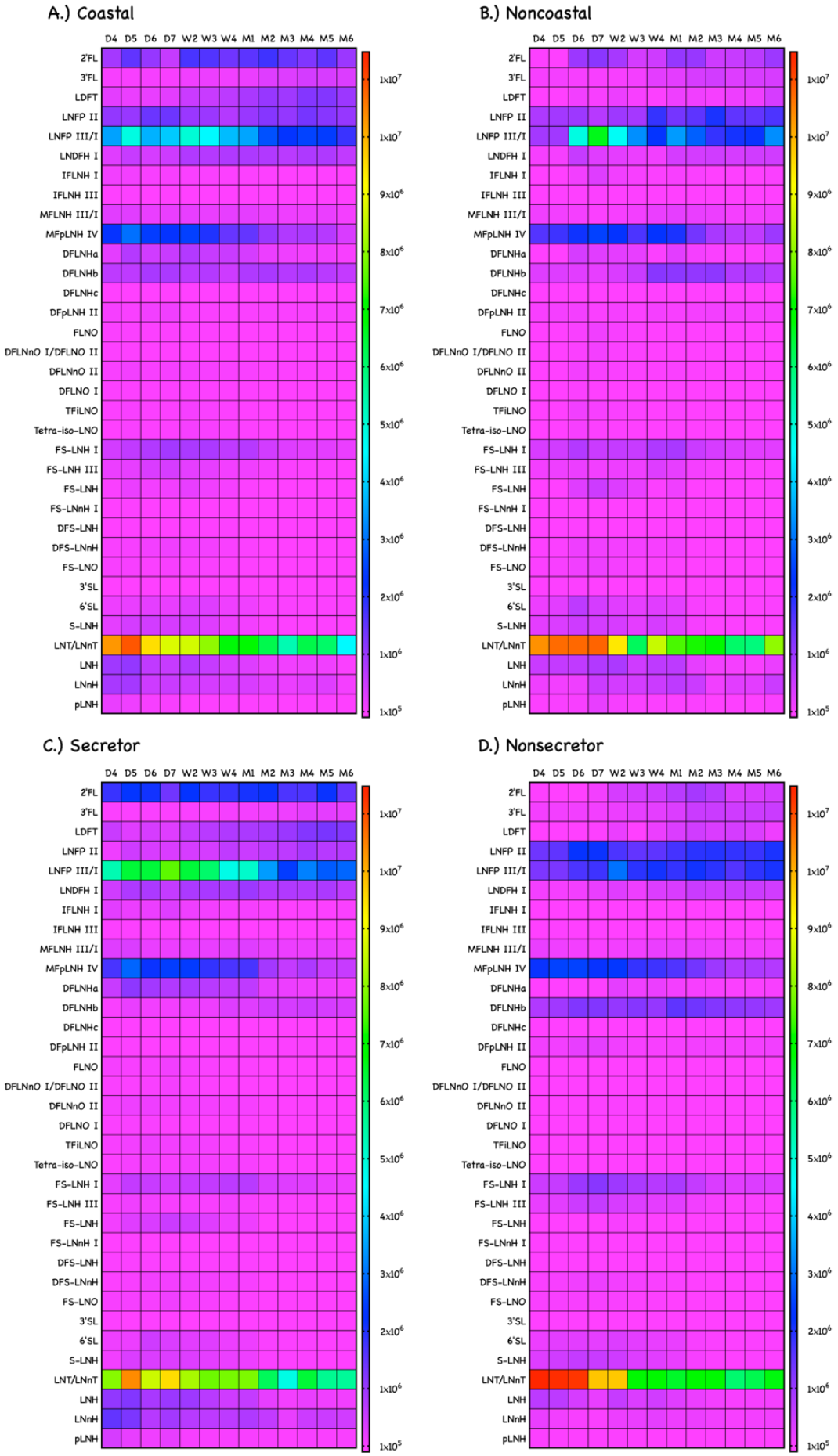


Figure 7: Relative Abundance of Annotated HMOs from A.) Coastal, B.) Noncoastal, C.) Secretor and, D.) Nonsecretor Mothers living in Baybay City, Leyte.

Secretor Mothers have the Highest Abundance of 2'FL

Secretor status has more influence on the abundance of annotated fucosylated HMOs (Thurl et al. 2010; Totten et al. 2014). We observed that secretor status has a significant influence ($P<0.05$) on the abundance of the annotated fucosylated HMOs, except for IFLNH III, LDFT, LNDFH I, MFLNH III/I, MFpLNH IV. These aforementioned fucosylated HMOs were not used as quantitative markers since their comparable abundances between milk types were not ideal for secretor status determination (Totten et al. 2012).

2'FL abundances were significantly higher ($P<0.05$) in secretor milk compared to nonsecretor milk throughout the first six months of lactation. Its isomer, 3'FL, remained constant in secretor milk throughout the lactation period. For nonsecretor milk, however, 3'FL abundances were significantly increasing at Day 5 to Month 2 and remained constant throughout the lactation period. This was not surprising since the highly expressed FUT2 (α1-2-fucoslyltransferase) in secretor mothers determines the high abundance of 2'FL in secretor milk (Bode 2012; Chaturvedi et al. 2001). For nonsecretor mothers, the expression of a different fucosyltransferase, FUT3 (α1-3/4-fucoslyltransferase), explains the increased abundance of the isomer 3'FL in nonsecretor milk (Smilowitz et al. 2014; Totten et al. 2012).

Even with the expression of an FUT3 in secretors, if not lacking completely, the decreasing activity of FUT3 in secretors as lactation progressed was evident on the decreasing abundance of branched annotated α1-3/4-fucosylated HMOs, namely FLNO, DFLNHa, DFLNHc, DFLNnO II, DFLNnO I/III DFLNO I, TFiLNO, and Tetra-iso-LNO. The abundance of these fucosylated branched HMOs significantly decreased ($P<0.001$) in secretor milk, while maintaining constant low levels in nonsecretor milk. It was observed that linear α1-3/4 fucosylated HMOs like LNFP II, DFLNHb, and DFpLNH II, were the predominant fucosylated HMOs in nonsecretor mothers, while these linear fucosylated HMOs were significantly lower ($P<0.001$) in secretor mothers. These results suggest the preference of fucosylation in branched HMOs for secretor mothers, while fucosylation in linear HMOs for nonsecretor mothers. It would be interesting to monitor in future studies the activities of possible fucosyltransferases that are responsible for the formation of the branched fucosylated HMOS, in the proposed biosynthetic pathway (Blank et al. 2012; Kellman et al. 2022). In addition, the observed preference of fucosylation in linear and branched HMOs gives a portrait on the interplay of fucosyltransferases in HMO production and expression. This leads to insights on the irregular fucosylation pattern at different lactation stages and on the hypothesis that responsible glycosyltransferases are switched on and off during lactation (De Leoz et al. 2012).

For the annotated sialylated HMOs, 3'SL, 6'SL, and S-LNH, no significant difference ($P>0.05$) in abundance was observed between secretor and nonsecretor mothers. However, 6'SL and S-LNH were significantly decreasing ($P<0.01$) in abundance at Month 2 in secretor mothers. Interestingly, 3'SL abundance remained constant throughout the six months lactation period for both secretor and nonsecretor mothers. The same was observed for fuc-sialylated HMOs and may be attributed to α1-3/4 L-fucose linkages present in fuc-sialylated HMOs, in contrast to the α1-2 linkages as influenced by secretor status. **SI Table 16** confirms the discrepancy of α1-2 linked fuc-sialylated HMOs between secretor and nonsecretor mothers with significantly higher ($P<0.01$) α1-2 linked fuc-sialylated HMOs, FS-LNH and DFS-LNH, in secretor milk. The same with other HMO types, all annotated fuc-sialylated HMOs were significantly decreasing ($P<0.01$) in starting at Month 2 until the end of the first six months lactation period.

For the annotated undecorated HMOs, such as LNH, LNnH, and pLNH, significantly higher abundances in secretor milk at the first week of lactation ($P<0.05$) were observed as compared to nonsecretor milk. The abundance of the most predominant undecorated HMOs, LNT/LNnT, with the highest abundance levels reaching 10^7, did not significantly differ between secretor and nonsecretor milk (**SI Table 26**). According to Sela and Mills (2010), HMOs have evolved in concert with both the human host and the infant-borne *Bifidobacterium*. It is not surprising then that the predominance of undecorated HMOs is a necessary feature for optimum colonization of Bifidobacteria in breastfed infants. However, LNT/LNnT was observed to decrease significantly ($P<0.05$) in abundance, along with LNnH and pLNH, in secretor milk as lactation progressed, while remaining at high levels in nonsecretor milk throughout the first six months of lactation.

SUMMARY AND CONCLUSION

A profile of 172 human milk oligosaccharides (HMOs) from the Filipino mothers of Baybay City, Leyte was elucidated using Q-TOF LC/MS, and thirty-eight (38) HMOs were annotated with glycan structures identified. The higher abundance of total HMOs from coastal and secretor mothers compared to noncoastal and nonsecretor ones were attributed to the higher expression of fucosylated and undecorated HMO species. There was no significant association ($P=0.608$) of secretor mothers living in coastal areas and does not influence the HMO profile of coastal mothers. For nonsecretor mothers, the major HMOs comprised primarily of undecorated HMOs, specifically LNT/LNnT. On the other hand, the coastal environmental condition and secretor status of Filipino mothers did not give any significant results ($P>0.05$) on its abundance of sialylated and fuc-sialylated HMOs.

We determined the total glycoprotein content, as well as dynamic glycosylation of these glycoproteins at a site-specific level using QqQ LC/MS - Multiple Reaction Monitoring (MRM), in the breastmilk of Filipino mothers living in Baybay City, Leyte throughout the first six months of lactation. A concentration range of 5-15 μg/μL total human milk protein, predominantly comprising of secretory IgA (64%), followed by α-Lactalbumin (14%), Lactoferrin (12%), Lysozyme (4%), β-Casein (3%), κ-Casein (2%), α-Casein, IgG, IgM, α1-Antitrypsin, and Osteopontin which compose the remaining 2%, were quantified and variations in site-specific glycosylation of respective glycoproteins monitored. These milk proteins were observed significantly decreasing ($P<0.05$) as lactation progresses while the peculiar significant increase of lysozyme concentration throughout the end of the study was attributed to its enzymatic function. In this preliminary study, the biological adaptation of Filipino mothers was demonstrated in the predominance of secretory IgA concentrations of their breastmilk and primarily function in the immune protection of the infant's intestinal mucosa. Our results confirm that breastmilk is an immunological integration between the mother's and the infant's immune systems.

The coastal environment has no significant influence ($P>0.05$) on protein expression in human milk. This was expected since protein synthesis is the translation product of an individual's gene, therefore the environmental factor will not contribute to protein expression. Surprisingly, lactoferrin was found significantly higher ($P<0.05$) in nonsecretor milk compared to secretor milk. It can be inferred that the higher concentration of lactoferrin in nonsecretor milk is a biological adaptation to provide milk glycans as substrates for immune protection and prebiotics on the colonization of beneficial gut microbiota, as

lactoferrin was observed to be highly glycosylated. Moreover, the site-specific glycosylation of secretory IgA sub-units, as well as other immunoglobulins: IgG and IgM, and the other dominant glycoproteins in human milk, specifically α-lactalbumin and lactoferrin, were also monitored. Most glycoforms monitored in this study were not influenced by both secretor status and coastal environment and exhibited dynamic variation across lactation. Glycosylation at N-glycosites were also not obligatory due to expected microheterogeneity of N-glycans, as observed in this study. Overall, the coastal environmental factor has a stronger influence in protein glycosylation compared to secretor status as the expression of more glycoforms were significantly influenced ($P<0.05$) by the coastal environment. The observed site-specific glycosylation heterogeneity can be attributed to the dynamic variation of these glycoforms which is biologically directed to the immunological, as well as the prebiotic function of these glycoproteins. The goal for human milk oligosaccharide profiling and site-specific glycosylation mapping in human milk glycoproteins not only to discover milk patterns associated to secretor status but also other factors, such as the nutritional and environmental influence. To our knowledge, no current studies support these hypotheses and offer an in-depth look at the milk glycans, as well as glycoprotein compositions as influenced by secretor status and the coastal environmental condition up until this study.

In summary, the study provided in-depth information on human milk oligosaccharides variations and glycoprotein expression, as well as revealed its association to secretor status, coastal environmental condition, and long-term seafood diet of lactating Filipino mothers living in Baybay City, Leyte. Considering all these protective effects conferred by human milk glycans, it is plausible to hypothesize that HMOs, as well as the antibodies present in human milk, could serve as anti-viral against SARS-CoV-2, the virus for COVID-19 and the causative pathogen of the global pandemic. The current pandemic has renewed interest in bioactive compounds with known or supposed antiviral activity. As many mammalian viruses have evolved to bind with receptor glycans expressed on host epithelial cell surfaces, the potential binding between SARS-CoV-2 and HMOs, as well as the predominant antibody in human milk (sIgA) that protects mucosal surfaces, may offer new frontiers in COVID-19 immunity and of certain great interest for future research.

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CONFLICT OF INTERESTS

The authors have no conflict of interest to disclose.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

The authors' responsibilities were as follows—GCJC: designed the research; RFK: conducted the research, analyzed the data, and interpreted the results; RFK and GCJC: wrote the manuscript; CBL: provided the equipment and facilities on the research analyses; and all authors: read and approved the final manuscript.

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SUPPLEMENTARY INFORMATION

Dynamic Changes of Glycoforms in Human Milk Glycoproteins

The predominant antibody in human milk, sIgA (secretory Immunoglobulin A), is a protein tetramer comprising of two identical IgA monomers (IgA1 and IgA2), linked together via a 16-kDa joint chain (J chain), and a secretory component (SC) which protects the immunoglobulin from proteolytic degradation (Huang et al. 2015). Glycopeptide abundance changes with protein concentration and affect the site-specific quantitation of glycoforms. Normalizing the glycopeptide signal to the protein abundance factors out protein concentration contribution and allowed determination of changes in glycosylation at a protein-specific level. This relative quantitation of protein glycosylation provided enough information in monitoring dynamic variations of glycoforms of human milk glycoproteins. With the MRM transition list provided by Huang et al. (2017), glycopeptides from the sIgA components were monitored and quantitated, as well as the glycopeptides of IgG, IgM, lactoferrin, and α1-Antitrypsin. Some glycosites of these glycoproteins share the same tryptic peptide backbone and therefore cannot be distinguished. IgA1 and IgA2 glycosites ^{144}N and ^{131}N, respectively, share the same tryptic peptide backbone, thus having identical masses and cannot be differentiated. The same was true for each respective glycosites, ^{227}N and ^{174}N, of the two IgG subclasses, IgG3 and IgG4, sharing the same tryptic peptide amino acid composition.

In this study, glycopeptide signals for IgA1/2 were also normalized to its common tryptic peptide and 18 complex glycan structures, including high mannose, sialylated and/or fucosylated glycans, were monitored on respective glycosites. In addition, 4 complex glycan structures were monitored on the ^{205}N glycosite of IgA2. For the J Chain, 8 glycans, mostly fucosylated and/or sialylated, were monitored on its single ^{71}N glycosite, while a total of 15 complex glycans were monitored on the SC glycosites ^{135}N, ^{186}N, ^{421}N, and ^{469}N. Huang et al. (2017) provided the complete list of glycoforms monitored in this study (**SI Table 7**), specifically sIgA and other glycoproteins including IgG, IgM, lactoferrin, and α1-Antitrypsin.

IgG has four subclasses, namely IgG1, IgG2, IgG3, and IgG4. The first two subclasses, IgG1(^{180}N) and IgG2 (^{176}N), have distinct tryptic peptide moieties and thus, its glycopeptides could be monitored individually. Eleven fucosylated glycans were monitored on the ^{180}N glycosite of IgG1, while nine were monitored on ^{176}N of IgG2. For IgG3 and IgG4, their glycopeptides could not be distinguished as their respective glycosites, ^{227}N and ^{174}N, share the same tryptic peptide moieties and have exact masses (Hong et al. 2013). In this study, 4 fucosylated glycans were monitored on the N-glycosites of IgG3/4 and normalized to its common tryptic peptide. For IgM, it has several N-glycosylation sites, specifically ^{46}N, ^{209}N, ^{272}N, ^{279}N, and ^{439}N. Due to the limitation of tryptic digestion, glycosites ^{272}N and ^{279}N would yield a single tryptic peptide and individual site information would be indistinguishable (Huang et al. 2017). Hence, only ^{46}N, ^{209}N, and ^{439}N glycosites with a total of 11 complex glycans, such as high-mannose, fucosylated and/or sialylated glycan structures, on glycosylation sites mentioned were monitored in this study.

In Lactoferrin, ten complex fucosylated and/or sialylated glycan structures were monitored for each glycosites, ^{156}N and ^{497}N, of lactoferrin in human milk. A minor glycoprotein, α1-Antitrypsin, has three N-glycosylation sites. Ten highly sialylated glycans were monitored on two of its N-glycosites, ^{107}N and ^{271}N. The third glycosite, ^{70}N, was not observed in the previous study of Huang et al. (2017) due to its low occupancy and hence, was not monitored.

SI Table 1: Odd Ratio of Seafood Diet of Coastal Mothers of Baybay City, Leyte.

P value and statistical significance			
Test	Fisher's exact test		
P value	<.001		
P value summary	***		
One- or two-sided	Two-sided		
Statistically significant (P < 0.05)?	Yes		
Effect size	**Value**	**95% CI**	
Odds ratio	3.151	1.915 to 5.176	
Reciprocal of odds ratio	0.3173	0.1932 to 0.5221	
Methods used to compute CIs			
Odds ratio	Baptista-Pike		
Data analyzed	**Coastal**	**Noncoastal**	**Total**
Seafood Diet	102	41	143
Nonseafood Diet	60	76	136
Total	162	117	279
Percentage of row total	**Coastal**	**Noncoastal**	
Seafood Diet	71.33%	28.67%	
Nonseafood Diet	44.12%	55.88%	
Percentage of column total	**Coastal**	**Noncoastal**	
Seafood Diet	62.96%	35.04%	
Nonseafood Diet	37.04%	64.96%	
Percentage of grand total	**Coastal**	**Noncoastal**	
Seafood Diet	36.56%	14.70%	
Nonseafood Diet	21.51%	27.24%	

SI Table 2: Odd Ratio of Secretor Mothers in Coastal Areas of Baybay City, Leyte.

P value and statistical significance			
Test	Fisher's exact test		
P value	.608		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant (P < 0.05)?	No		
Effect size	**Value**	**95% CI**	
Odds ratio	2.500	0.2627 to 17.49	
Reciprocal of odds ratio	0.4000	0.05718 to 3.807	
Methods used to compute CIs			
Odds ratio	Baptista-Pike		
Data analyzed	**Coastal**	**Noncoastal**	**Total**
Secretor	5	2	7
Nonsecretor	4	4	8
Total	9	6	15
Percentage of row total	**Coastal**	**Noncoastal**	
Secretor	71.43%	28.57%	
Nonsecretor	50.00%	50.00%	
Percentage of column total	**Coastal**	**Noncoastal**	
Secretor	55.56%	33.33%	
Nonsecretor	44.44%	66.67%	
Percentage of grand total	**Coastal**	**Noncoastal**	
Secretor	33.33%	13.33%	
Nonsecretor	26.67%	26.67%	

SI Table 3: MRM Transitions of Targeted Peptides Monitored in the Quantitation of Human Milk Glycoprotein Concentration using UPLC-QqQ MS.

Glycoprotein	Peptide Sqn	Precursor Ion	Product Ion	Identifier	RT	D RT
a-Casein	CAEQFCR	485.697	739.319	Qualifier	1.6	0.5
	CAEQFCR	485.697	204.08	Quantifier	1.6	0.5
a1-Antitrypsin	LSITGTYDLK	555.8	910.5	Qualifier	3.1	0.5
	LSITGTYDLK	555.8	797.4	Quantifier	3.1	0.5
b-Casein	VLPIPQQVVPYPQR	545.3196	660.3	Quantifier	3.8	0.5
	VLPIPQQVVPYPQR	545.3196	606.3	Qualifier	3.8	0.5
Immunoglobulin A	VAAEDWK	409.7	719.4	Qualifier	1.9	0.5
	VAAEDWK	409.7	648.3	Quantifier	1.9	0.5
Immunoglobulin G	DTLMISR	418.2	619.4	Qualifier	2.5	0.5
	DTLMISR	418.2	506.3	Quantifier	2.5	0.5
Immunoglobulin M	FTCTVTHTDLPSPLK	572.95	734.9	Quantifier	2.9	0.5
	FTCTVTHTDLPSPLK	572.95	654.9	Qualifier	2.9	0.5
k-Casein	TYYANPAVVRPHAQIPQ	521.3	827.458	Qualifier	2.2	0.5
	TYYANPAVVRPHAQIPQ	521.3	734.925	Quantifier	2.2	0.5
a -Lactalbumin	FLDDDITDDIMCAK	836.4	953.4	Quantifier	3.1	0.5
	FLDDDITDDIMCAK	551.8	654.4	Qualifier	3.1	0.5
Lactoferrin	LRPVAAEVYGTER	487.6	737.4	Qualifier	2.2	0.5
	LRPVAAEVYGTER	487.6	625.3	Quantifier	2.2	0.5
Lysozyme	STDYGIFQINSR	700.8	764.4	Quantifier	3.45	0.5
	STDYGIFQINSR	700.8	489.3	Qualifier	3.45	0.5
Osteopontin	GDSVVYGLR	483.256	607.356	Quantifier	2.6	0.5
	GDSVVYGLR	483.256	508.288	Qualifier	2.6	0.5

SI Table 4: Mixed-Effects Analysis of Variance (ANOVA) on Human Milk Glycoproteins at Different Lactation Points from Coastal, Noncoastal, Secretor, and Nonsecretor Mothers living in Baybay City, Leyte.

Mixed-effects model (REML)	Matching: Stacked
Assume sphericity?	Yes
Alpha	0.05

a-Casein

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 119) = 5.049
Coastal vs. Noncoastal	.147	ns	No	F (1, 119) = 2.135
Lactation Points x Coastal vs. Noncoastal	.080	ns	No	F (12, 119) = 1.676

Was the matching effective?	
Chi-square, df	
P value	
P value summary	
Is there significant matching (P < 0.05)?	No

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 5.227
Secretor Status	.452	ns	No	F (1, 13) = 0.6004
Lactation Points x Secretor Status	.010	*	Yes	F (12, 106) = 2.344

Was the matching effective?	
Chi-square, df	0.05634, 1
P value	.812
P value summary	ns
Is there significant matching (P < 0.05)?	No

b-Casein

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.653	ns	No	F (12, 119) = 0.7964
Coastal vs. Noncoastal	.445	ns	No	F (1, 119) = 0.5864
Lactation Points x Coastal vs. Noncoastal	.211	ns	No	F (12, 119) = 1.330

Was the matching effective?	
Chi-square, df	
P value	
P value summary	
Is there significant matching (P < 0.05)?	No

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.352	ns	No	F (12, 106) = 1.121
Secretor Status	.216	ns	No	F (1, 13) = 1.693
Lactation Points x Secretor Status	.072	ns	No	F (12, 106) = 1.724

Was the matching effective?	
Chi-square, df	0.4367, 1
P value	.509

P value summary	ns
Is there significant matching (P < 0.05)?	No

k-Casein

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 4.814
Coastal vs. Noncoastal	.573	ns	No	F (1, 13) = 0.3347
Lactation Points x Coastal vs. Noncoastal	.061	ns	No	F (12, 106) = 1.778

Was the matching effective?	
Chi-square, df	12.05, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 6.449
Secretor Status	.144	ns	No	F (1, 13) = 2.421
Lactation Points x Secretor Status	.011	*	Yes	F (12, 106) = 2.326

Was the matching effective?	
Chi-square, df	16.04, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

a1-AntiTrypsin

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.003	**	Yes	F (12, 106) = 2.721
Coastal vs. Noncoastal	.864	ns	No	F (1, 13) = 0.03048
Lactation Points x Coastal vs. Noncoastal	.396	ns	No	F (12, 106) = 1.066

Was the matching effective?	
Chi-square, df	15.07, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 3.748
Secretor Status	.635	ns	No	F (1, 13) = 0.2363
Lactation Points x Secretor Status	.037	*	Yes	F (12, 106) = 1.940

Was the matching effective?	
Chi-square, df	16.77, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Immunoglobulin A

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.012	*	Yes	F (12, 106) = 2.305
Coastal vs. Noncoastal	.993	ns	No	F (1, 13) = 9.047e-005
Lactation Points x Coastal vs. Noncoastal	.899	ns	No	F (12, 106) = 0.5174

Was the matching effective?	
Chi-square, df	55.25, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.005	**	Yes	F (12, 106) = 2.579
Secretor Status	.905	ns	No	F (1, 13) = 0.01478
Lactation Points x Secretor Status	.027	*	Yes	F (12, 106) = 2.048

Was the matching effective?	
Chi-square, df	64.32, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Immunoglobulin G

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.911	ns	No	F (12, 106) = 0.4995
Coastal vs. Noncoastal	.571	ns	No	F (1, 13) = 0.3378
Lactation Points x Coastal vs. Noncoastal	.970	ns	No	F (12, 106) = 0.3729

Was the matching effective?	
Chi-square, df	13.48, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.337	ns	No	F (12, 106) = 1.139
Secretor Status	.432	ns	No	F (1, 13) = 0.6571
Lactation Points x Secretor Status	.329	ns	No	F (12, 106) = 1.150

Was the matching effective?	
Chi-square, df	14.91, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Immunoglobulin M

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)

Lactation Points	.002	**	Yes	F (12, 106) = 2.865
Coastal vs. Noncoastal	.900	ns	No	F (1, 13) = 0.01645
Lactation Points x Coastal vs. Noncoastal	.755	ns	No	F (12, 106) = 0.6927

Was the matching effective?	
Chi-square, df	27.54, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 3.755
Secretor Status	.697	ns	No	F (1, 13) = 0.1587
Lactation Points x Secretor Status	.135	ns	No	F (12, 106) = 1.502

Was the matching effective?	
Chi-square, df	31.79, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

a-Lactalbumin

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.787	ns	No	F (12, 106) = 0.6586
Coastal vs. Noncoastal	.934	ns	No	F (1, 13) = 0.007155
Lactation Points x Coastal vs. Noncoastal	.426	ns	No	F (12, 106) = 1.031

Was the matching effective?	
Chi-square, df	5.352, 1
P value	.021
P value summary	*
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.639	ns	No	F (12, 106) = 0.8103
Secretor Status	.471	ns	No	F (1, 13) = 0.5519
Lactation Points x Secretor Status	.436	ns	No	F (12, 106) = 1.020

Was the matching effective?	
Chi-square, df	4.093, 1
P value	.043
P value summary	*
Is there significant matching (P < 0.05)?	Yes

Lactoferrin

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 4.950
Coastal vs. Noncoastal	.789	ns	No	F (1, 13) = 0.07479

Lactation Points x Coastal vs. Noncoastal	.588	ns	No	F (12, 106) = 0.8609
Was the matching effective?				
Chi-square, df	21.33, 1			
P value	<.001			
P value summary	***			
Is there significant matching (P < 0.05)?	Yes			

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 6.709
Secretor Status	.045	*	Yes	F (1, 13) = 4.935
Lactation Points x Secretor Status	.002	**	Yes	F (12, 106) = 2.914
Was the matching effective?				
Chi-square, df	19.38, 1			
P value	<.001			
P value summary	***			
Is there significant matching (P < 0.05)?	Yes			

Lysozyme

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 10.54
Coastal vs. Noncoastal	.782	ns	No	F (1, 13) = 0.07952
Lactation Points x Coastal vs. Noncoastal	.594	ns	No	F (12, 106) = 0.8548
Was the matching effective?				
Chi-square, df	55.74, 1			
P value	<.001			
P value summary	***			
Is there significant matching (P < 0.05)?	Yes			

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 10.59
Secretor Status	.590	ns	No	F (1, 13) = 0.3060
Lactation Points x Secretor Status	.242	ns	No	F (12, 106) = 1.279
Was the matching effective?				
Chi-square, df	53.94, 1			
P value	<.001			
P value summary	***			
Is there significant matching (P < 0.05)?	Yes			

Osteopontin

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.072	ns	No	F (12, 106) = 1.721
Coastal vs. Noncoastal	.085	ns	No	F (1, 13) = 3.477
Lactation Points x Coastal vs. Noncoastal	.620	ns	No	F (12, 106) = 0.8299
Was the matching effective?				

Chi-square, df	3.924, 1
P value	.048
P value summary	*
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.090	ns	No	F (12, 106) = 1.646
Secretor Status	.485	ns	No	F (1, 13) = 0.5175
Lactation Points x Secretor Status	.238	ns	No	F (12, 106) = 1.286

Was the matching effective?	
Chi-square, df	4.464, 1
P value	.035
P value summary	*
Is there significant matching (P < 0.05)?	Yes

Total Glycoprotein

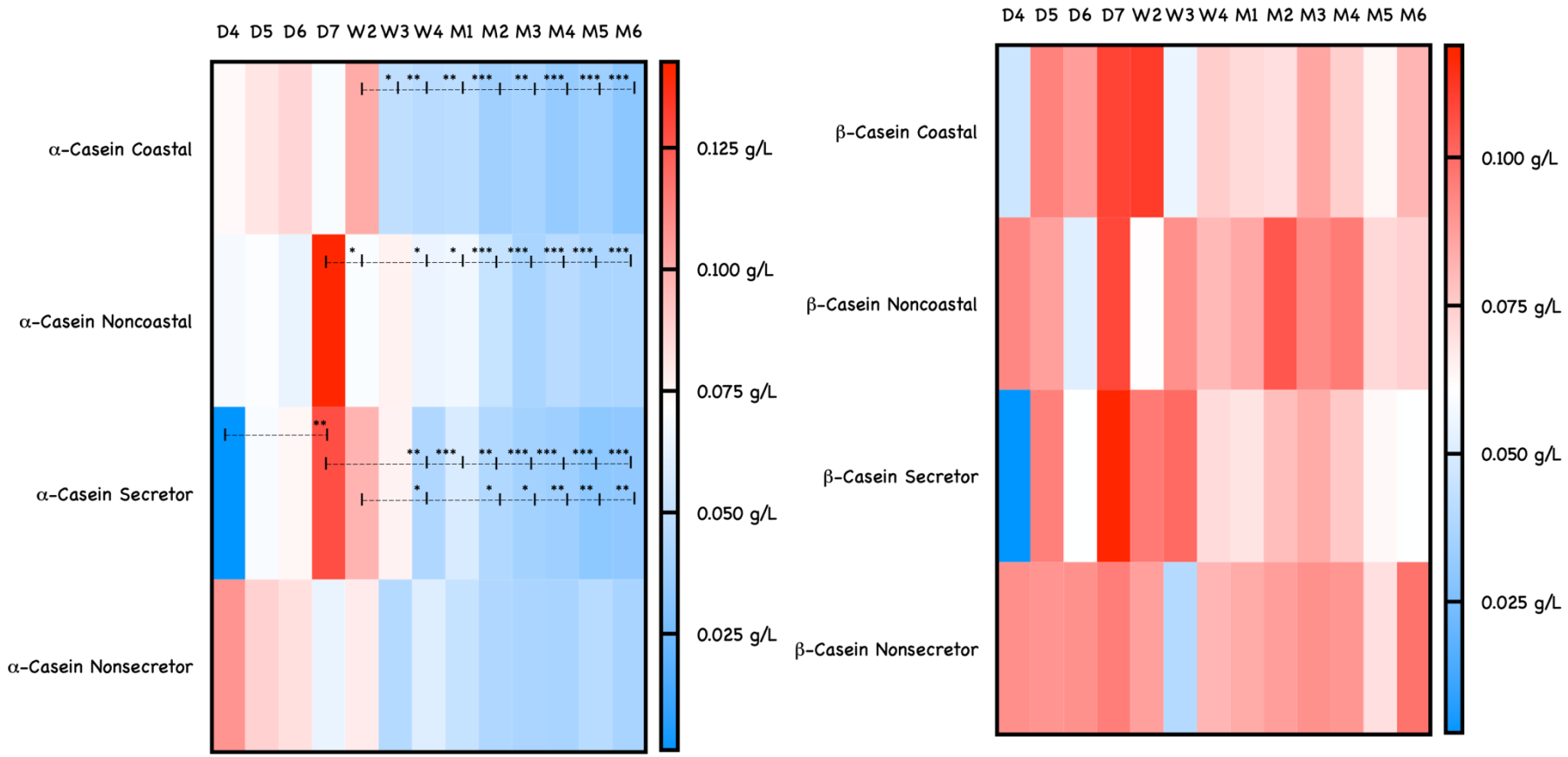
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.014	*	Yes	F (12, 106) = 2.244
Coastal vs. Noncoastal	.980	ns	No	F (1, 13) = 0.0006484
Lactation Points x Coastal vs. Noncoastal	.944	ns	No	F (12, 106) = 0.4395

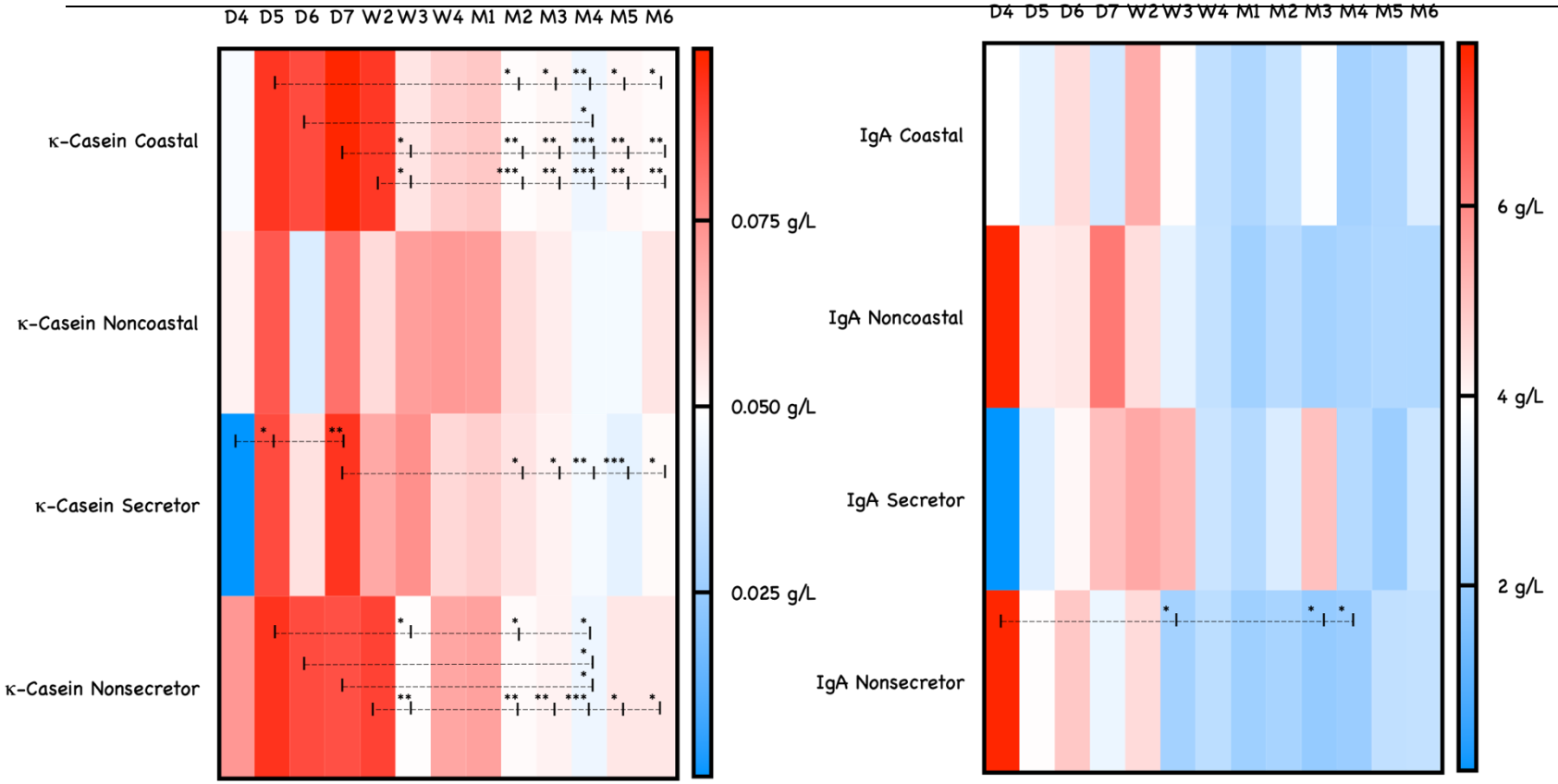
Was the matching effective?	
Chi-square, df	42.54, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

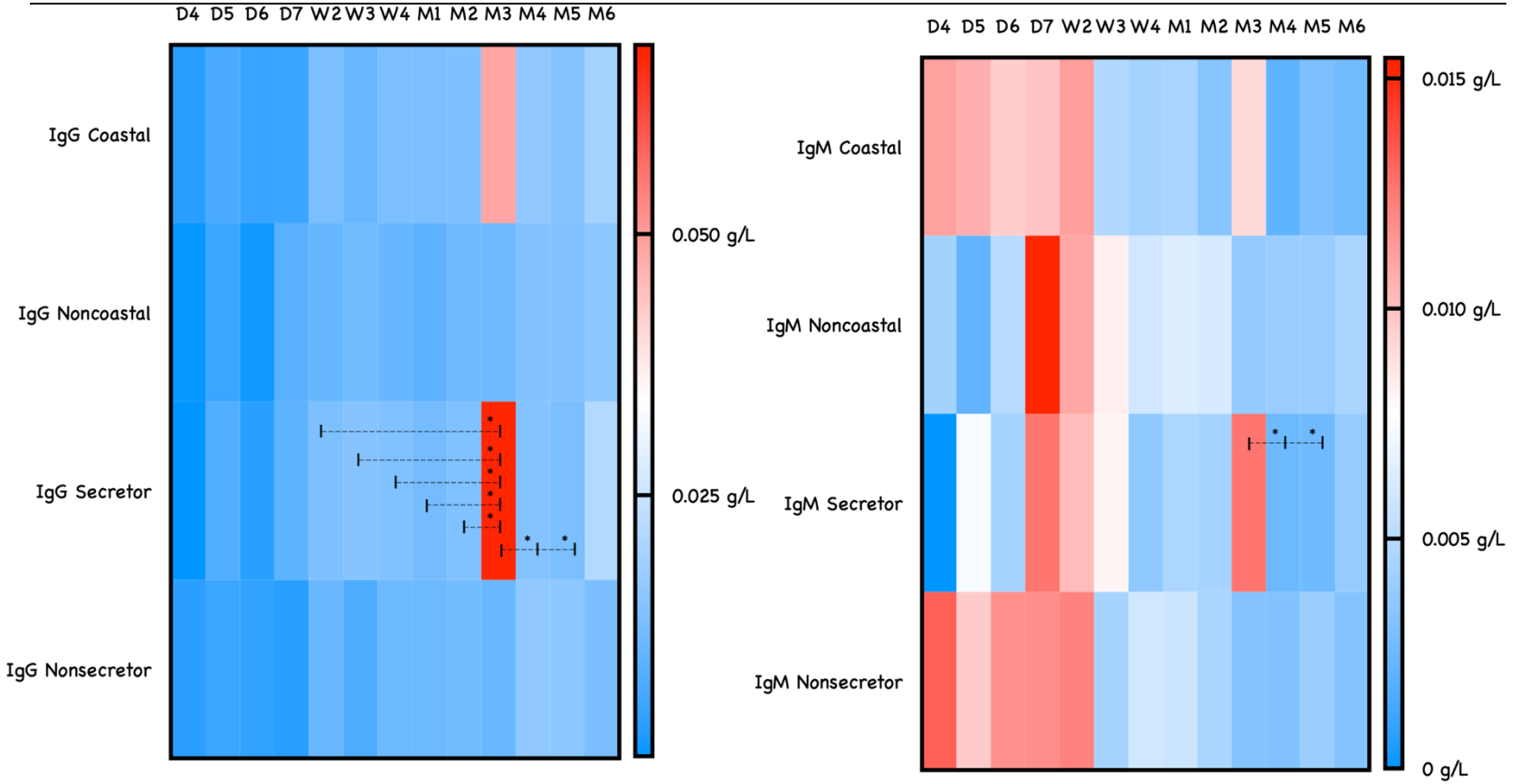
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.002	**	Yes	F (12, 106) = 2.813
Secretor Status	.971	ns	No	F (1, 13) = 0.001362
Lactation Points x Secretor Status	.013	*	Yes	F (12, 106) = 2.267

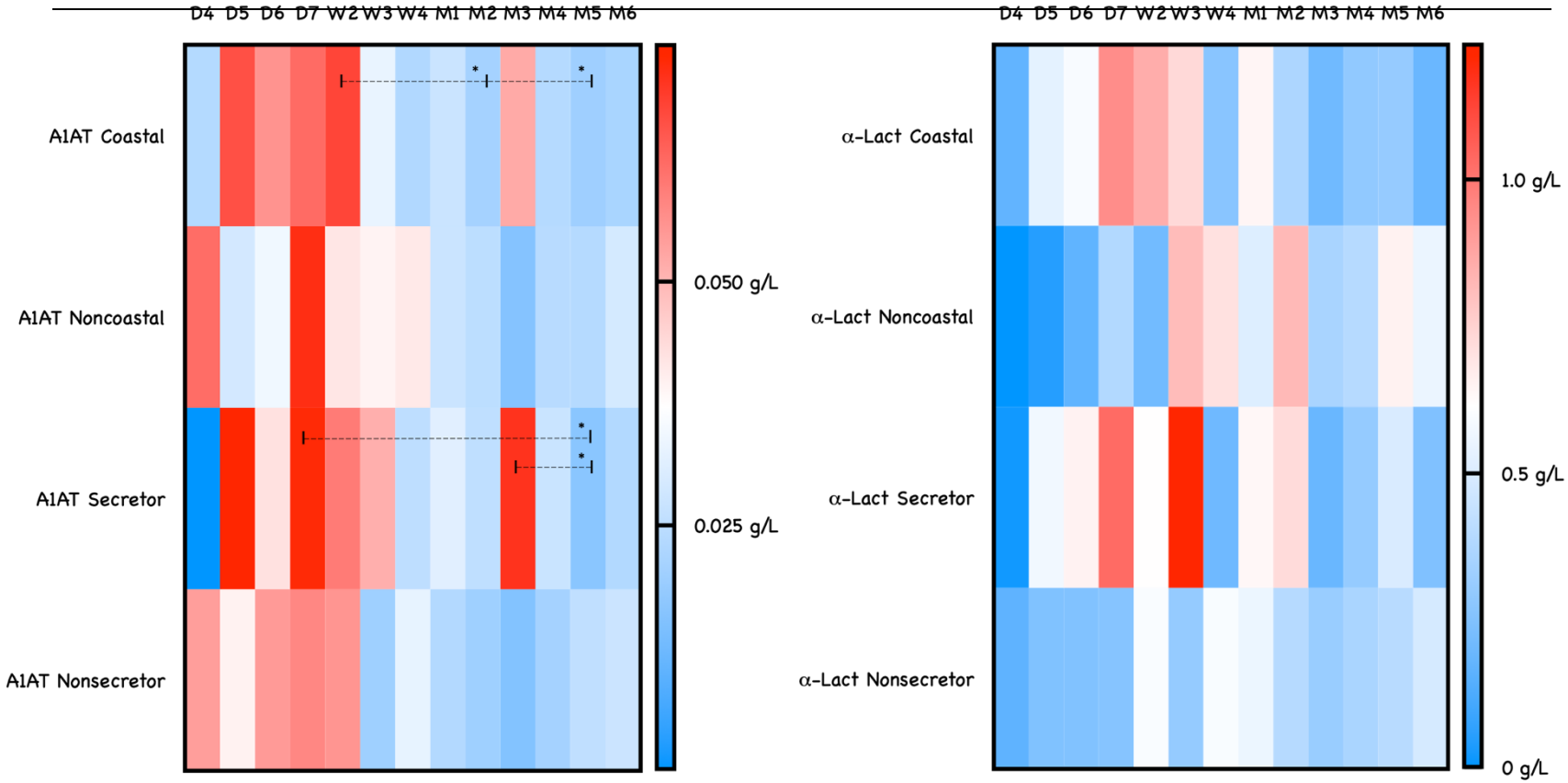
Was the matching effective?	
Chi-square, df	52.21, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

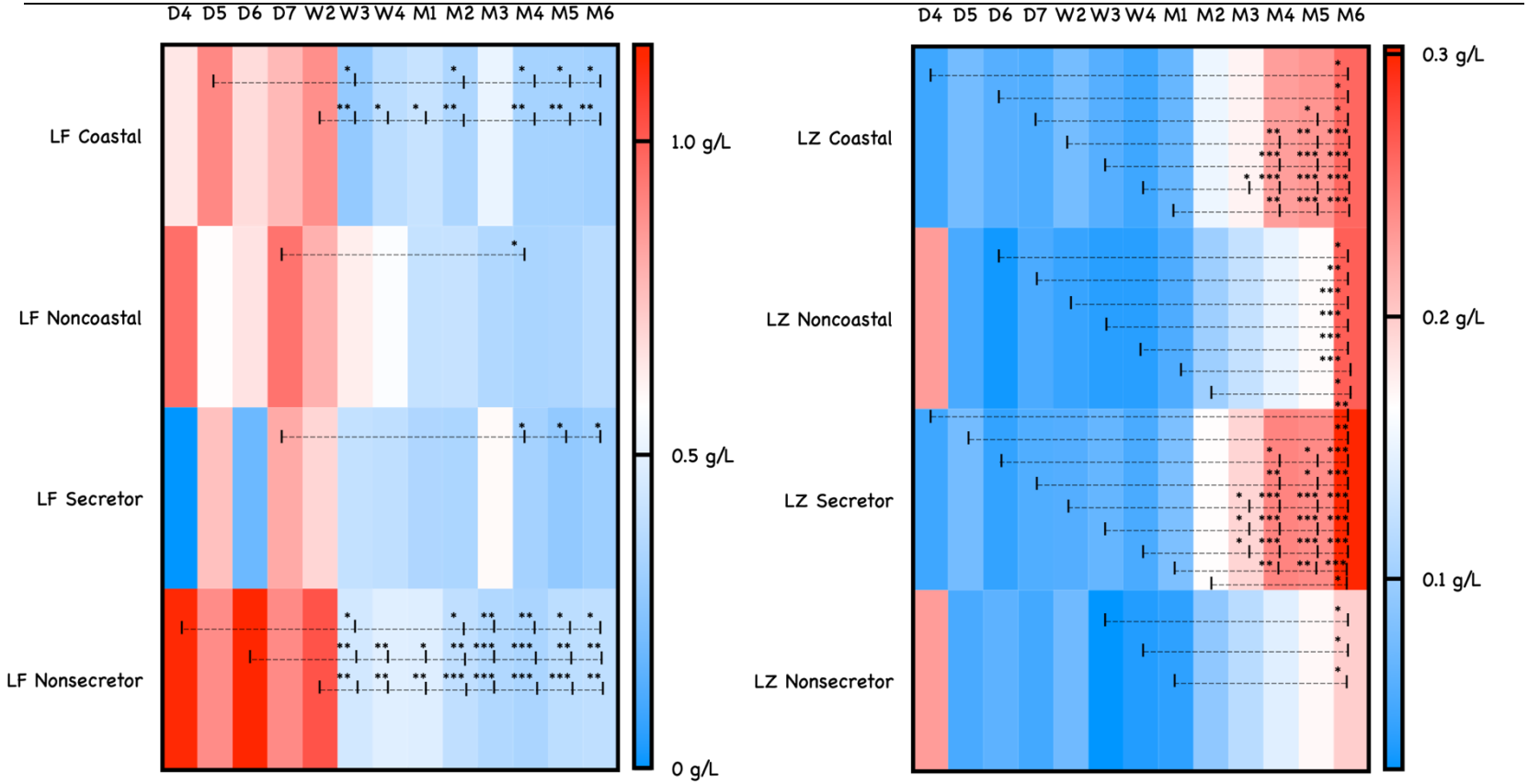
SI Table 5: Tukey's Multiple Comparisons Test on Human Milk Glycoproteins at Different Lactation Points from Coastal, Noncoastal, Secretor, and Nonsecretor Mothers living in Baybay City, Leyte.

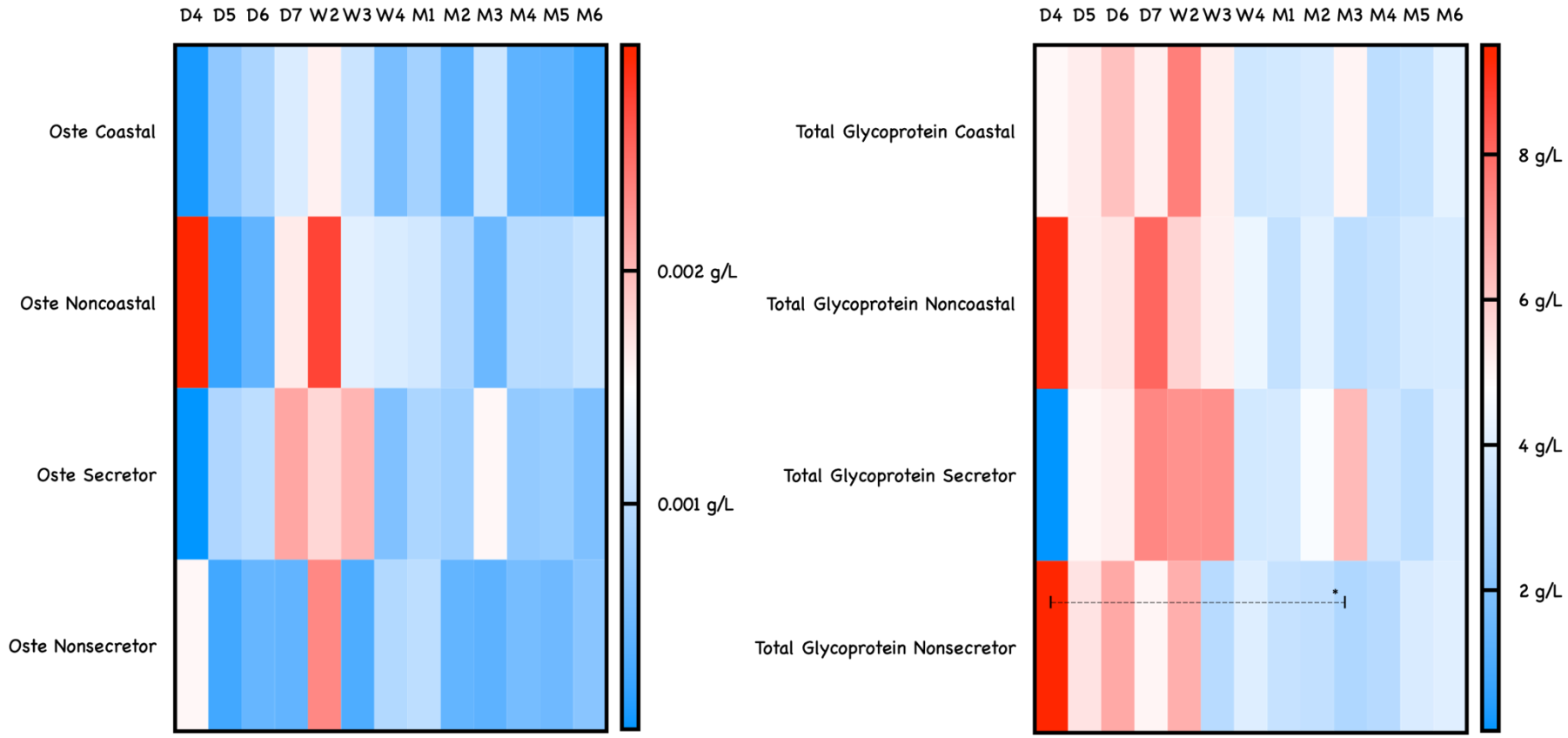



D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
κ-Casein Coastal
κ-Casein Noncoastal
κ-Casein Secretor
κ-Casein Nonsecretor
0.075 g/L
0.050 g/L
0.025 g/L
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IgA Coastal
IgA Noncoastal
IgA Secretor
IgA Nonsecretor
6 g/L
4 g/L
2 g/L


D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IgG Coastal
IgG Noncoastal
IgG Secretor
IgG Nonsecretor
0.050 g/L
0.025 g/L
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IgM Coastal
IgM Noncoastal
IgM Secretor
IgM Nonsecretor
0.015 g/L
0.010 g/L
0.005 g/L
0 g/L

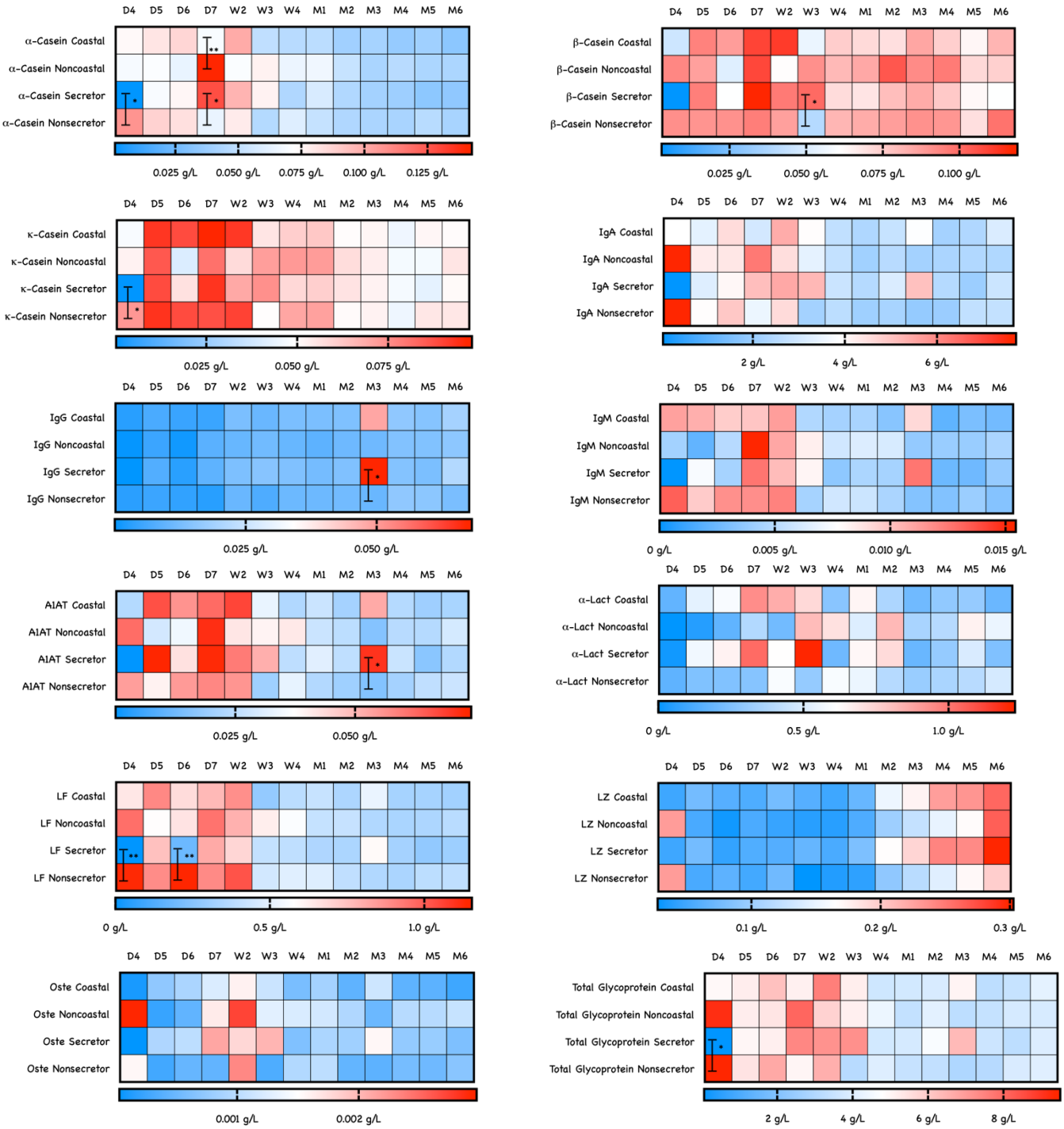

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
A1AT Coastal
A1AT Noncoastal
A1AT Secretor
A1AT Nonsecretor
0.050 g/L
0.025 g/L
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
α-Lact Coastal
α-Lact Noncoastal
α-Lact Secretor
α-Lact Nonsecretor
1.0 g/L
0.5 g/L
0 g/L


D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF Coastal
LF Noncoastal
LF Secretor
LF Nonsecretor
1.0 g/L
0.5 g/L
0 g/L
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LZ Coastal
LZ Noncoastal
LZ Secretor
LZ Nonsecretor
0.3 g/L
0.2 g/L
0.1 g/L



* identifies significant results at P values ≤ 0.05
** identifies highly significant results at P values ≤ 0.01
*** identifies very high significant results at P values ≤ 0

SI Table 6: Sidak's Multiple Comparisons Test on Coastal, Noncoastal, Secretor, and Nonsecretor Human Milk Glycoproteins at Different Lactation Points from Mothers living in Baybay City, Leyte.



* identifies significant results at P values ≤ 0.05;
** identifies highly significant results at P values ≤ 0.01

SI Table 7: Optimized MRM Transition List of Huang et al. (2017) for Targeted Glycopeptide Monitoring and Quantitation of Human Milk Glycoprotein Glycoforms using UPLC-QqQ MS.

Glycoprotein	Glycopeptide	Precursor Ion	Product Ion	RT	D RT
a-Antitrypsin	AT- 107 H5N4F1S2	1209.33	204.1	9.7	0.8
a-Antitrypsin	AT- 107 H5N4S2	1180.12	204.1	9	1
a-Antitrypsin	AT- 107 H6N5F1S3	1340.59	204.1	9.81	0.8
a-Antitrypsin	AT- 107 H6N5S3	1311.34	204.1	10.6	0.8
a-Antitrypsin	AT- 107 H7N6F2S3	1202.51	204.1	9.7	0.8
a-Antitrypsin	AT- 271 H4N3S1	1102.2	204.1	4.8	0.8
a-Antitrypsin	AT- 271 H5N4F1S2	1027.4	204.1	5.1	0.8
a-Antitrypsin	AT- 271 H5N4S1	1223.9	366.1	4.9	0.8
a-Antitrypsin	AT- 271 H5N4S2	990.9	204.1	4.7	0.5
a-Antitrypsin	AT- 271 H6N5S3	1154.98	204.1	5.2	0.7
sIgA	IGA-131/144 H3N3	1015.5	204.1	5.2	0.8
sIgA	IGA-131/144 H3N4	1066.3	204.1	5.1	0.8
sIgA	IGA-131/144 H3N5	1117.1	366.1	5.1	0.8
sIgA	IGA-131/144 H3N5F1	1102.8	204.1	5.4	0.8
sIgA	IGA-131/144 H4N4S1	1179.6	204.1	7.5	0.8
sIgA	IGA-131/144 H4N5	1157.6	204.1	5.1	0.8
sIgA	IGA-131/144 H4N5S1	984.5	204.1	5.7	0.8
sIgA	IGA-131/144 H5N2	1045.8	204.1	5	0.8
sIgA	IGA-131/144 H5N3S1	1169.3	204.1	5.5	0.8
sIgA	IGA-131/144 H5N4	1147.3	204.1	4.9	0.8
sIgA	IGA-131/144 H5N4S1	976.3	204.1	5.7	1
sIgA	IGA-131/144 H5N4S2	1034.5	204.1	5.5	1
sIgA	IGA-131/144 H5N5	1198.1	366.1	6.2	0.8
sIgA	IGA-131/144 H5N5S1	1016.9	366.1	5.6	0.8
sIgA	IGA-131/144 H5N5S2	1075.1	366.1	6.8	0.8
sIgA	IGA-131/144 H6N2	1168	204.1	5	0.8
sIgA	IGA-131/144 H8N2	934.1	204.1	5.1	1
sIgA	IGA-131/144 H9N2	966.5	204.1	4.6	0.8
sIgA	IGA2-205 H3N5F1	869.4	204.1	5.15	1
sIgA	IGA2-205 H5N4F1S1	1006.8	366.1	6.8	0.8
sIgA	IGA2-205 H5N5F1	977.5	366.1	5.7	1

Glycoprotein	Glycopeptide	Precursor Ion	Product Ion	RT	D RT
sIgA	IGA2-205 H5N5F1S1	1074.5	366.1	6.8	1
sIgA	J-71 H4N5	1271.3	366.1	5.7	0.8
sIgA	J-71 H4N5F1	990.2	204.1	5.1	0.8
sIgA	J-71 H5N3F1	1238.6	204.1	5.4	0.8
sIgA	J-71 H5N3F1S1	1002	366.1	5.7	0.8
sIgA	J-71 H5N4	1257.6	366.1	5.8	0.8
sIgA	J-71 H5N4F1S1	1052.8	366.1	4.85	0.5
sIgA	J-71 H5N4F1S2	1125.5	366.1	6.65	0.8
sIgA	J-71 H5N4S1	1016.2	204.1	5.1	0.8
sIgA	SC-135 H5N4F1S2	906.2	204.1	5.1	0.8
sIgA	SC-135 H6N6	1092.5	204.1	4.9	0.8
sIgA	SC-135 H6N7	1143.3	204.1	5.6	0.8
sIgA	SC-135 H7N6	1133	366.1	5.5	0.8
sIgA	SC-135 H7N7	1173.5	204.1	5.5	0.8
sIgA	SC-186 H6N6S1	1252.6	366.1	5.8	0.8
sIgA	SC-421 H5N4F1S1	1116.3	366.1	7.3	1
sIgA	SC-469 H4N5F1	1069.8	366.1	6.8	0.8
sIgA	SC-469 H4N5F1S1	1019.7	204.1	4.2	1
sIgA	SC-469 H5N2	872.1	204.1	4.85	0.5
sIgA	SC-469 H5N4F1S1	1153.2	366.1	7.2	0.8
sIgA	SC-469 H5N4F1S2	937.9	204.1	5.15	1
sIgA	SC-469 H5N5F1S1	1060.2	366.1	4.25	0.5
sIgA	SC-469 H5N5F1S2	1132.9	366.1	4.4	0.5
sIgA	SC-469 H6N2	926.1	366.1	4.9	1
IgG	IGG H3N4F1_1	878.8	204.1	1.7	0.5
IgG	IGG H3N4F1_2	868.1	204.1	2	0.5
IgG	IGG H3N5F1_1	946.5	204.1	1.42	0.5
IgG	IGG H3N5F1_2	935.8	204.1	2.1	0.8
IgG	IGG H3N5F1_3_4	941.1	204.1	1.4	0.5
IgG	IGG H4N4_1	884.1	204.1	1.7	0.5

Glycoprotein	Glycopeptide	Precursor Ion	Product Ion	RT	D RT
IgG	IGG H4N4_2	873.4	204.1	2.1	0.5
IgG	IGG H4N4F1_1	932.8	204.1	1.5	0.5
IgG	IGG H4N4F1_2	922.1	204.1	2	0.5
IgG	IGG H4N4F1_3_4	927.4	204.1	1.6	0.5
IgG	IGG H4N4F1S1_1	1029.8	204.1	1.77	0.5
IgG	IGG H4N4F1S1_2	1019.1	204.1	2.1	0.5
IgG	IGG H4N4F1S1_3_4	1024.5	204.1	1.65	0.5
IgG	IGG H4N5_1	951.7	204.1	2	0.5
IgG	IGG H4N5_2	941.1	204.1	2.1	0.5
IgG	IGG H4N5F1_1	1000.5	204.1	1.5	0.5
IgG	IGG H4N5F1_2	989.9	204.1	2.1	0.5
IgG	IGG H4N5F1_3_4	995.1	204.1	1.7	0.5
IgG	IGG H5N4_1	938.1	366.1	1.6	0.5
IgG	IGG H5N4F1_1	986.8	366.1	1.5	0.5
IgG	IGG H5N4F1_2	976.1	366.1	2.1	0.5
IgG	IGG H5N4F1S1_1	1083.8	366.1	1.5	0.5
IgG	IGG H5N4F1S1_2	1073.1	366.1	2.1	0.5
IgG	IGG H5N5F1_1	1054.5	366.1	1.4	0.5
IgM	IGM-209 H4N5F1S1	1110.8	366.1	3.12	0.5
IgM	IGM-209 H5N4F1S1	1100.5	366.1	4.8	0.8
IgM	IGM-209 H5N4F1S2	1173.3	366.1	3.6	0.8
IgM	IGM-209 H5N5F1S1	1151.3	366.1	3.1	0.5
IgM	IGM-209 H5N5F1S2	1224.1	366.1	3	0.5
IgM	IGM-439 H5N2	1194.5	1284.7	3.7	0.5
IgM	IGM-439 H6N2	1248.5	1284.7	3.65	0.5
IgM	IGM-439 H7N2	1302.6	1284.7	3.6	0.5
IgM	IGM-439 H8N2	1356.6	1284.7	3.65	0.5
IgM	IGM-439 H9N2	1058.3	1284.7	4.2	0.5
IgM	IGM-46 H5N4F1S1	1115.5	366.1	2.9	0.5
Lactoferrin	LF-156 H5N4F1	1250.6	204.1	5.8	0.8
Lactoferrin	LF-156 H5N4F1S1	1058.9	204.1	6.8	0.8
Lactoferrin	LF-156 H5N4F1S2	1117.1	204.1	7.4	0.8

Glycoprotein	Glycopeptide	Precursor Ion	Product Ion	RT	D RT
Lactoferrin	LF-156 H5N4F2	1287.1	204.1	5.8	0.8
Lactoferrin	LF-156 H5N4F2S1	1088.1	204.1	6.7	0.8
Lactoferrin	LF-156 H5N4F3	1323.6	204.1	5.6	0.8
Lactoferrin	LF-156 H5N4F3S1	1117.3	204.1	7.35	0.8
Lactoferrin	LF-156 H5N4F3S2	1175.3	204.1	9	0.8
Lactoferrin	LF-156 H6N5F2S1	1161.1	204.1	6.4	0.8
Lactoferrin	LF-156 H6N5F3S1	1190.3	366.2	6.3	0.8
Lactoferrin	LF-497 H5N4F1	966.7	204.1	4.85	0.8
Lactoferrin	LF-497 H5N4F1S1	1039.4	204.1	4.86	0.8
Lactoferrin	LF-497 H5N4F1S2	1112.2	204.1	5.05	0.8
Lactoferrin	LF-497 H5N4F2	1003.2	204.1	4.9	0.8
Lactoferrin	LF-497 H5N4F2S1	1075.9	204.1	4.86	0.8
Lactoferrin	LF-497 H5N4F2S2	1148.7	204.1	5.1	0.8
Lactoferrin	LF-497 H5N4F3	1039.7	204.1	4.8	0.8
Lactoferrin	LF-497 H5N4F3S1	1112.4	204.1	5.1	0.8
Lactoferrin	LF-497 H5N4S1	1002.9	204.1	4.9	0.5
Lactoferrin	LF-497 H5N4S2	1075.7	204.1	4.8	0.8

SI Table 8: Mixed-Effects Analysis of Variance (ANOVA) on Glycoforms of Human Milk Glycoproteins at Different Lactation Points from Coastal, Noncoastal, Secretor, and Nonsecretor Mothers living in Baybay City, Leyte.

Mixed-effects model (REML)	Matching: Stacked
Assume sphericity?	Yes
Alpha	0.05

AT-271 H6N5S3

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.213	ns	No	F (11, 32) = 1.416
Coastal vs. Noncoastal	.628	ns	No	F (1, 12) = 0.2470
Lactation Points x Coastal vs. Noncoastal	.218	ns	No	F (11, 32) = 1.405

Was the matching effective?	
Chi-square, df	0.3333, 1
P value	.564
P value summary	ns
Is there significant matching (P < 0.05)?	No

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.102	ns	No	F (11, 32) = 1.773
Secretor Status	.301	ns	No	F (1, 12) = 1.166
Lactation Points x Secretor Status	.387	ns	No	F (11, 32) = 1.108

Was the matching effective?	
Chi-square, df	2.913, 1
P value	.088
P value summary	ns
Is there significant matching (P < 0.05)?	No

IGA131/144 H3N5

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (11, 66) = 3.732
Coastal vs. Noncoastal	.126	ns	No	F (1, 13) = 2.680
Lactation Points x Coastal vs. Noncoastal	.002	**	Yes	F (11, 66) = 3.171

Was the matching effective?	
Chi-square, df	19.20, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.013	*	Yes	F (11, 68) = 2.426
Secretor Status	.913	ns	No	F (1, 13) = 0.01233
Lactation Points x Secretor Status	.007	**	Yes	F (11, 68) = 2.665

Was the matching effective?	
Chi-square, df	16.55, 1
P value	<.001
P value summary	***

Is there significant matching (P < 0.05)?	Yes

IGA131/144 H5N5

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (9, 47) = 5.750
Coastal vs. Noncoastal	.347	ns	No	F (1, 13) = 0.9515
Lactation Points x Coastal vs. Noncoastal	<.001	***	Yes	F (9, 47) = 4.222

Was the matching effective?	
Chi-square, df	32.23, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (10, 48) = 4.303
Secretor Status	.354	ns	No	F (1, 13) = 0.9241
Lactation Points x Secretor Status	.336	ns	No	F (10, 48) = 1.166

Was the matching effective?	
Chi-square, df	26.02, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

IGA-131/144 H3N3

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.304	ns	No	F (10, 48) = 1.218
Coastal vs. Noncoastal	.384	ns	No	F (1, 13) = 0.8132
Lactation Points x Coastal vs. Noncoastal	.257	ns	No	F (10, 48) = 1.301

Was the matching effective?	
Chi-square, df	20.70, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.222	ns	No	F (11, 48) = 1.362
Secretor Status	.975	ns	No	F (1, 13) = 0.001011
Lactation Points x Secretor Status	.317	ns	No	F (11, 48) = 1.193

Was the matching effective?	
Chi-square, df	18.76, 1
P value	<.001
P value summary	***

Is there significant matching (P < 0.05)?	Yes

IGA-131/144 H3N4

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 97) = 3.458
Coastal vs. Noncoastal	.017	*	Yes	F (1, 13) = 7.560
Lactation Points x Coastal vs. Noncoastal	<.001	***	Yes	F (12, 97) = 4.038

Was the matching effective?	
Chi-square, df	71.82, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.677	ns	No	F (11, 97) = 0.7617
Secretor Status	.759	ns	No	F (1, 13) = 0.09819
Lactation Points x Secretor Status	.085	ns	No	F (11, 97) = 1.699

Was the matching effective?	
Chi-square, df	81.91, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

IGA-131/144 H4N4S1

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.714	ns	No	F (4, 4) = 0.5457
Coastal vs. Noncoastal	.162	ns	No	F (1, 11) = 2.247
Lactation Points x Coastal vs. Noncoastal	.054	ns	No	F (4, 4) = 6.127

Was the matching effective?	
Chi-square, df	7.082, 1
P value	.008
P value summary	**
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.562	ns	No	F (3, 4) = 0.7835
Secretor Status	.955	ns	No	F (1, 11) = 0.003372
Lactation Points x Secretor Status	.250	ns	No	F (3, 4) = 2.049

Was the matching effective?	
Chi-square, df	8.362, 1
P value	.004
P value summary	**
Is there significant matching (P < 0.05)?	Yes

IGA-131/144 H4N5

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)

Lactation Points	<.001	***	Yes	F (12, 88) = 4.598
Coastal vs. Noncoastal	.076	ns	No	F (1, 13) = 3.713
Lactation Points x Coastal vs. Noncoastal	<.001	***	Yes	F (12, 88) = 3.242

Was the matching effective?	
Chi-square, df	36.49, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (11, 88) = 5.107
Secretor Status	.813	ns	No	F (1, 13) = 0.05820
Lactation Points x Secretor Status	.129	ns	No	F (11, 88) = 1.547

Was the matching effective?	
Chi-square, df	45.99, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

IGA-131/144 H4N5S1

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (11, 23) = 7.954
Coastal vs. Noncoastal	.312	ns	No	F (1, 12) = 1.115
Lactation Points x Coastal vs. Noncoastal	.023	*	Yes	F (11, 23) = 2.653

Was the matching effective?	
Chi-square, df	12.38, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (10, 23) = 9.156
Secretor Status	.215	ns	No	F (1, 12) = 1.711
Lactation Points x Secretor Status	.268	ns	No	F (10, 23) = 1.340

Was the matching effective?	
Chi-square, df	8.723, 1
P value	.003
P value summary	**
Is there significant matching (P < 0.05)?	Yes

IGA-131/144 H5N2

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 90) = 4.505

Coastal vs. Noncoastal	.457	ns	No	F (1, 13) = 0.5870
Lactation Points x Coastal vs. Noncoastal	.108	ns	No	F (12, 90) = 1.592

Was the matching effective?	
Chi-square, df	63.79, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (11, 90) = 6.662
Secretor Status	.838	ns	No	F (1, 13) = 0.04343
Lactation Points x Secretor Status	.052	ns	No	F (11, 90) = 1.886

Was the matching effective?	
Chi-square, df	67.85, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

IGA-131/144 H5N4

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.022	*	Yes	F (10, 59) = 2.319
Coastal vs. Noncoastal	.778	ns	No	F (1, 12) = 0.08350
Lactation Points x Coastal vs. Noncoastal	.072	ns	No	F (10, 59) = 1.844

Was the matching effective?	
Chi-square, df	8.102, 1
P value	.004
P value summary	**
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.153	ns	No	F (11, 59) = 1.508
Secretor Status	.107	ns	No	F (1, 12) = 3.035
Lactation Points x Secretor Status	.582	ns	No	F (11, 59) = 0.8605

Was the matching effective?	
Chi-square, df	5.509, 1
P value	.019
P value summary	*
Is there significant matching (P < 0.05)?	Yes

IGA-131/144 H5N4S1

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.021	*	Yes	F (8, 18) = 3.137
Coastal vs. Noncoastal	.007	**	Yes	F (1, 12) = 10.39
Lactation Points x Coastal vs. Noncoastal	.109	ns	No	F (8, 18) = 1.979

Was the matching effective?	
Chi-square, df	2.016, 1
P value	.156
P value summary	ns
Is there significant matching (P < 0.05)?	No

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.247	ns	No	F (10, 21) = 1.401
Secretor Status	.975	ns	No	F (1, 12) = 0.001052
Lactation Points x Secretor Status	.342	ns	No	F (10, 21) = 1.205

Was the matching effective?	
Chi-square, df	9.961, 1
P value	.002
P value summary	**
Is there significant matching (P < 0.05)?	Yes

IGA-131/144 H5N5S1

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.059	ns	No	F (9, 11) = 2.744
Coastal vs. Noncoastal	.351	ns	No	F (1, 11) = 0.9487
Lactation Points x Coastal vs. Noncoastal	.179	ns	No	F (9, 11) = 1.792

Was the matching effective?	
Chi-square, df	1.907, 1
P value	.167
P value summary	ns
Is there significant matching (P < 0.05)?	No

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.182	ns	No	F (9, 9) = 1.873
Secretor Status	.784	ns	No	F (1, 11) = 0.07920
Lactation Points x Secretor Status	.860	ns	No	F (9, 9) = 0.4726

Was the matching effective?	
Chi-square, df	2.049, 1
P value	.152
P value summary	ns
Is there significant matching (P < 0.05)?	No

IGA-131/144 H8N2

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.049	*	Yes	F (8, 21) = 2.437
Coastal vs. Noncoastal	.797	ns	No	F (1, 12) = 0.06924
Lactation Points x Coastal vs. Noncoastal	.004	**	Yes	F (8, 21) = 4.257

Was the matching effective?	
Chi-square, df	13.73, 1
P value	<.001

P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.018	*	Yes	F (11, 22) = 2.829
Secretor Status	.556	ns	No	F (1, 12) = 0.3666
Lactation Points x Secretor Status	.021	*	Yes	F (11, 22) = 2.759

Was the matching effective?	
Chi-square, df	11.10, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

IGA-131/144 H9N2

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.009	**	Yes	F (9, 43) = 2.894
Coastal vs. Noncoastal	.503	ns	No	F (1, 13) = 0.4744
Lactation Points x Coastal vs. Noncoastal	.125	ns	No	F (9, 43) = 1.673

Was the matching effective?	
Chi-square, df	37.05, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.026	*	Yes	F (9, 42) = 2.416
Secretor Status	.840	ns	No	F (1, 13) = 0.04260
Lactation Points x Secretor Status	.026	*	Yes	F (9, 42) = 2.410

Was the matching effective?	
Chi-square, df	39.44, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

J-71 H4N5

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.162	ns	No	F (11, 23) = 1.610
Coastal vs. Noncoastal	.393	ns	No	F (1, 13) = 0.7802
Lactation Points x Coastal vs. Noncoastal	.225	ns	No	F (11, 23) = 1.432

Was the matching effective?	
Chi-square, df	17.72, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.344	ns	No	F (10, 23) = 1.195
Secretor Status	.309	ns	No	F (1, 13) = 1.123
Lactation Points x Secretor Status	.901	ns	No	F (10, 23) = 0.4558

Was the matching effective?	
Chi-square, df	11.17, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

J-71 H5N4F1S1

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (10, 31) = 7.698
Coastal vs. Noncoastal	.525	ns	No	F (1, 12) = 0.4282
Lactation Points x Coastal vs. Noncoastal	.046	*	Yes	F (10, 31) = 2.191

Was the matching effective?	
Chi-square, df	9.661, 1
P value	.002
P value summary	**
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (11, 33) = 7.680
Secretor Status	.459	ns	No	F (1, 12) = 0.5854
Lactation Points x Secretor Status	.777	ns	No	F (11, 33) = 0.6449

Was the matching effective?	
Chi-square, df	8.672, 1
P value	.003
P value summary	**
Is there significant matching (P < 0.05)?	Yes

J-71 H5N4S1

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.288	ns	No	F (12, 58) = 1.226
Coastal vs. Noncoastal	.162	ns	No	F (1, 13) = 2.193
Lactation Points x Coastal vs. Noncoastal	.174	ns	No	F (12, 58) = 1.441

Was the matching effective?	
Chi-square, df	34.01, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.478	ns	No	F (11, 58) = 0.9759
Secretor Status	.370	ns	No	F (1, 13) = 0.8627
Lactation Points x Secretor Status	.510	ns	No	F (11, 58) = 0.9393

Was the matching effective?	
Chi-square, df	31.61, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

SC-135 H5N4F1S2

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.719	ns	No	F (12, 83) = 0.7293
Coastal vs. Noncoastal	.122	ns	No	F (1, 13) = 2.729
Lactation Points x Coastal vs. Noncoastal	.295	ns	No	F (12, 83) = 1.203

Was the matching effective?	
Chi-square, df	25.46, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.857	ns	No	F (11, 83) = 0.5587
Secretor Status	.408	ns	No	F (1, 13) = 0.7300
Lactation Points x Secretor Status	.785	ns	No	F (11, 83) = 0.6448

Was the matching effective?	
Chi-square, df	23.90, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

SC-421 H5N4F1S1

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.390	ns	No	F (11, 33) = 1.103
Coastal vs. Noncoastal	.649	ns	No	F (1, 11) = 0.2191
Lactation Points x Coastal vs. Noncoastal	.423	ns	No	F (11, 33) = 1.056

Was the matching effective?	
Chi-square, df	16.94, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)

Lactation Points	.400	ns	No	F (11, 33) = 1.089
Secretor Status	.332	ns	No	F (1, 11) = 1.032
Lactation Points x Secretor Status	.224	ns	No	F (11, 33) = 1.389

Was the matching effective?	
Chi-square, df	13.41, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

SC-469 H4N5F1

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (11, 65) = 19.67
Coastal vs. Noncoastal	.981	ns	No	F (1, 13) = 0.0005631
Lactation Points x Coastal vs. Noncoastal	.951	ns	No	F (11, 65) = 0.3994

Was the matching effective?	
Chi-square, df	28.47, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (11, 66) = 21.12
Secretor Status	.357	ns	No	F (1, 13) = 0.9121
Lactation Points x Secretor Status	.872	ns	No	F (11, 66) = 0.5363

Was the matching effective?	
Chi-square, df	29.64, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

SC-469 H5N4F1S1

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (11, 66) = 5.490
Coastal vs. Noncoastal	.491	ns	No	F (1, 13) = 0.5033
Lactation Points x Coastal vs. Noncoastal	.752	ns	No	F (11, 66) = 0.6808

Was the matching effective?	
Chi-square, df	64.69, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)

Lactation Points	<.001	***	Yes	F (11, 67) = 4.643
Secretor Status	.507	ns	No	F (1, 13) = 0.4661
Lactation Points x Secretor Status	.596	ns	No	F (11, 67) = 0.8455

Was the matching effective?	
Chi-square, df	64.59, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

SC-469 H5N4F1S2

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.030	*	Yes	F (10, 19) = 2.705
Coastal vs. Noncoastal	.484	ns	No	F (1, 11) = 0.5249
Lactation Points x Coastal vs. Noncoastal	.363	ns	No	F (10, 19) = 1.177

Was the matching effective?	
Chi-square, df	10.99, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.038	*	Yes	F (11, 20) = 2.465
Secretor Status	.751	ns	No	F (1, 12) = 0.1053
Lactation Points x Secretor Status	.949	ns	No	F (11, 20) = 0.3808

Was the matching effective?	
Chi-square, df	6.993, 1
P value	.008
P value summary	**
Is there significant matching (P < 0.05)?	Yes

SC-469 H5N5F1S1

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (9, 21) = 6.189
Coastal vs. Noncoastal	.108	ns	No	F (1, 12) = 3.014
Lactation Points x Coastal vs. Noncoastal	.889	ns	No	F (9, 21) = 0.4533

Was the matching effective?	
Chi-square, df	15.63, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (10, 24) = 12.30
Secretor Status	.069	ns	No	F (1, 11) = 4.051

Lactation Points x Secretor Status	.017	*	Yes	F (10, 24) = 2.861
Was the matching effective?				
Chi-square, df	28.27, 1			
P value	<.001			
P value summary	***			
Is there significant matching (P < 0.05)?	Yes			

SC-469 H5N5F1S2

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.037	*	Yes	F (7, 15) = 2.963
Coastal vs. Noncoastal	.281	ns	No	F (1, 13) = 1.263
Lactation Points x Coastal vs. Noncoastal	.142	ns	No	F (7, 15) = 1.894
Was the matching effective?				
Chi-square, df	2.376, 1			
P value	.123			
P value summary	ns			
Is there significant matching (P < 0.05)?	No			

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (7, 17) = 7.961
Secretor Status	.717	ns	No	F (1, 13) = 0.1377
Lactation Points x Secretor Status	.606	ns	No	F (7, 17) = 0.7890
Was the matching effective?				
Chi-square, df	7.728, 1			
P value	.005			
P value summary	**			
Is there significant matching (P < 0.05)?	Yes			

IGG H4N5_2

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (9, 37) = 4.965
Coastal vs. Noncoastal	.897	ns	No	F (1, 13) = 0.01733
Lactation Points x Coastal vs. Noncoastal	.749	ns	No	F (9, 37) = 0.6483
Was the matching effective?				
Chi-square, df	19.12, 1			
P value	<.001			
P value summary	***			
Is there significant matching (P < 0.05)?	Yes			

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (11, 38) = 5.202
Secretor Status	.405	ns	No	F (1, 13) = 0.7396
Lactation Points x Secretor Status	.625	ns	No	F (11, 38) = 0.8151

Was the matching effective?	
Chi-square, df	18.21, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

IGG H4N4F1_2

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.355	ns	No	F (8, 29) = 1.162
Coastal vs. Noncoastal	.385	ns	No	F (1, 12) = 0.8119
Lactation Points x Coastal vs. Noncoastal	.983	ns	No	F (8, 29) = 0.2284

Was the matching effective?	
Chi-square, df	17.46, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.243	ns	No	F (11, 32) = 1.351
Secretor Status	.682	ns	No	F (1, 12) = 0.1767
Lactation Points x Secretor Status	.883	ns	No	F (11, 32) = 0.5090

Was the matching effective?	
Chi-square, df	26.09, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LF-156 H5N4F1

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.155	ns	No	F (12, 50) = 1.502
Coastal vs. Noncoastal	.423	ns	No	F (1, 13) = 0.6840
Lactation Points x Coastal vs. Noncoastal	.796	ns	No	F (12, 50) = 0.6417

Was the matching effective?	
Chi-square, df	9.575, 1
P value	.002
P value summary	**
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.066	ns	No	F (12, 50) = 1.846
Secretor Status	.286	ns	No	F (1, 13) = 1.237
Lactation Points x Secretor Status	.065	ns	No	F (12, 50) = 1.849

Was the matching effective?	
Chi-square, df	6.415, 1
P value	.011
P value summary	*

Is there significant matching (P < 0.05)?	Yes

LF-156 H5N4F1S1

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 85) = 21.17
Coastal vs. Noncoastal	.376	ns	No	F (1, 13) = 0.8392
Lactation Points x Coastal vs. Noncoastal	.030	*	Yes	F (12, 85) = 2.040

Was the matching effective?	
Chi-square, df	<.001
P value	.376
P value summary	.030
Is there significant matching (P < 0.05)?	<.001

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (11, 85) = 20.97
Secretor Status	.124	ns	No	F (1, 13) = 2.706
Lactation Points x Secretor Status	.107	ns	No	F (11, 85) = 1.621

Was the matching effective?	
Chi-square, df	52.85, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LF-156 H5N4F1S2

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.015	*	Yes	F (12, 45) = 2.453
Coastal vs. Noncoastal	.202	ns	No	F (1, 13) = 1.802
Lactation Points x Coastal vs. Noncoastal	.087	ns	No	F (12, 45) = 1.752

Was the matching effective?	
Chi-square, df	38.25, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.552	ns	No	F (11, 45) = 0.8949
Secretor Status	.050	ns	No	F (1, 13) = 4.659
Lactation Points x Secretor Status	.650	ns	No	F (11, 45) = 0.7888

Was the matching effective?	
Chi-square, df	35.75, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LF-156 H5N4F2

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 69) = 25.14
Coastal vs. Noncoastal	.192	ns	No	F (1, 13) = 1.896
Lactation Points x Coastal vs. Noncoastal	.149	ns	No	F (12, 69) = 1.491

Was the matching effective?	
Chi-square, df	12.46, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (11, 69) = 23.59
Secretor Status	.093	ns	No	F (1, 13) = 3.279
Lactation Points x Secretor Status	.609	ns	No	F (11, 69) = 0.8316

Was the matching effective?	
Chi-square, df	10.31, 1
P value	.001
P value summary	**
Is there significant matching (P < 0.05)?	Yes

LF-156 H5N4F2S1

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.103	ns	No	F (12, 95) = 1.607
Coastal vs. Noncoastal	.928	ns	No	F (1, 13) = 0.008591
Lactation Points x Coastal vs. Noncoastal	.915	ns	No	F (12, 95) = 0.4911

Was the matching effective?	
Chi-square, df	100.5, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.056	ns	No	F (11, 95) = 1.850
Secretor Status	.033	*	Yes	F (1, 13) = 5.688
Lactation Points x Secretor Status	.522	ns	No	F (11, 95) = 0.9228

Was the matching effective?	
Chi-square, df	76.38, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LF-156 H5N4F3

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)

Lactation Points	<.001	***	Yes	F (5, 31) = 19.07
Coastal vs. Noncoastal	.752	ns	No	F (1, 12) = 0.1046
Lactation Points x Coastal vs. Noncoastal	.375	ns	No	F (5, 31) = 1.111

Was the matching effective?	
Chi-square, df	10.62, 1
P value	.001
P value summary	**
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (4, 31) = 14.64
Secretor Status	.193	ns	No	F (1, 12) = 1.900
Lactation Points x Secretor Status	.952	ns	No	F (4, 31) = 0.1702

Was the matching effective?	
Chi-square, df	12.50, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LF-156 H5N4F3S1

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.014	*	Yes	F (11, 26) = 2.827
Coastal vs. Noncoastal	.564	ns	No	F (1, 11) = 0.3531
Lactation Points x Coastal vs. Noncoastal	.055	ns	No	F (11, 26) = 2.132

Was the matching effective?	
Chi-square, df	28.17, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.789	ns	No	F (11, 27) = 0.6277
Secretor Status	.102	ns	No	F (1, 11) = 3.190
Lactation Points x Secretor Status	.529	ns	No	F (11, 27) = 0.9288

Was the matching effective?	
Chi-square, df	13.34, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LF-156 H5N4F3S2

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.024	*	Yes	F (12, 79) = 2.123
Coastal vs. Noncoastal	.158	ns	No	F (1, 13) = 2.249

Lactation Points x Coastal vs. Noncoastal	.003	**	Yes	F (12, 79) = 2.767

Was the matching effective?	
Chi-square, df	53.41, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.094	ns	No	F (11, 79) = 1.679
Secretor Status	.748	ns	No	F (1, 13) = 0.1077
Lactation Points x Secretor Status	.336	ns	No	F (11, 79) = 1.150

Was the matching effective?	
Chi-square, df	49.50, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LF-156 H6N5F2S1

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.042	*	Yes	F (6, 4) = 6.821
Coastal vs. Noncoastal	.207	ns	No	F (1, 10) = 1.820
Lactation Points x Coastal vs. Noncoastal	.108	ns	No	F (6, 4) = 3.809

Was the matching effective?	
Chi-square, df	5.436, 1
P value	.020
P value summary	*
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.128	ns	No	F (5, 3) = 4.354
Secretor Status	.383	ns	No	F (1, 10) = 0.8328
Lactation Points x Secretor Status	.241	ns	No	F (5, 3) = 2.499

Was the matching effective?	
Chi-square, df	3.601, 1
P value	.058
P value summary	ns
Is there significant matching (P < 0.05)?	No

LF-497 H5N4F1

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (9, 45) = 6.173
Coastal vs. Noncoastal	.531	ns	No	F (1, 12) = 0.4160
Lactation Points x Coastal vs. Noncoastal	.098	ns	No	F (9, 45) = 1.786

Was the matching effective?	
Chi-square, df	14.37, 1
P value	<.001
P value summary	***

Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.002	**	Yes	F (4, 31) = 5.408
Secretor Status	.641	ns	No	F (1, 12) = 0.2285
Lactation Points x Secretor Status	.537	ns	No	F (4, 31) = 0.7957

Was the matching effective?	
Chi-square, df	4.198, 1
P value	.040
P value summary	*
Is there significant matching (P < 0.05)?	Yes

LF-497 H5N4F1S1

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 82) = 8.290
Coastal vs. Noncoastal	.353	ns	No	F (1, 13) = 0.9264
Lactation Points x Coastal vs. Noncoastal	.285	ns	No	F (12, 82) = 1.218

Was the matching effective?	
Chi-square, df	31.28, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (10, 80) = 9.797
Secretor Status	.210	ns	No	F (1, 13) = 1.741
Lactation Points x Secretor Status	.063	ns	No	F (10, 80) = 1.862

Was the matching effective?	
Chi-square, df	31.42, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LF-497 H5N4F1S2

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.033	*	Yes	F (6, 8) = 4.218
Coastal vs. Noncoastal	.264	ns	No	F (1, 10) = 1.399
Lactation Points x Coastal vs. Noncoastal	.588	ns	No	F (6, 8) = 0.8142

Was the matching effective?	
	0.000386
Chi-square, df	2, 1
P value	.984
P value summary	ns
Is there significant matching (P < 0.05)?	No

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.003	**	Yes	F (5, 6) = 14.48
Secretor Status	.028	*	Yes	F (1, 10) = 6.543
Lactation Points x Secretor Status	.047	*	Yes	F (5, 6) = 4.509

Was the matching effective?	
Chi-square, df	3.129, 1
P value	.077
P value summary	ns
Is there significant matching (P < 0.05)?	No

LF-497 H5N4F2

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (9, 48) = 4.785
Coastal vs. Noncoastal	.084	ns	No	F (1, 13) = 3.499
Lactation Points x Coastal vs. Noncoastal	.343	ns	No	F (9, 48) = 1.158

Was the matching effective?	
Chi-square, df	28.80, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.002	**	Yes	F (11, 50) = 3.320
Secretor Status	.306	ns	No	F (1, 13) = 1.136
Lactation Points x Secretor Status	.142	ns	No	F (11, 50) = 1.555

Was the matching effective?	
Chi-square, df	28.47, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LF-497 H5N4F2S1

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.472	ns	No	F (10, 16) = 1.015
Coastal vs. Noncoastal	.713	ns	No	F (1, 11) = 0.1429
Lactation Points x Coastal vs. Noncoastal	.653	ns	No	F (10, 16) = 0.7736

Was the matching effective?	
Chi-square, df	0.2737, 1
P value	.601
P value summary	ns
Is there significant matching (P < 0.05)?	No

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.509	ns	No	F (9, 12) = 0.9666
Secretor Status	.496	ns	No	F (1, 11) = 0.4952
Lactation Points x Secretor Status	.149	ns	No	F (9, 12) = 1.899

Was the matching effective?	
Chi-square, df	1.740, 1
P value	.187
P value summary	ns
Is there significant matching (P < 0.05)?	No

LF-497 H5N4F2S2

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.422	ns	No	F (12, 46) = 1.051
Coastal vs. Noncoastal	.503	ns	No	F (1, 12) = 0.4771
Lactation Points x Coastal vs. Noncoastal	.072	ns	No	F (12, 46) = 1.824

Was the matching effective?	
Chi-square, df	12.29, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.464	ns	No	F (11, 46) = 0.9961
Secretor Status	.073	ns	No	F (1, 12) = 3.861
Lactation Points x Secretor Status	.168	ns	No	F (11, 46) = 1.490

Was the matching effective?	
Chi-square, df	7.900, 1
P value	.005
P value summary	**
Is there significant matching (P < 0.05)?	Yes

LF-497 H5N4F3

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 95) = 11.07
Coastal vs. Noncoastal	.262	ns	No	F (1, 13) = 1.376
Lactation Points x Coastal vs. Noncoastal	.134	ns	No	F (12, 95) = 1.510

Was the matching effective?	
Chi-square, df	40.23, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 95) = 11.70
Secretor Status	.399	ns	No	F (1, 13) = 0.7612
Lactation Points x Secretor Status	.082	ns	No	F (12, 95) = 1.685

Was the matching effective?	
Chi-square, df	45.42, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LF-497 H5N4F3S1

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.075	ns	No	F (6, 22) = 2.263
Coastal vs. Noncoastal	.217	ns	No	F (1, 12) = 1.702
Lactation Points x Coastal vs. Noncoastal	.829	ns	No	F (6, 22) = 0.4621

Was the matching effective?	
Chi-square, df	5.773, 1
P value	.016
P value summary	*
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.009	**	Yes	F (7, 22) = 3.662
Secretor Status	.764	ns	No	F (1, 12) = 0.09448
Lactation Points x Secretor Status	.044	*	Yes	F (7, 22) = 2.547

Was the matching effective?	
Chi-square, df	8.554, 1
P value	.003
P value summary	**
Is there significant matching (P < 0.05)?	Yes

LF-497 H5N4S1

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.178	ns	No	F (10, 25) = 1.556
Coastal vs. Noncoastal	.504	ns	No	F (1, 12) = 0.4740
Lactation Points x Coastal vs. Noncoastal	.816	ns	No	F (10, 25) = 0.5787

Was the matching effective?	
Chi-square, df	12.41, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.274	ns	No	F (11, 28) = 1.303
Secretor Status	.234	ns	No	F (1, 12) = 1.568
Lactation Points x Secretor Status	.592	ns	No	F (11, 28) = 0.8534

Was the matching effective?	
Chi-square, df	9.916, 1
P value	.002
P value summary	**
Is there significant matching (P < 0.05)?	Yes

LF-497 H5N4S2

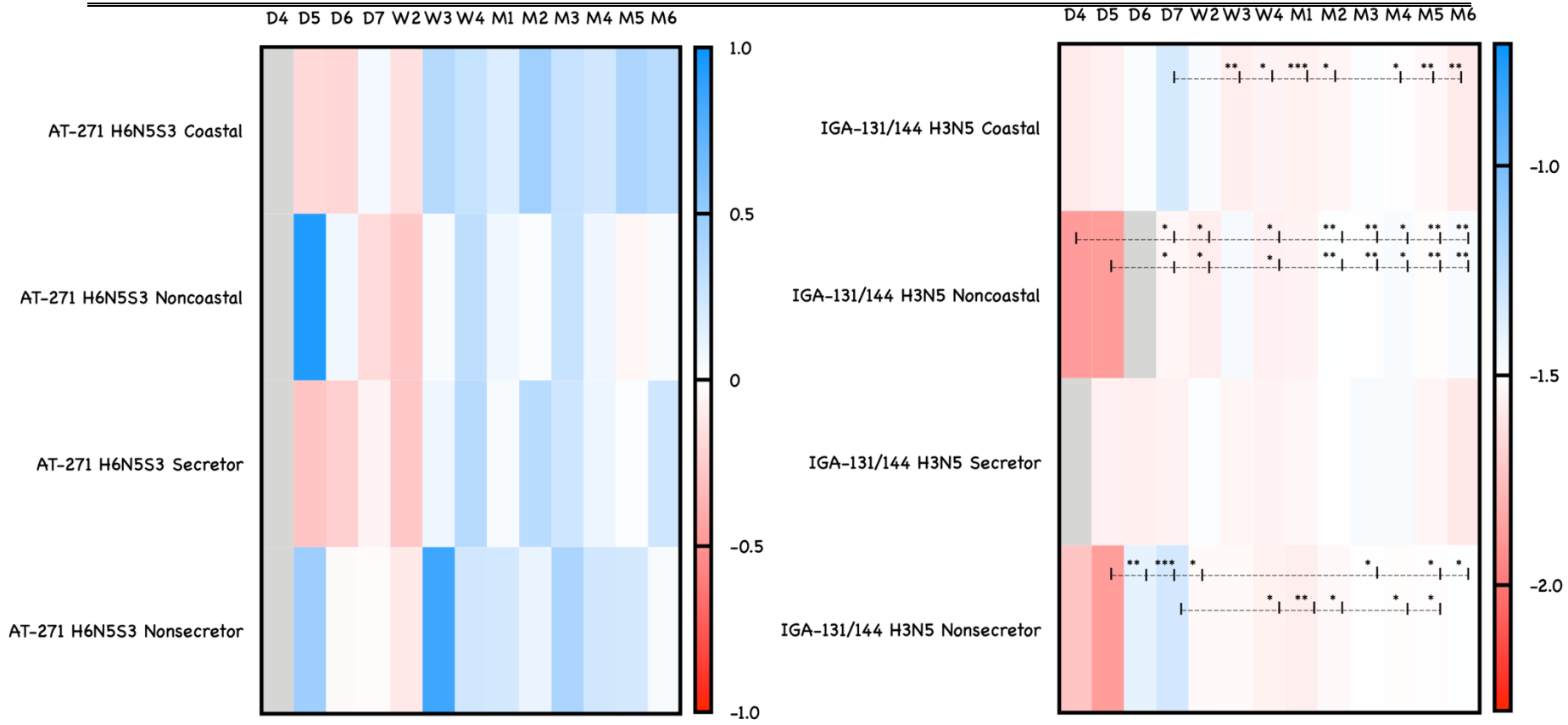
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.201	ns	No	F (5, 3) = 2.951
Coastal vs. Noncoastal	.652	ns	No	F (1, 9) = 0.2170
Lactation Points x Coastal vs. Noncoastal	.895	ns	No	F (5, 3) = 0.2849

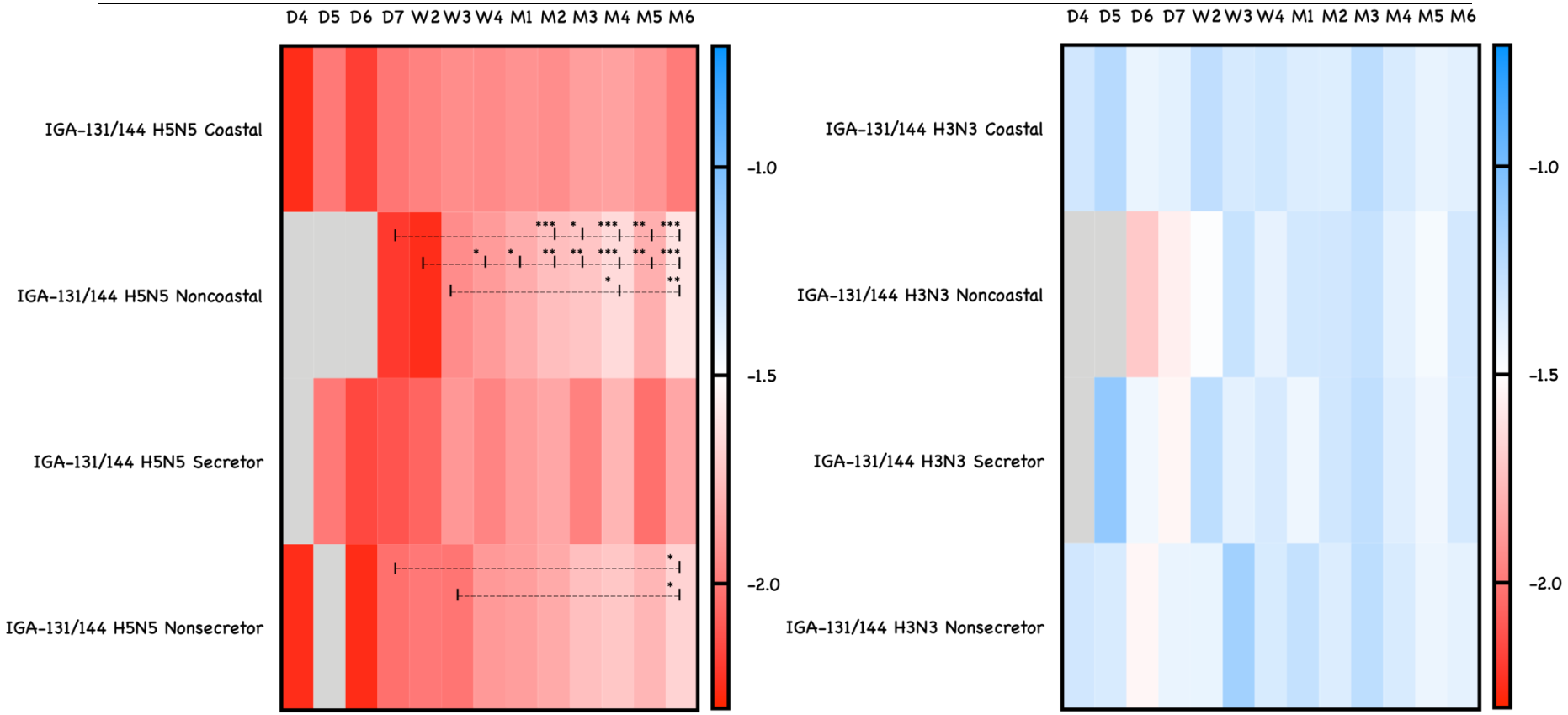
Was the matching effective?	
Chi-square, df	1.548, 1
P value	.213
P value summary	ns
Is there significant matching (P < 0.05)?	No

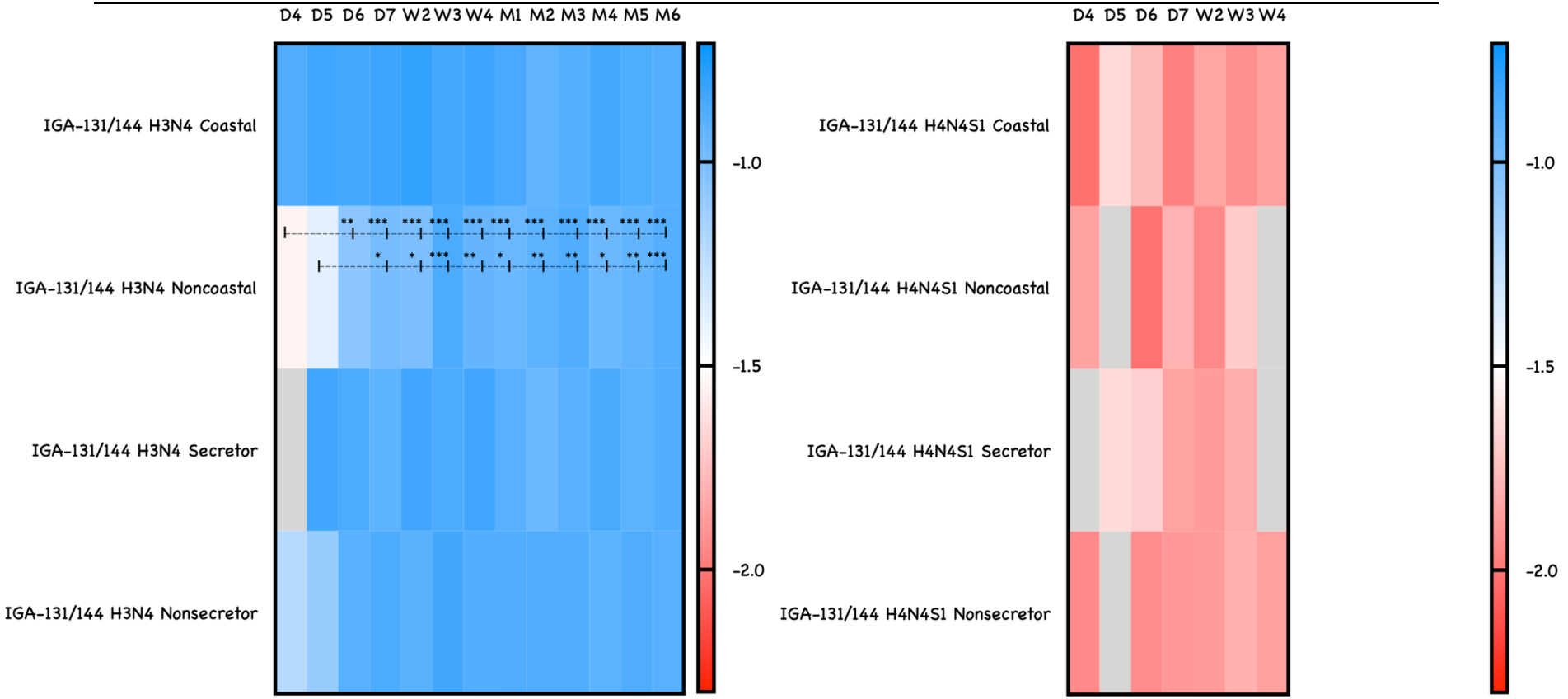
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.256	ns	No	F (3, 3) = 2.296
Secretor Status	.470	ns	No	F (1, 9) = 0.5699
Lactation Points x Secretor Status	.605	ns	No	F (3, 3) = 0.7165

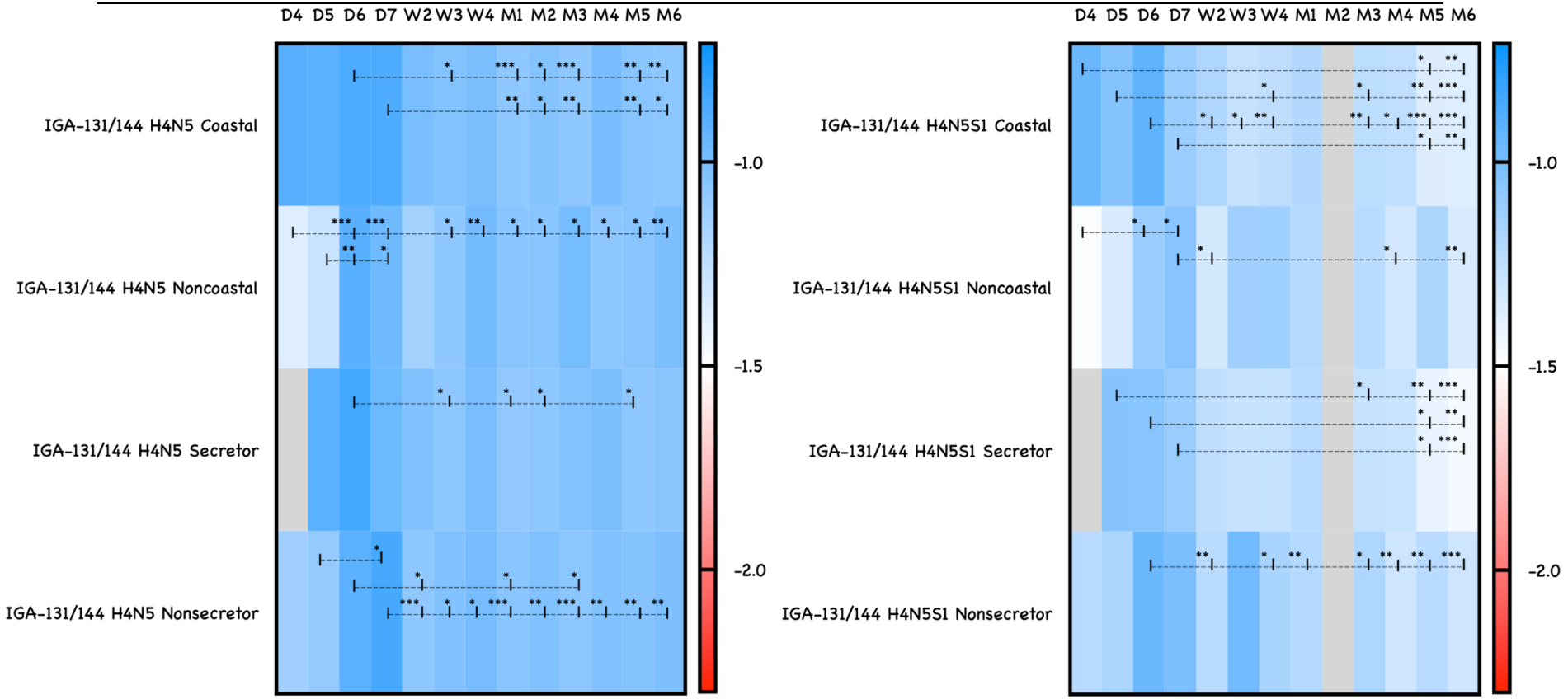
Was the matching effective?	
Chi-square, df	3.002, 1
P value	.083
P value summary	ns
Is there significant matching (P < 0.05)?	No

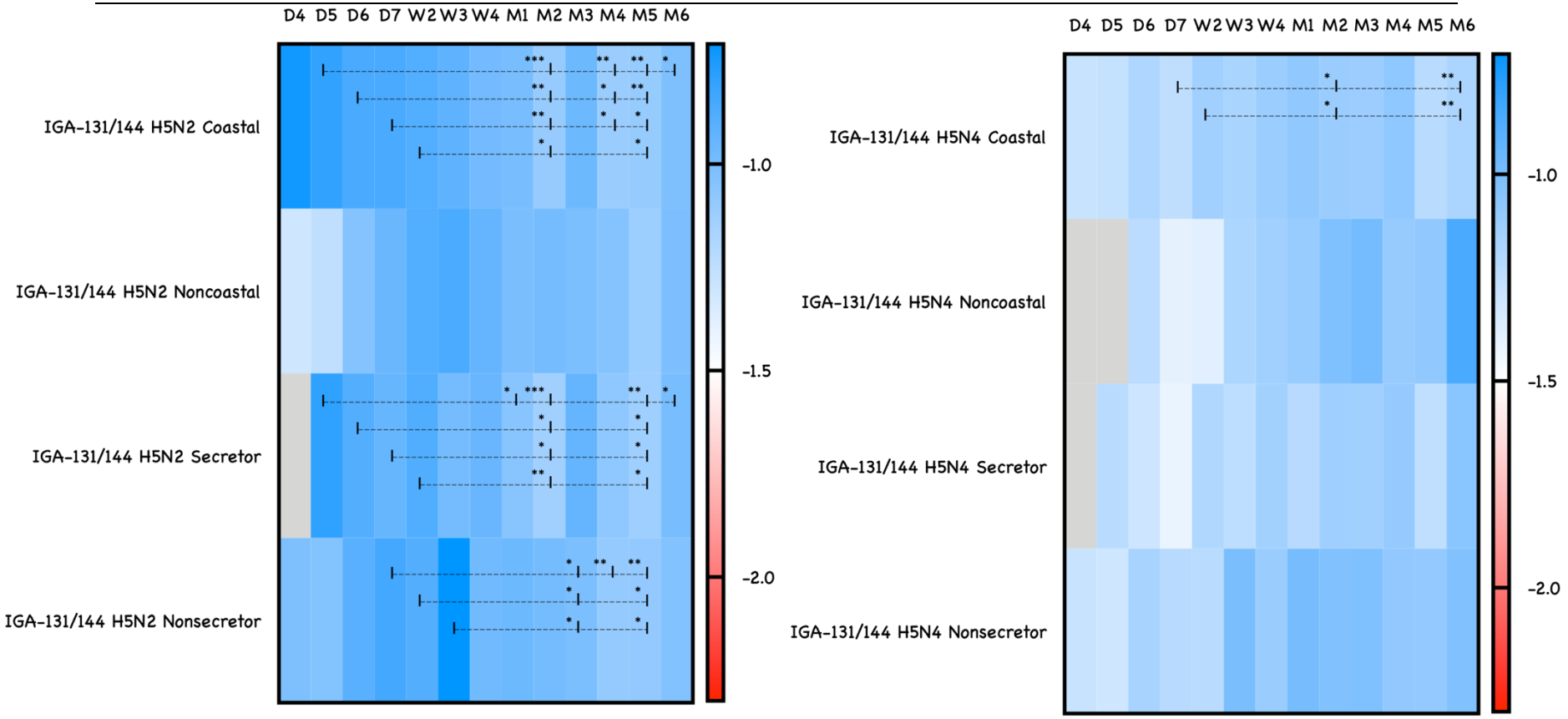
SI Table 9: Tukey's Multiple Comparisons Test on Glycoforms of Human Milk Glycoproteins at Different Lactation Points from Coastal, Noncoastal, Secretor, and Nonsecretor Mothers living in Baybay City, Leyte.

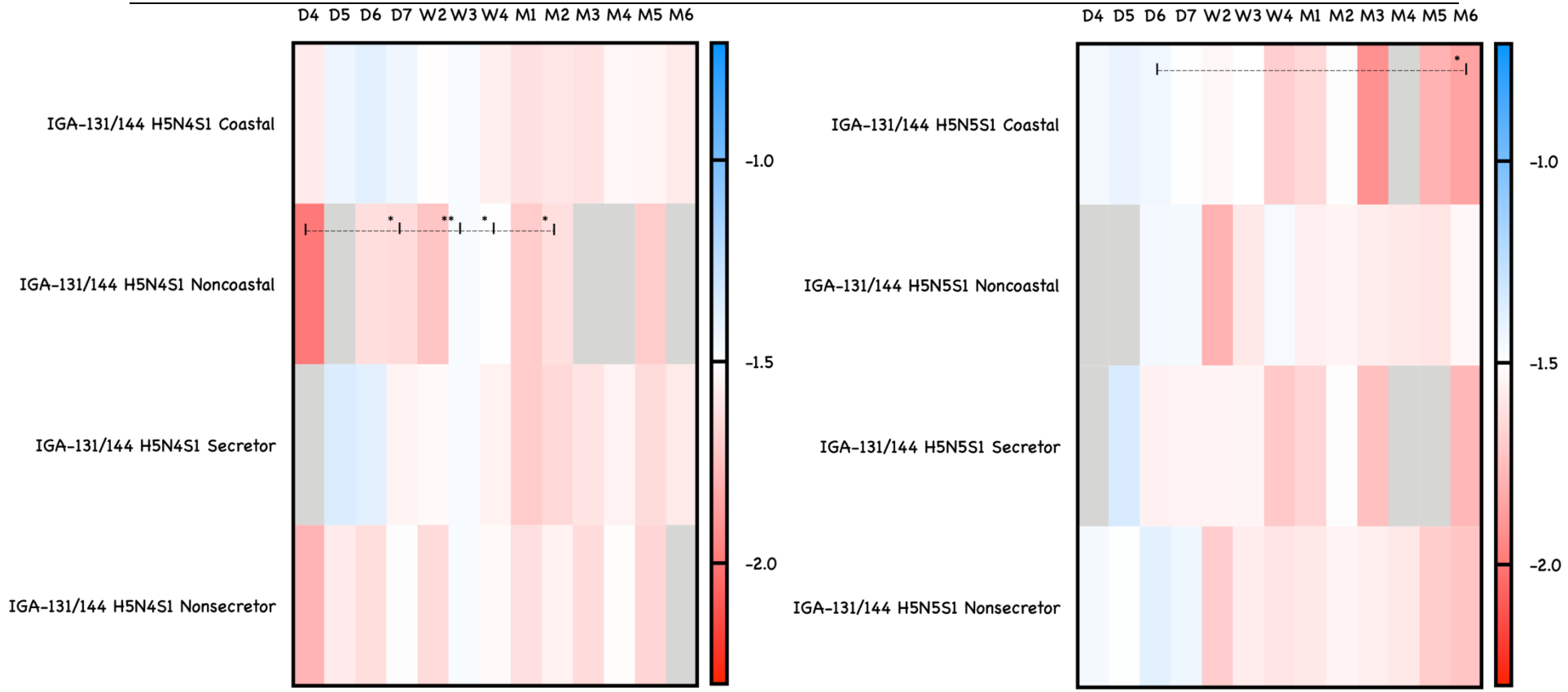


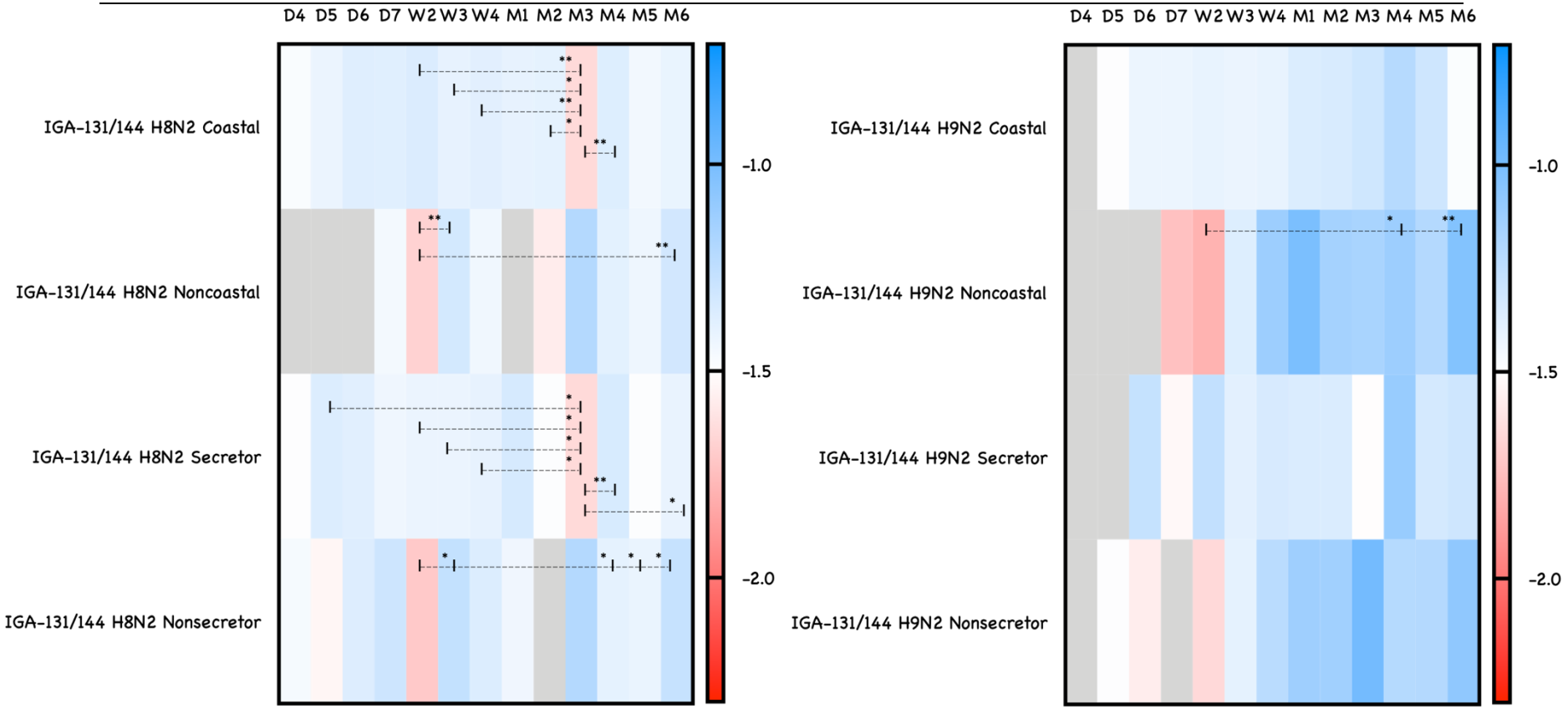

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H5N5 Coastal
IGA-131/144 H5N5 Noncoastal
IGA-131/144 H5N5 Secretor
IGA-131/144 H5N5 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H3N3 Coastal
IGA-131/144 H3N3 Noncoastal
IGA-131/144 H3N3 Secretor
IGA-131/144 H3N3 Nonsecretor
-1.0
-1.5
-2.0

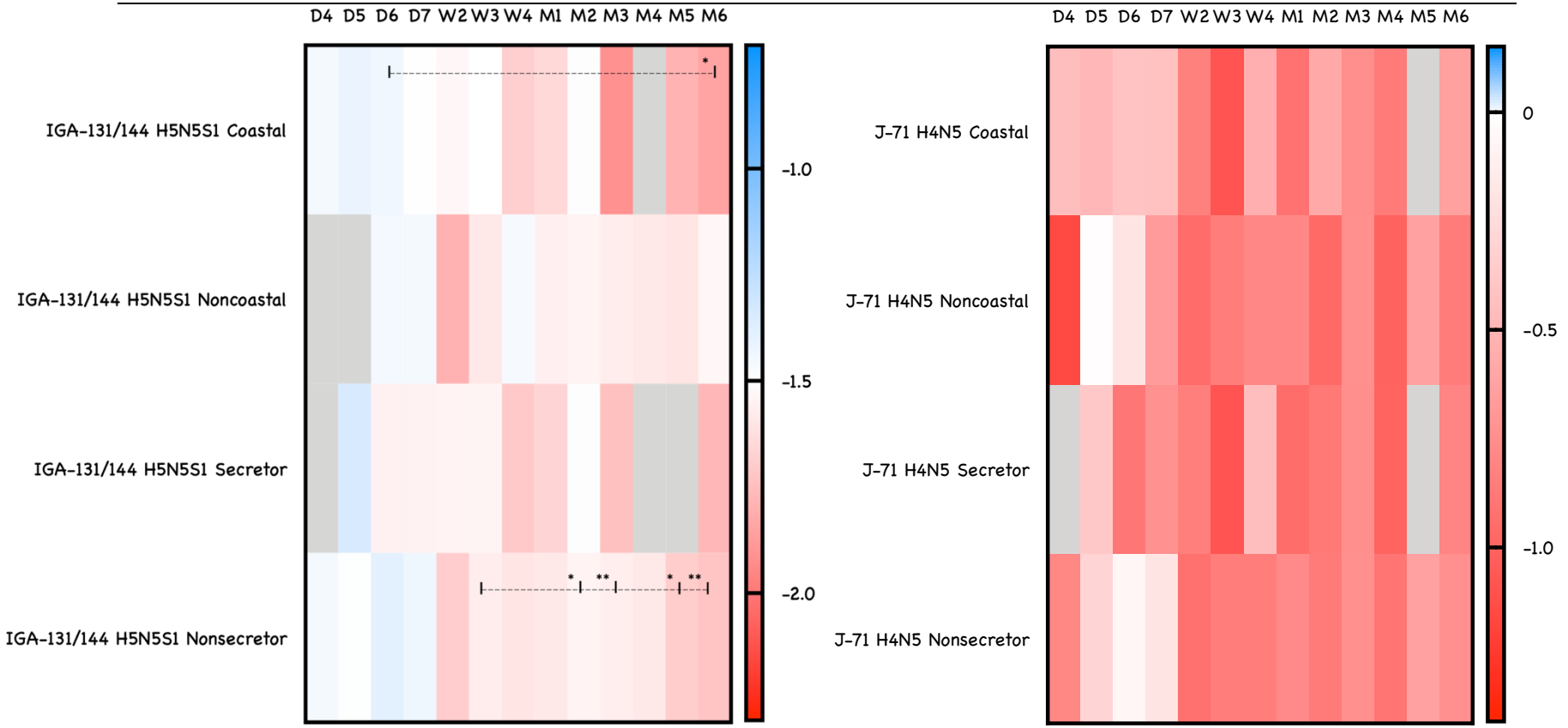

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H3N4 Coastal
IGA-131/144 H3N4 Noncoastal
IGA-131/144 H3N4 Secretor
IGA-131/144 H3N4 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4
IGA-131/144 H4N4S1 Coastal
IGA-131/144 H4N4S1 Noncoastal
IGA-131/144 H4N4S1 Secretor
IGA-131/144 H4N4S1 Nonsecretor
-1.0
-1.5
-2.0

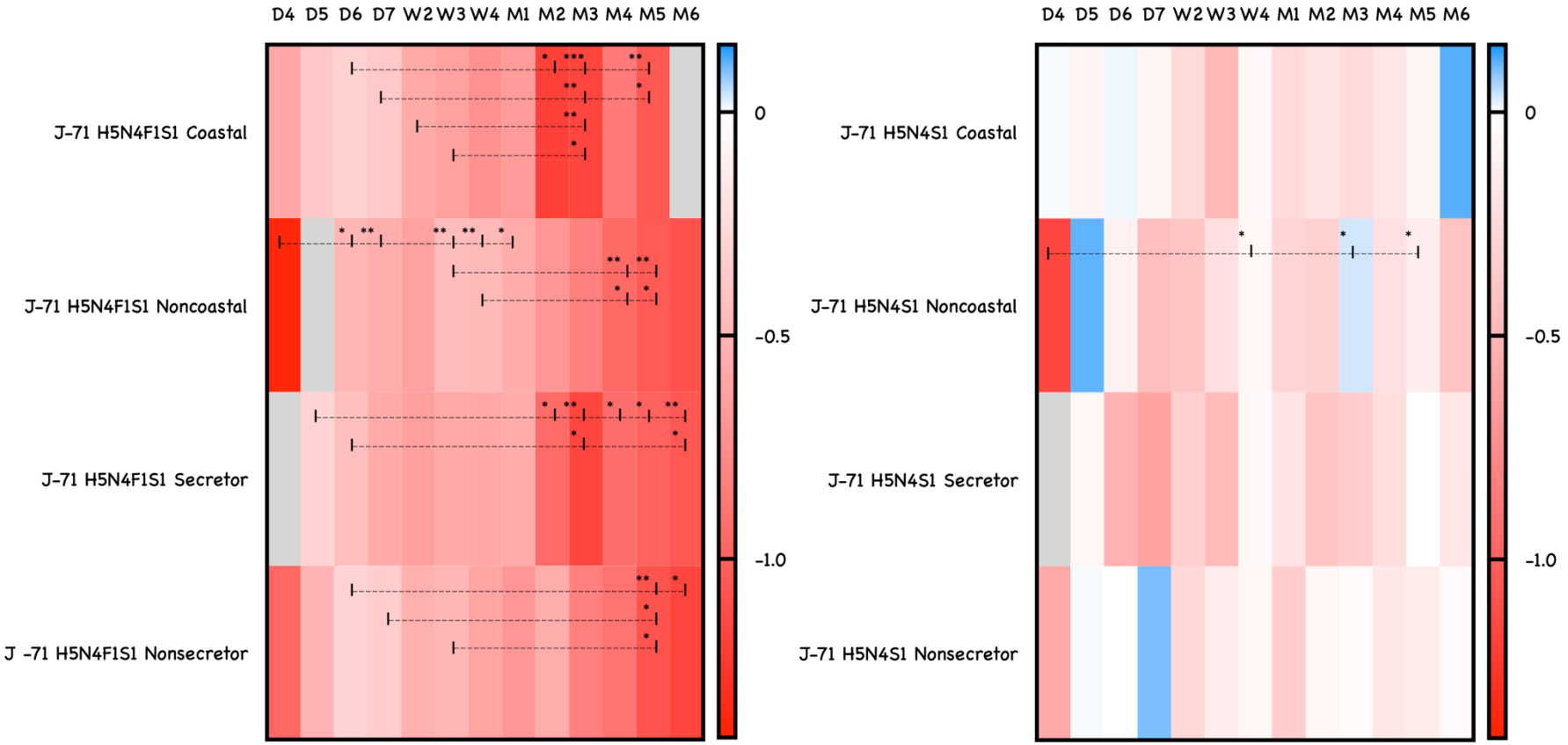

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H4N5 Coastal
IGA-131/144 H4N5 Noncoastal
IGA-131/144 H4N5 Secretor
IGA-131/144 H4N5 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H4N5S1 Coastal
IGA-131/144 H4N5S1 Noncoastal
IGA-131/144 H4N5S1 Secretor
IGA-131/144 H4N5S1 Nonsecretor
-1.0
-1.5
-2.0

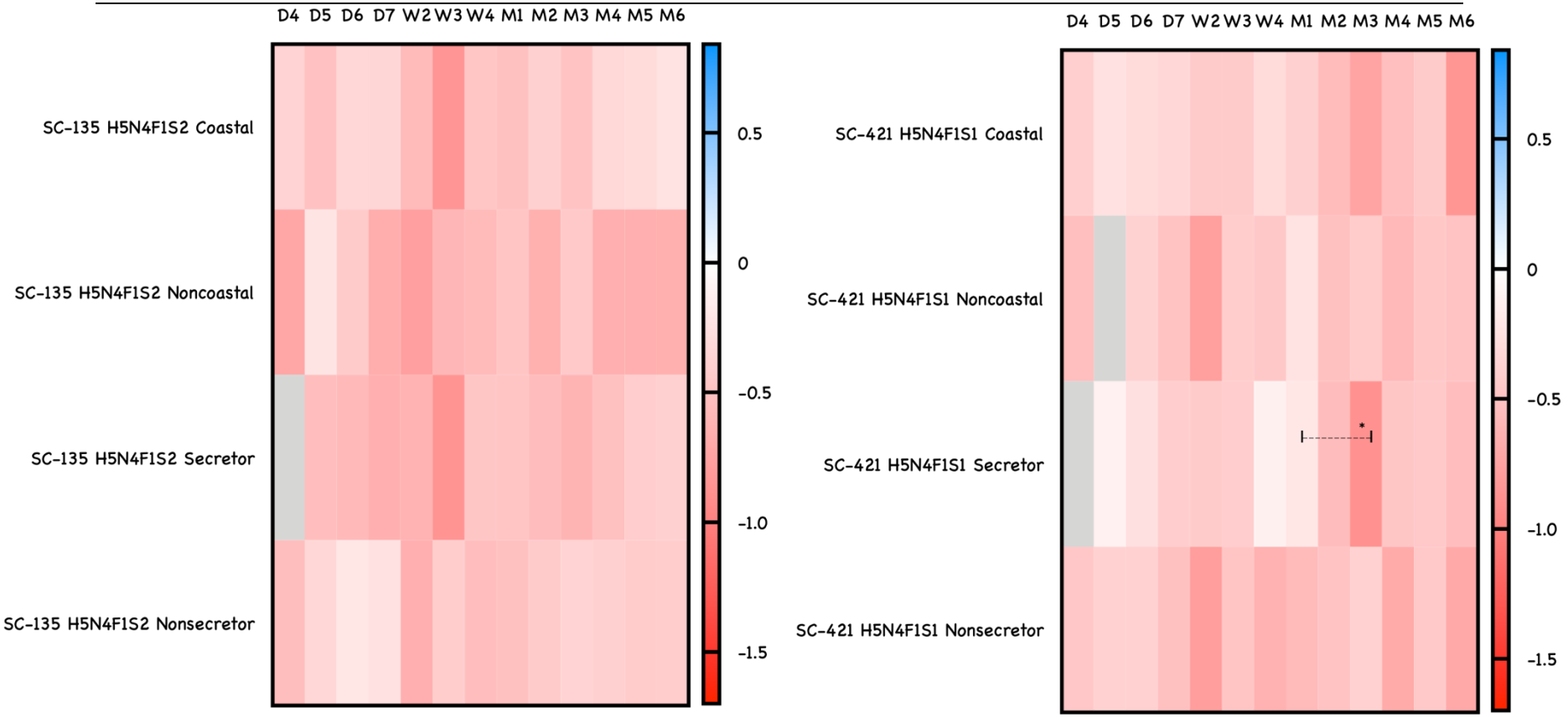

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H5N2 Coastal
IGA-131/144 H5N2 Noncoastal
IGA-131/144 H5N2 Secretor
IGA-131/144 H5N2 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H5N4 Coastal
IGA-131/144 H5N4 Noncoastal
IGA-131/144 H5N4 Secretor
IGA-131/144 H5N4 Nonsecretor
-1.0
-1.5
-2.0

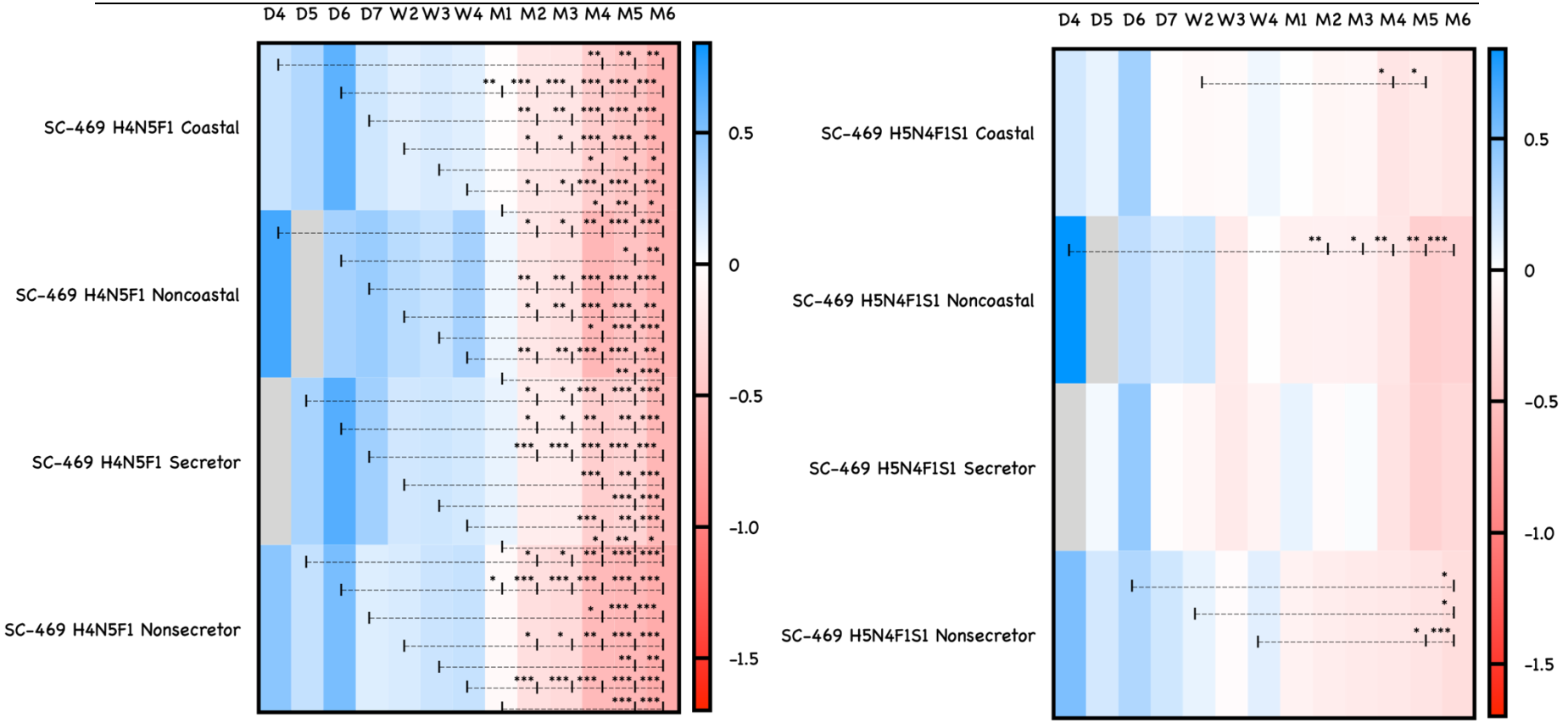

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H5N4S1 Coastal
IGA-131/144 H5N4S1 Noncoastal
IGA-131/144 H5N4S1 Secretor
IGA-131/144 H5N4S1 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H5N5S1 Coastal
IGA-131/144 H5N5S1 Noncoastal
IGA-131/144 H5N5S1 Secretor
IGA-131/144 H5N5S1 Nonsecretor
-1.0
-1.5
-2.0

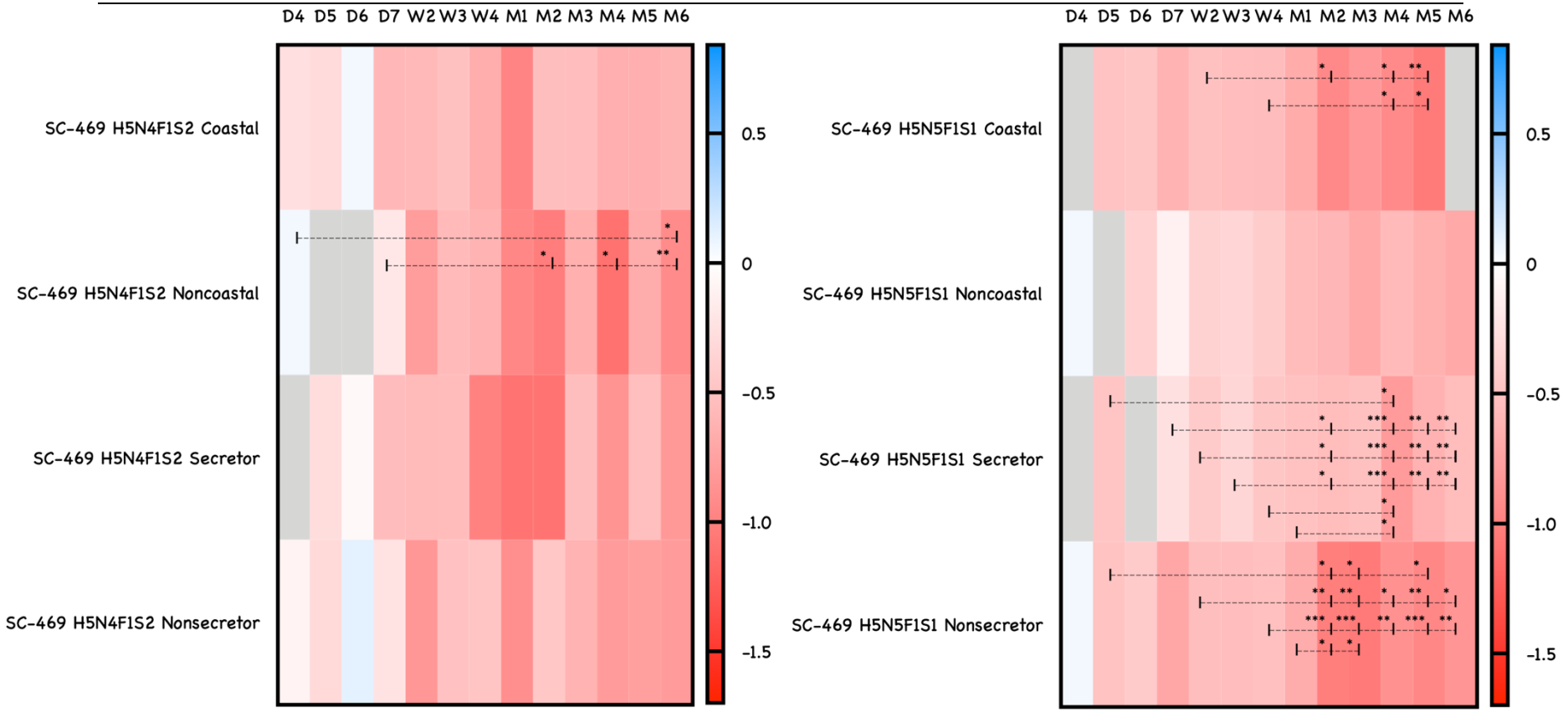

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H8N2 Coastal
IGA-131/144 H8N2 Noncoastal
IGA-131/144 H8N2 Secretor
IGA-131/144 H8N2 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H9N2 Coastal
IGA-131/144 H9N2 Noncoastal
IGA-131/144 H9N2 Secretor
IGA-131/144 H9N2 Nonsecretor
-1.0
-1.5
-2.0

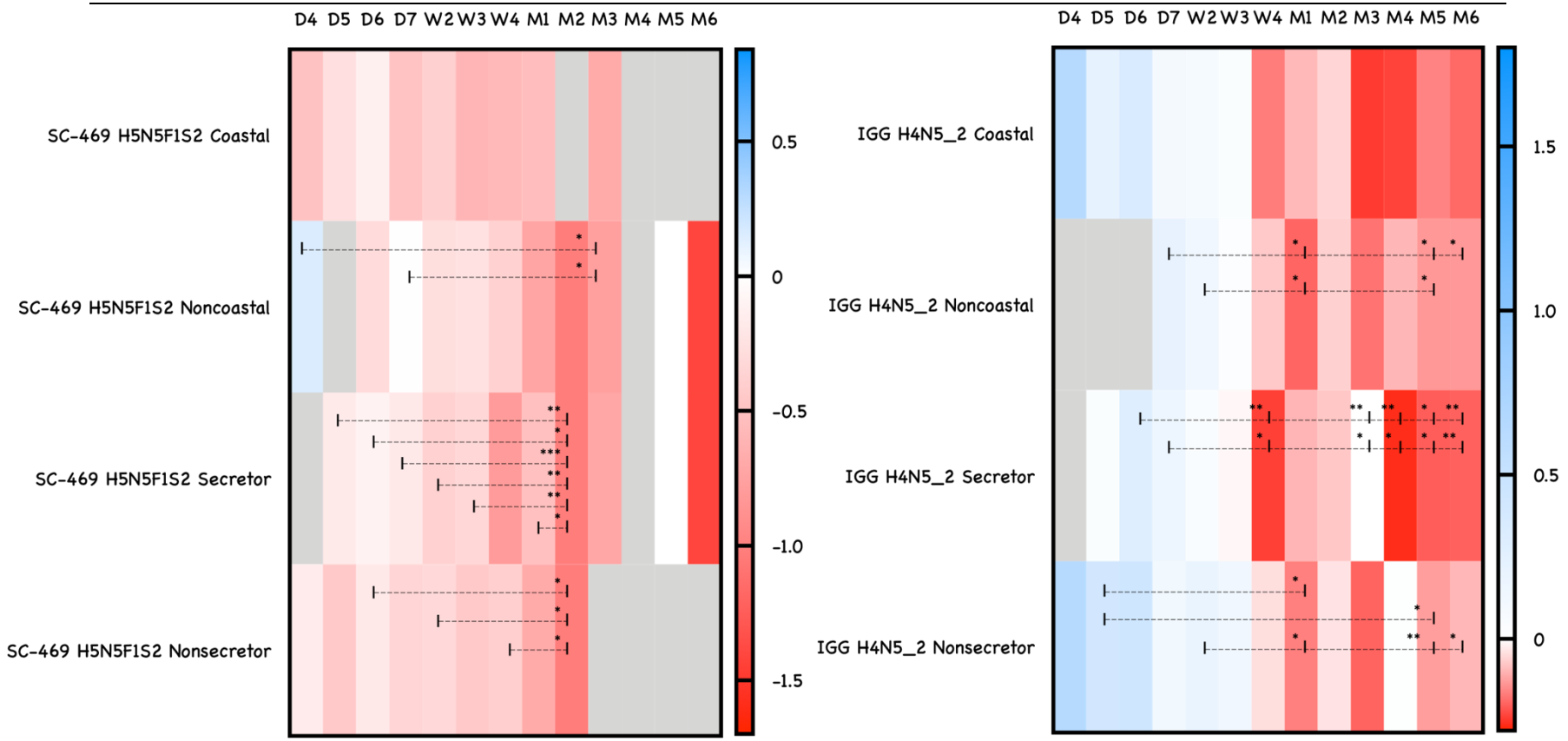

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H5N5S1 Coastal
IGA-131/144 H5N5S1 Noncoastal
IGA-131/144 H5N5S1 Secretor
IGA-131/144 H5N5S1 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
J-71 H4N5 Coastal
J-71 H4N5 Noncoastal
J-71 H4N5 Secretor
J-71 H4N5 Nonsecretor
0
-0.5
-1.0

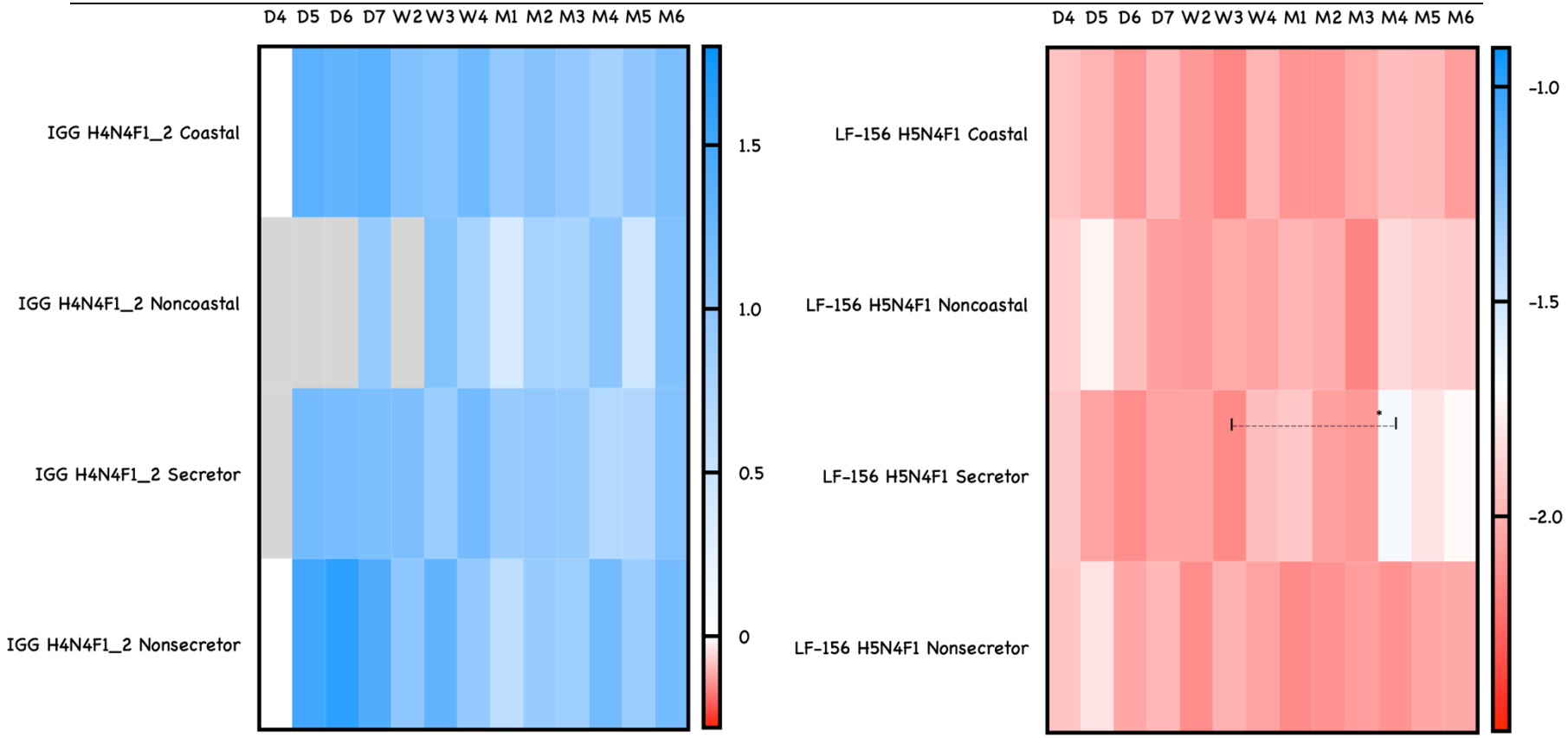

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
J-71 H5N4F1S1 Coastal
J-71 H5N4F1S1 Noncoastal
J-71 H5N4F1S1 Secretor
J -71 H5N4F1S1 Nonsecretor
0
-0.5
-1.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
J-71 H5N4S1 Coastal
J-71 H5N4S1 Noncoastal
J-71 H5N4S1 Secretor
J-71 H5N4S1 Nonsecretor
0
-0.5
-1.0

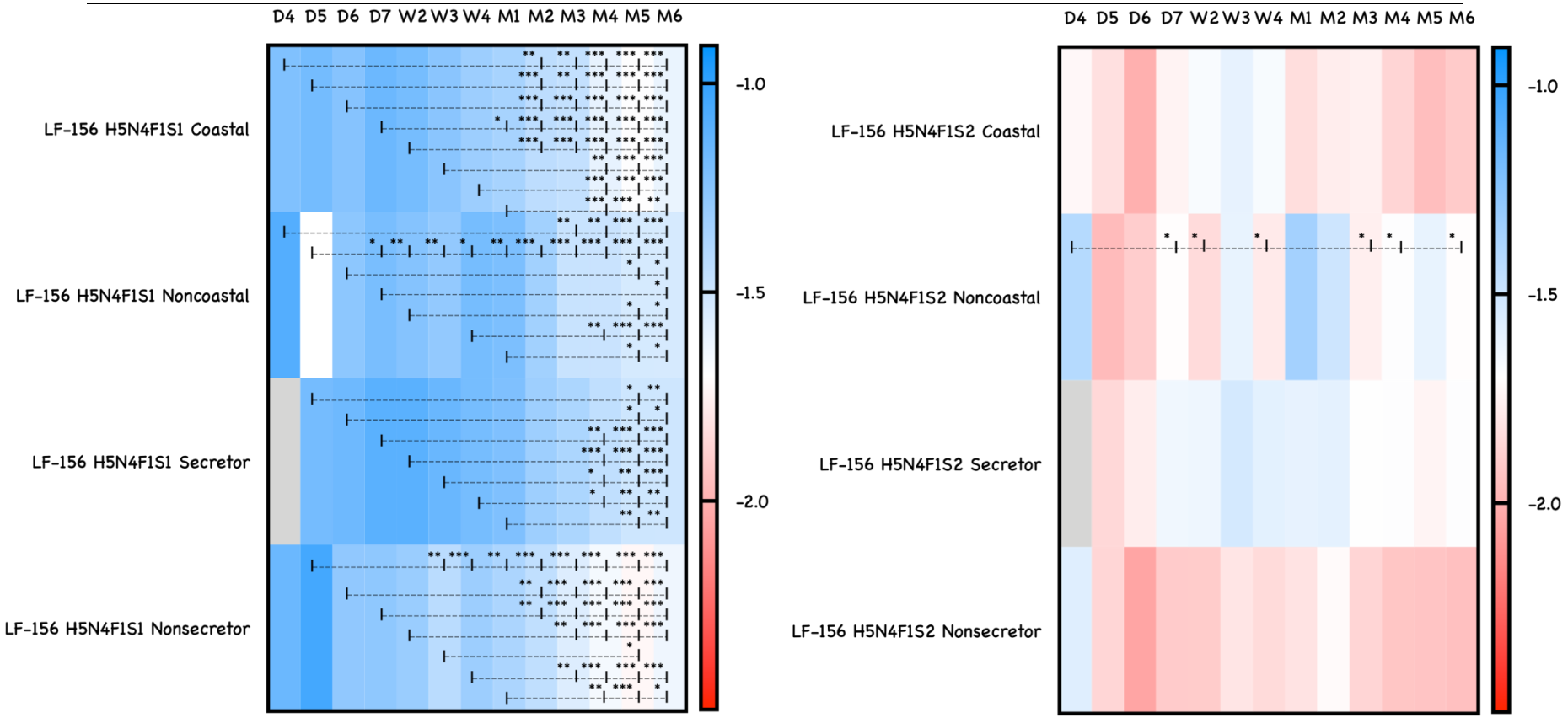

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
SC-135 H5N4F1S2 Coastal
SC-135 H5N4F1S2 Noncoastal
SC-135 H5N4F1S2 Secretor
SC-135 H5N4F1S2 Nonsecretor
0.5
0
-0.5
-1.0
-1.5
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
SC-421 H5N4F1S1 Coastal
SC-421 H5N4F1S1 Noncoastal
SC-421 H5N4F1S1 Secretor
SC-421 H5N4F1S1 Nonsecretor
*
0.5
0
-0.5
-1.0
-1.5

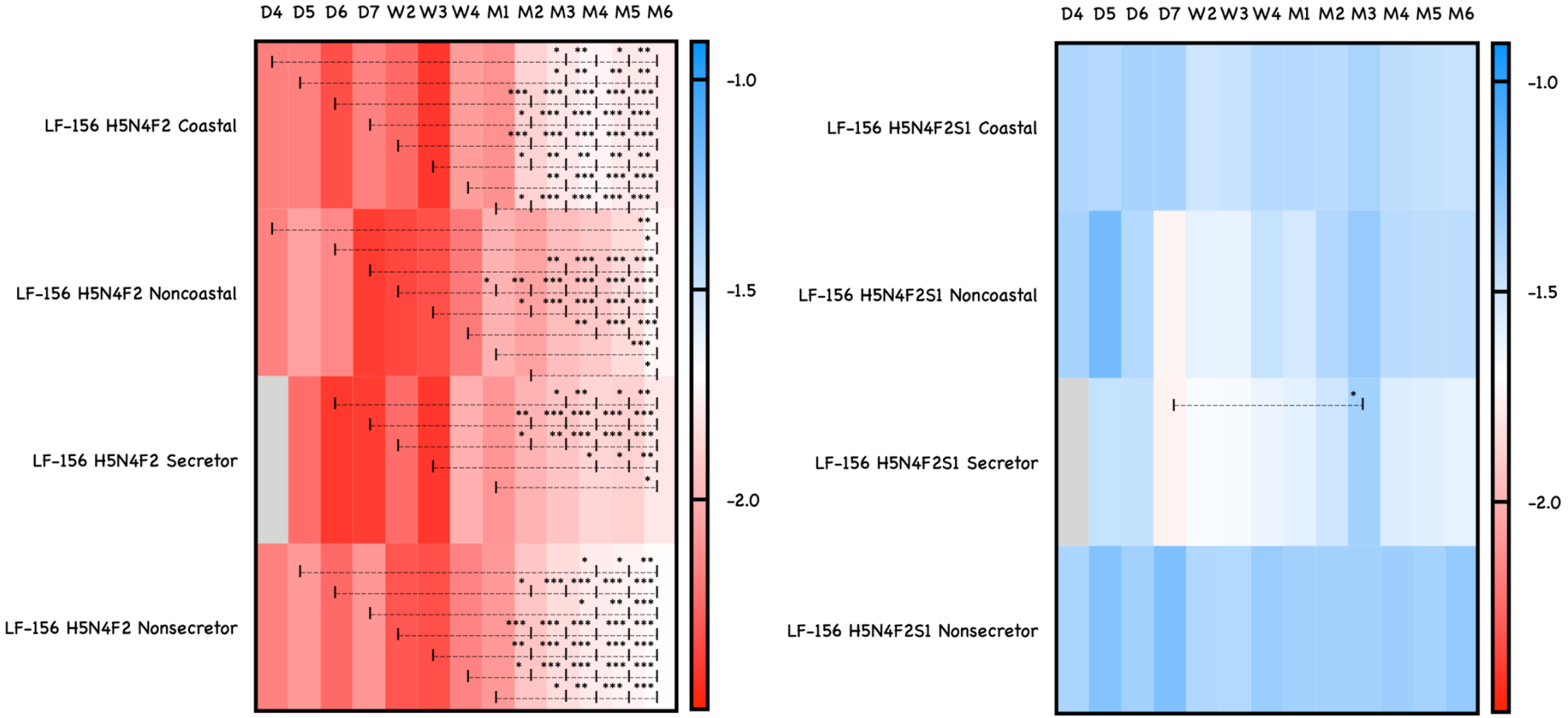

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
SC-469 H4N5F1 Coastal
SC-469 H4N5F1 Noncoastal
SC-469 H4N5F1 Secretor
SC-469 H4N5F1 Nonsecretor
0.5
0
-0.5
-1.0
-1.5
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
SC-469 H5N4F1S1 Coastal
SC-469 H5N4F1S1 Noncoastal
SC-469 H5N4F1S1 Secretor
SC-469 H5N4F1S1 Nonsecretor
0.5
0
-0.5
-1.0
-1.5

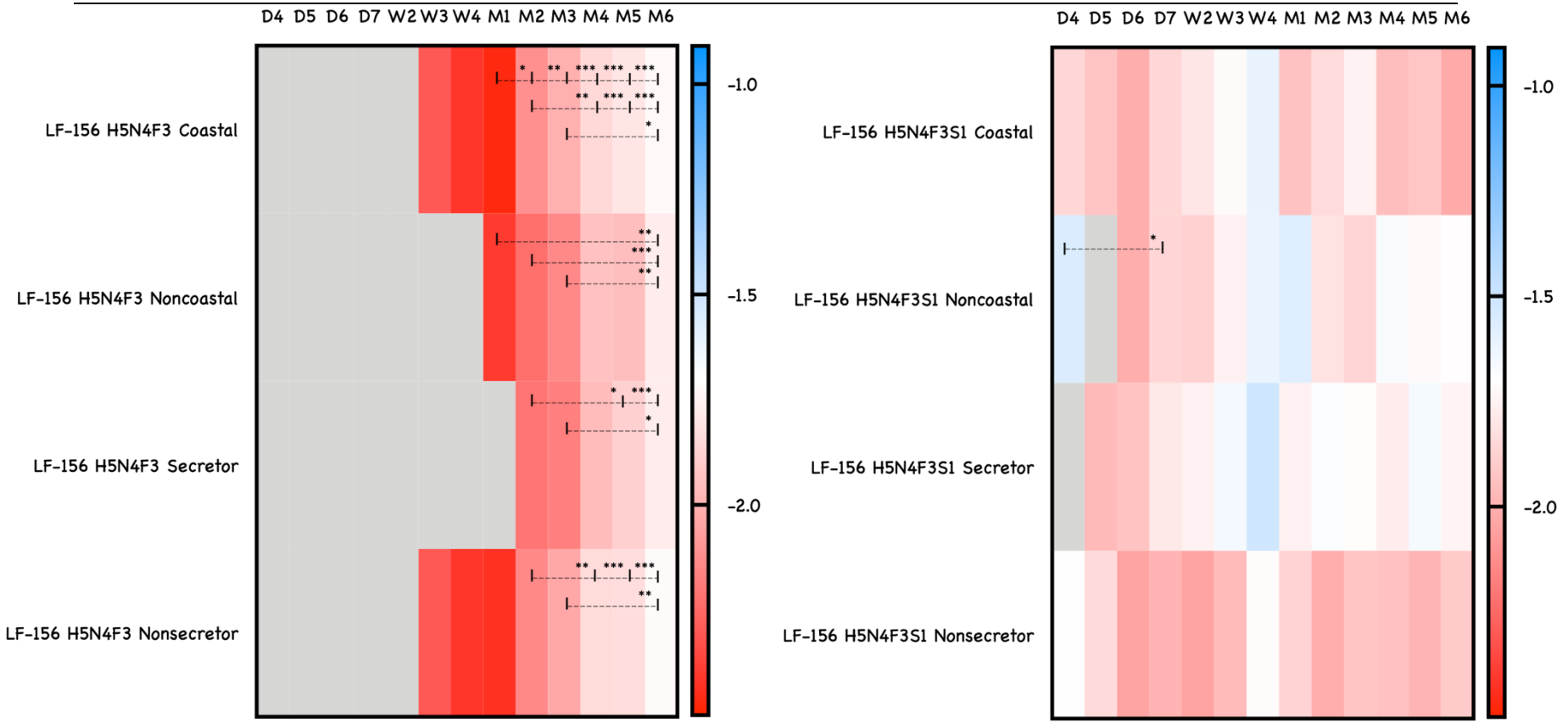

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
SC-469 H5N4F1S2 Coastal
SC-469 H5N4F1S2 Noncoastal
SC-469 H5N4F1S2 Secretor
SC-469 H5N4F1S2 Nonsecretor
0.5
0
-0.5
-1.0
-1.5
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
SC-469 H5N5F1S1 Coastal
SC-469 H5N5F1S1 Noncoastal
SC-469 H5N5F1S1 Secretor
SC-469 H5N5F1S1 Nonsecretor
0.5
0
-0.5
-1.0
-1.5

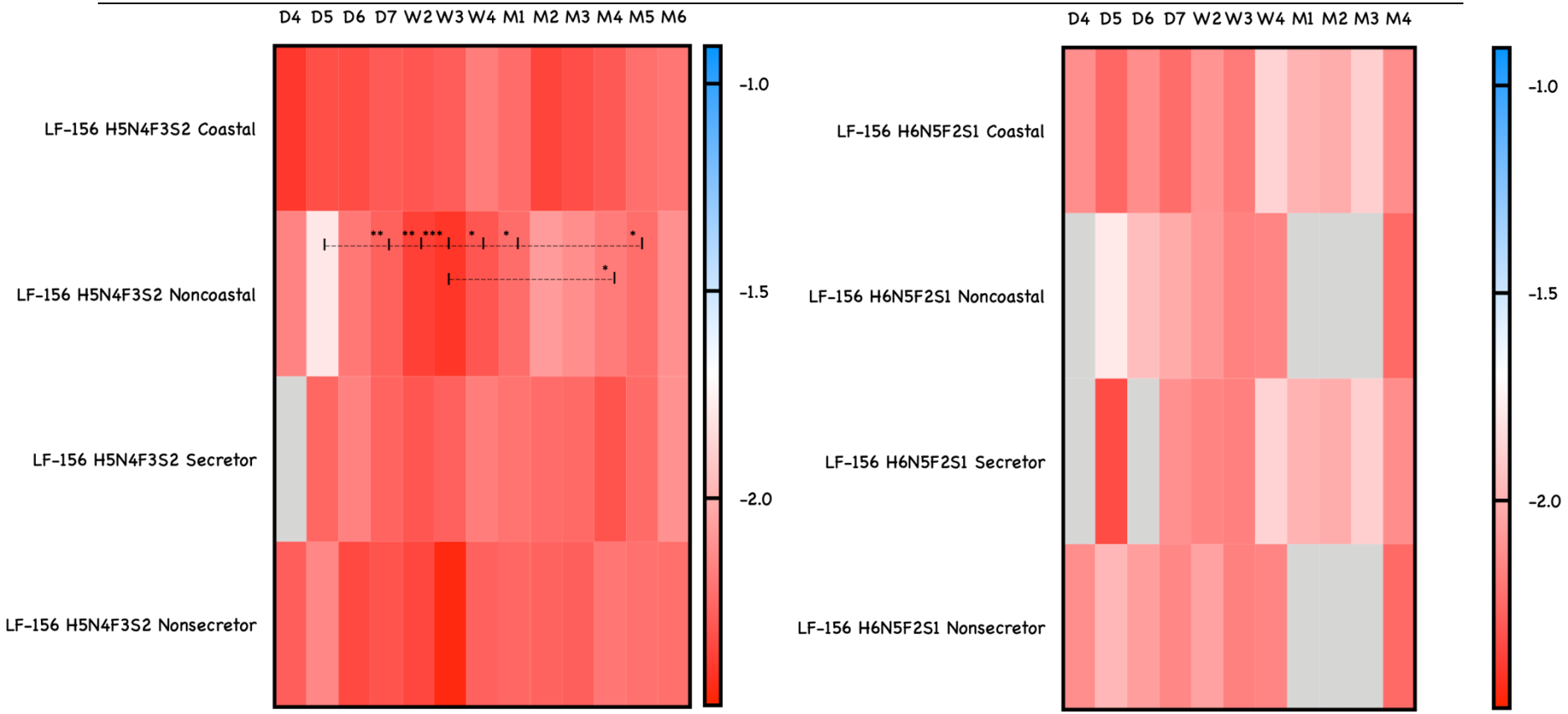

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
SC-469 H5N5F1S2 Coastal
SC-469 H5N5F1S2 Noncoastal
SC-469 H5N5F1S2 Secretor
SC-469 H5N5F1S2 Nonsecretor
0.5
0
-0.5
-1.0
-1.5
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGG H4N5_2 Coastal
IGG H4N5_2 Noncoastal
IGG H4N5_2 Secretor
IGG H4N5_2 Nonsecretor
1.5
1.0
0.5
0

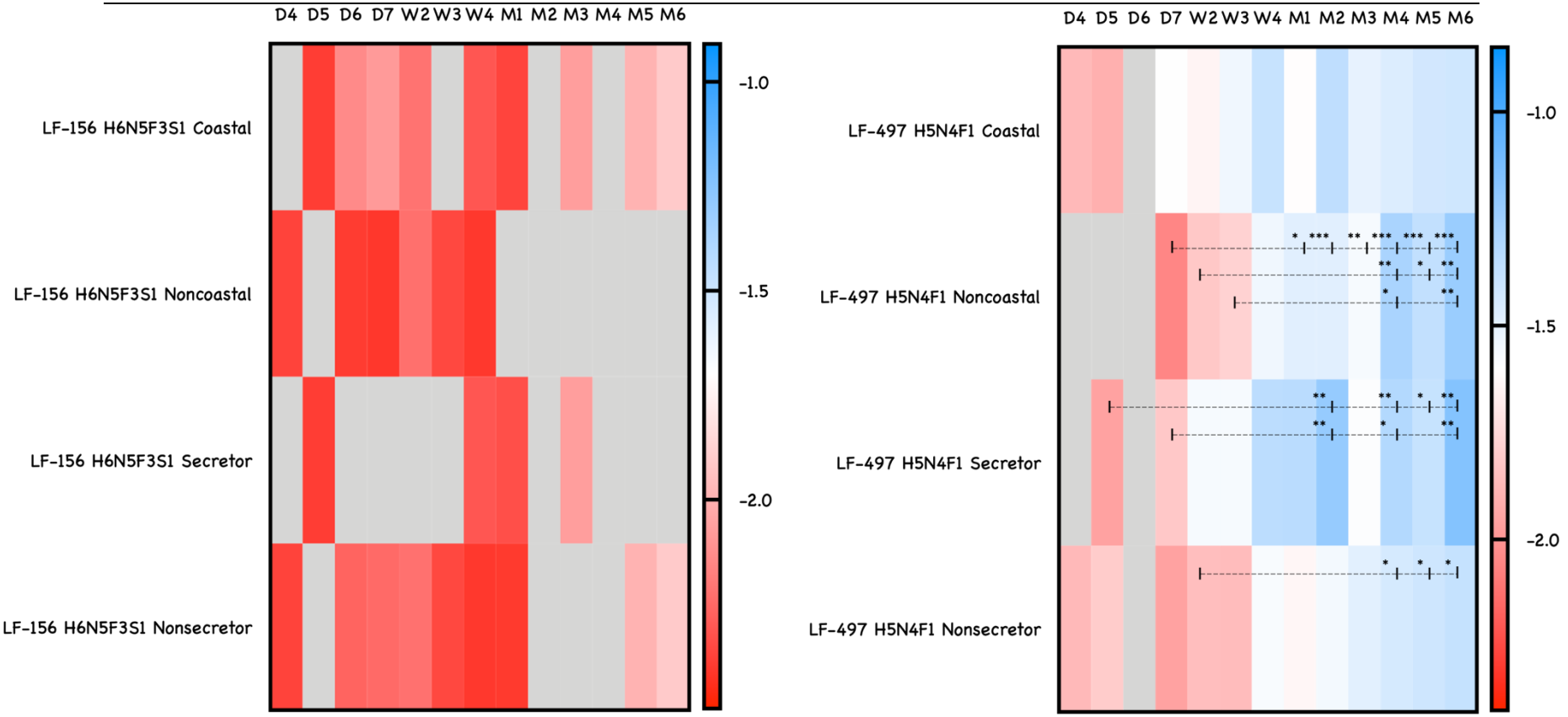

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGG H4N4F1_2 Coastal
IGG H4N4F1_2 Noncoastal
IGG H4N4F1_2 Secretor
IGG H4N4F1_2 Nonsecretor
1.5
1.0
0.5
0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F1 Coastal
LF-156 H5N4F1 Noncoastal
LF-156 H5N4F1 Secretor
LF-156 H5N4F1 Nonsecretor
*
-1.0
-1.5
-2.0

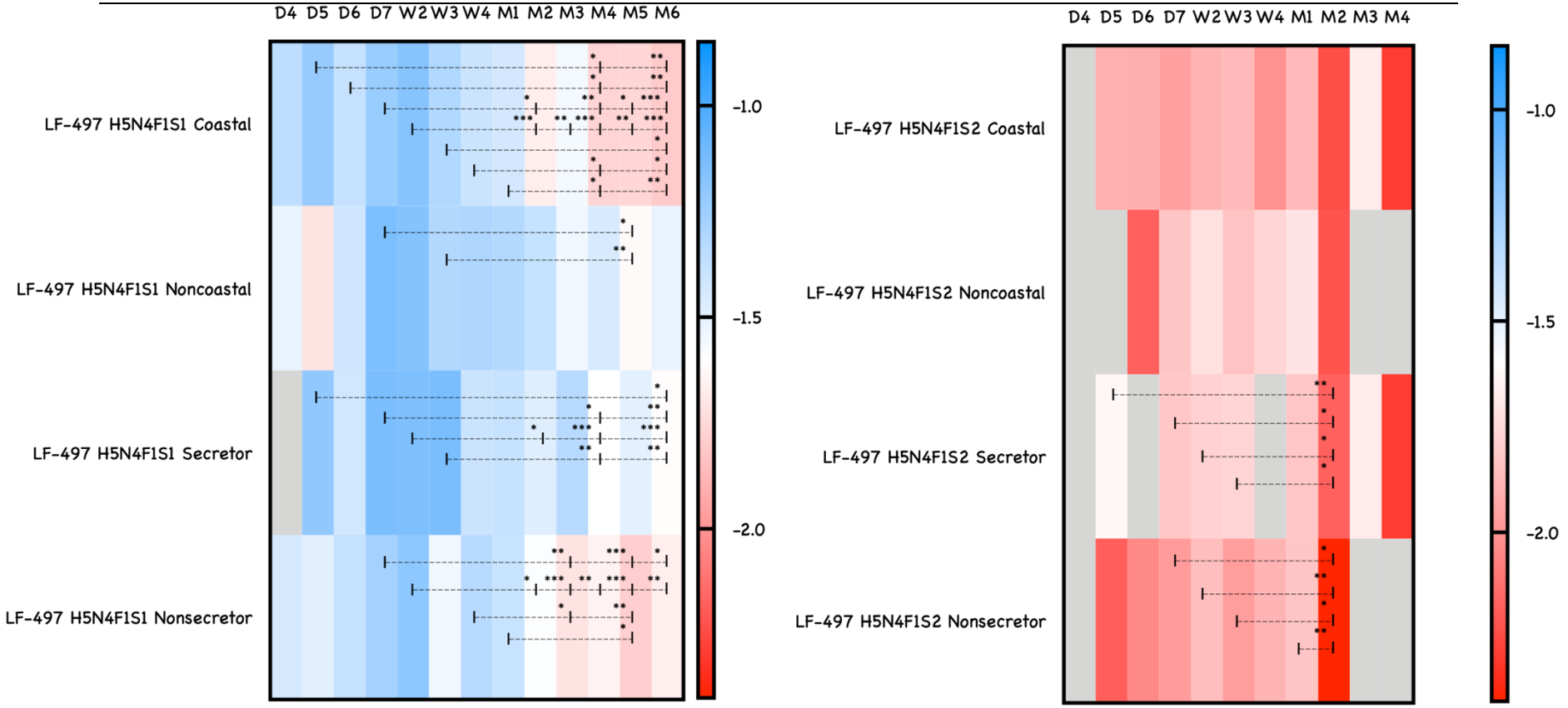

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F1S1 Coastal
LF-156 H5N4F1S1 Noncoastal
LF-156 H5N4F1S1 Secretor
LF-156 H5N4F1S1 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F1S2 Coastal
LF-156 H5N4F1S2 Noncoastal
LF-156 H5N4F1S2 Secretor
LF-156 H5N4F1S2 Nonsecretor
-1.0
-1.5
-2.0

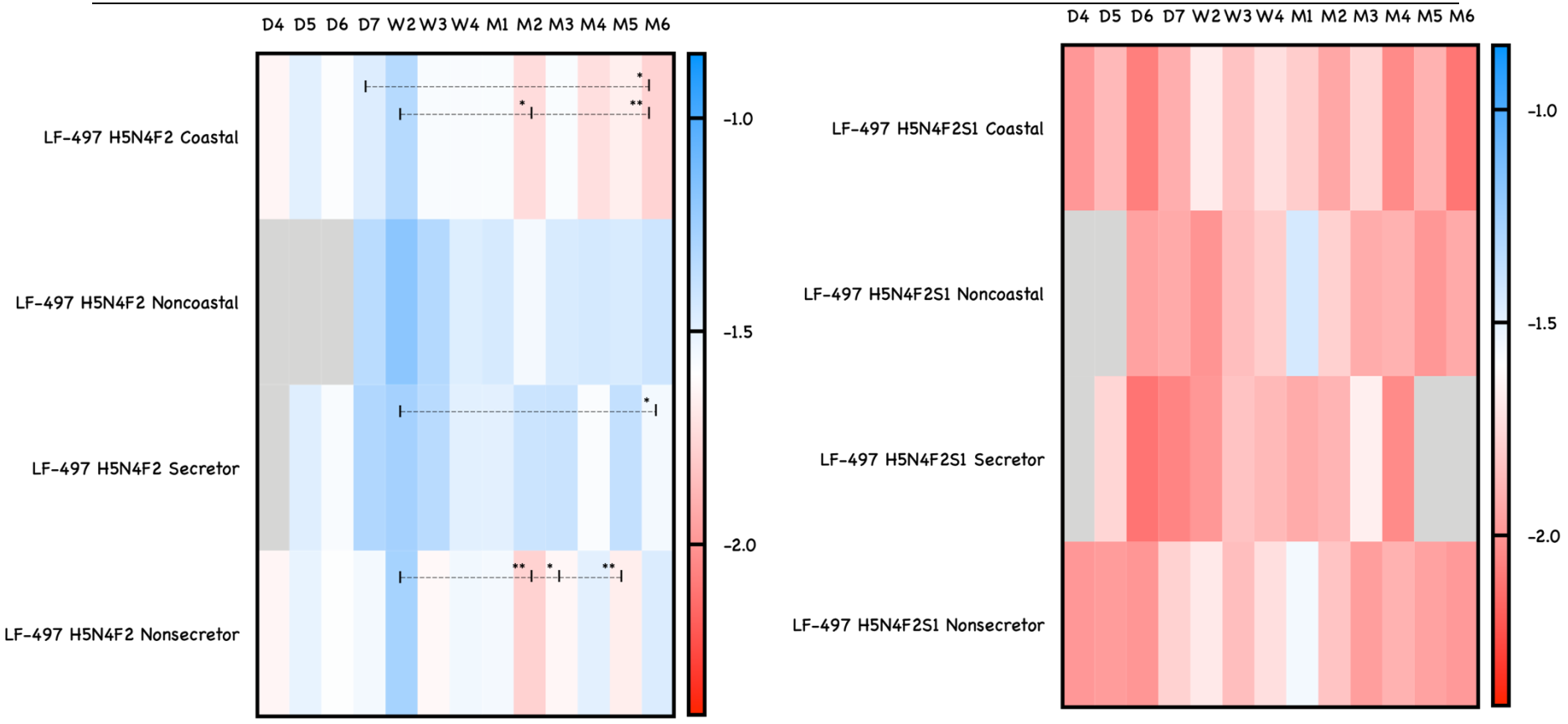

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F2 Coastal
LF-156 H5N4F2 Noncoastal
LF-156 H5N4F2 Secretor
LF-156 H5N4F2 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F2S1 Coastal
LF-156 H5N4F2S1 Noncoastal
LF-156 H5N4F2S1 Secretor
LF-156 H5N4F2S1 Nonsecretor
-1.0
-1.5
-2.0

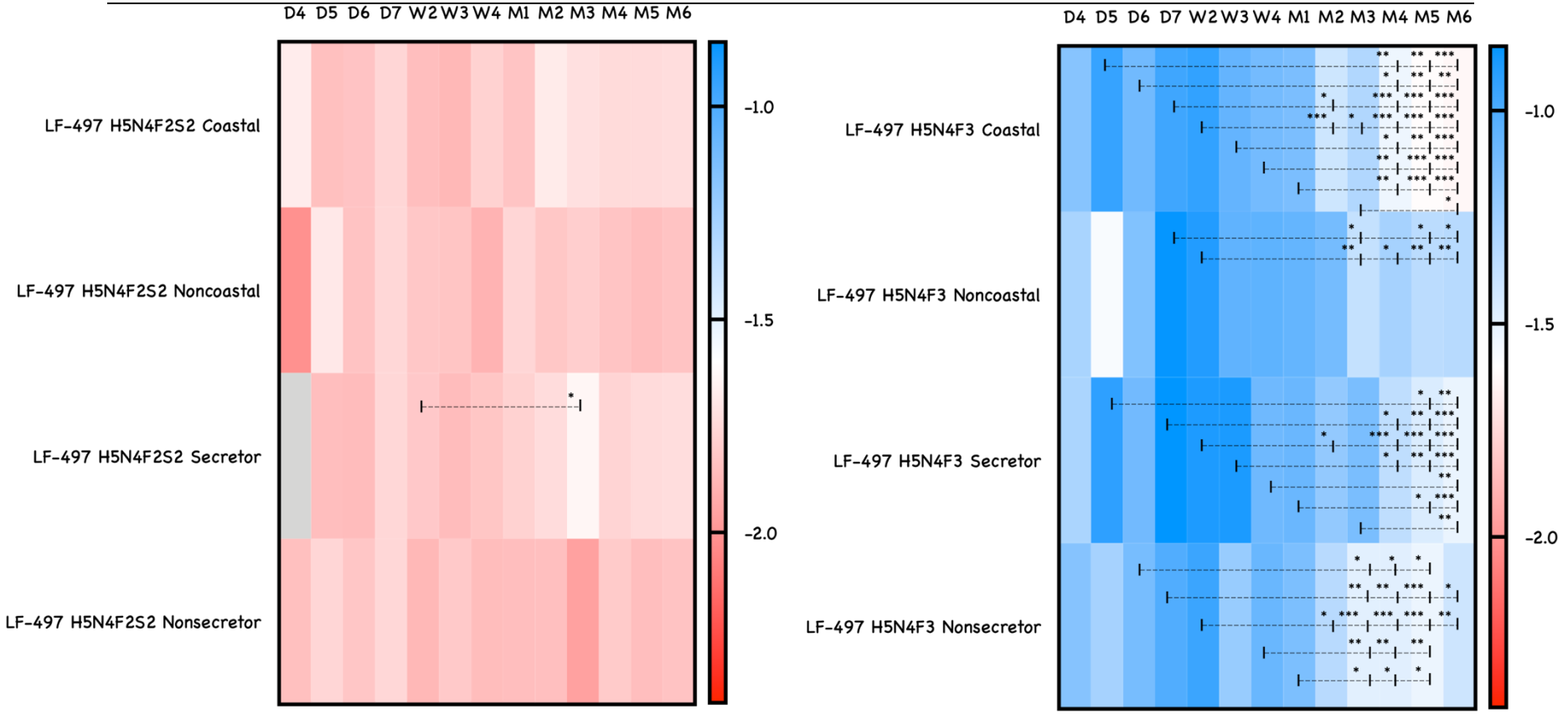

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F3 Coastal
LF-156 H5N4F3 Noncoastal
LF-156 H5N4F3 Secretor
LF-156 H5N4F3 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F3S1 Coastal
LF-156 H5N4F3S1 Noncoastal
LF-156 H5N4F3S1 Secretor
LF-156 H5N4F3S1 Nonsecretor
-1.0
-1.5
-2.0

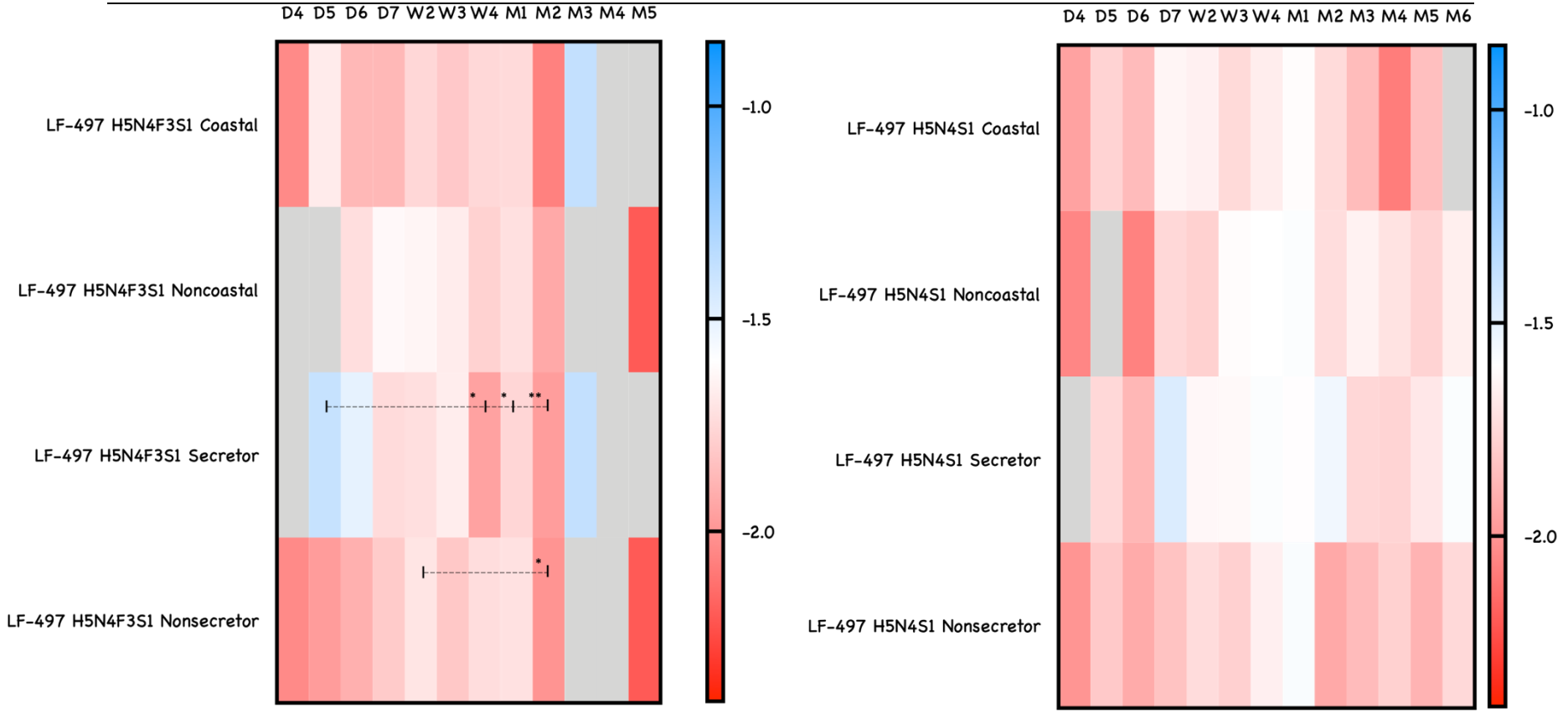

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F3S2 Coastal
LF-156 H5N4F3S2 Noncoastal
LF-156 H5N4F3S2 Secretor
LF-156 H5N4F3S2 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4
LF-156 H6N5F2S1 Coastal
LF-156 H6N5F2S1 Noncoastal
LF-156 H6N5F2S1 Secretor
LF-156 H6N5F2S1 Nonsecretor
-1.0
-1.5
-2.0

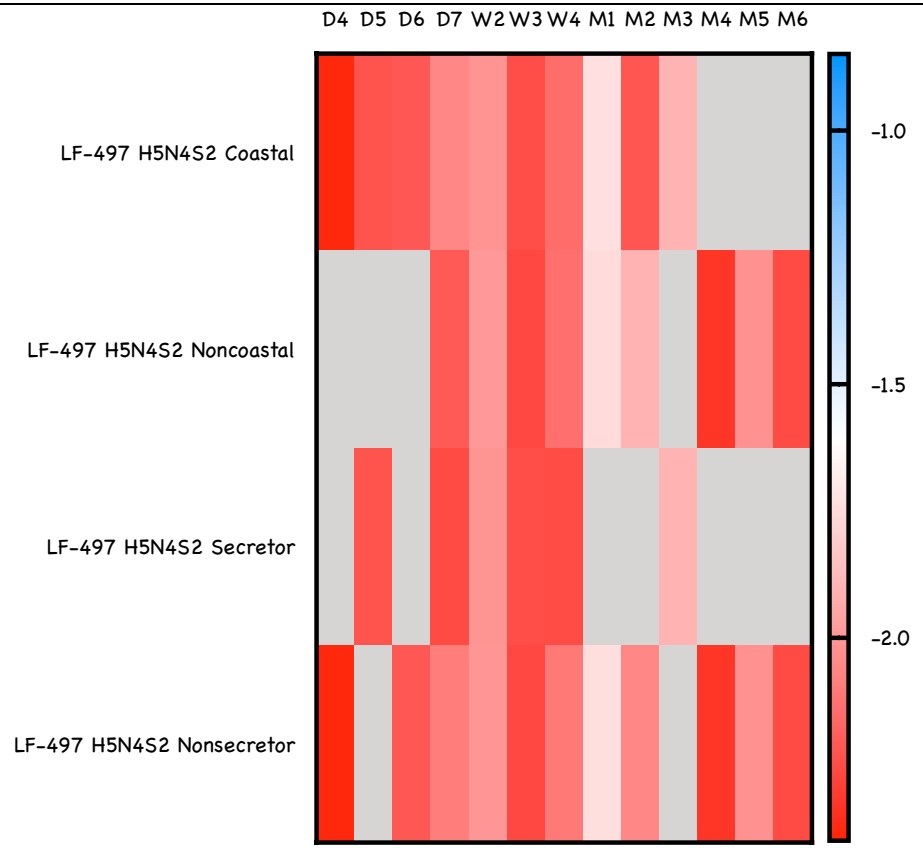

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H6N5F3S1 Coastal
LF-156 H6N5F3S1 Noncoastal
LF-156 H6N5F3S1 Secretor
LF-156 H6N5F3S1 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4F1 Coastal
LF-497 H5N4F1 Noncoastal
LF-497 H5N4F1 Secretor
LF-497 H5N4F1 Nonsecretor
-1.0
-1.5
-2.0


D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4F1S1 Coastal
LF-497 H5N4F1S1 Noncoastal
LF-497 H5N4F1S1 Secretor
LF-497 H5N4F1S1 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4
LF-497 H5N4F1S2 Coastal
LF-497 H5N4F1S2 Noncoastal
LF-497 H5N4F1S2 Secretor
LF-497 H5N4F1S2 Nonsecretor
-1.0
-1.5
-2.0


D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4F2 Coastal
LF-497 H5N4F2 Noncoastal
LF-497 H5N4F2 Secretor
LF-497 H5N4F2 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4F2S1 Coastal
LF-497 H5N4F2S1 Noncoastal
LF-497 H5N4F2S1 Secretor
LF-497 H5N4F2S1 Nonsecretor
-1.0
-1.5
-2.0

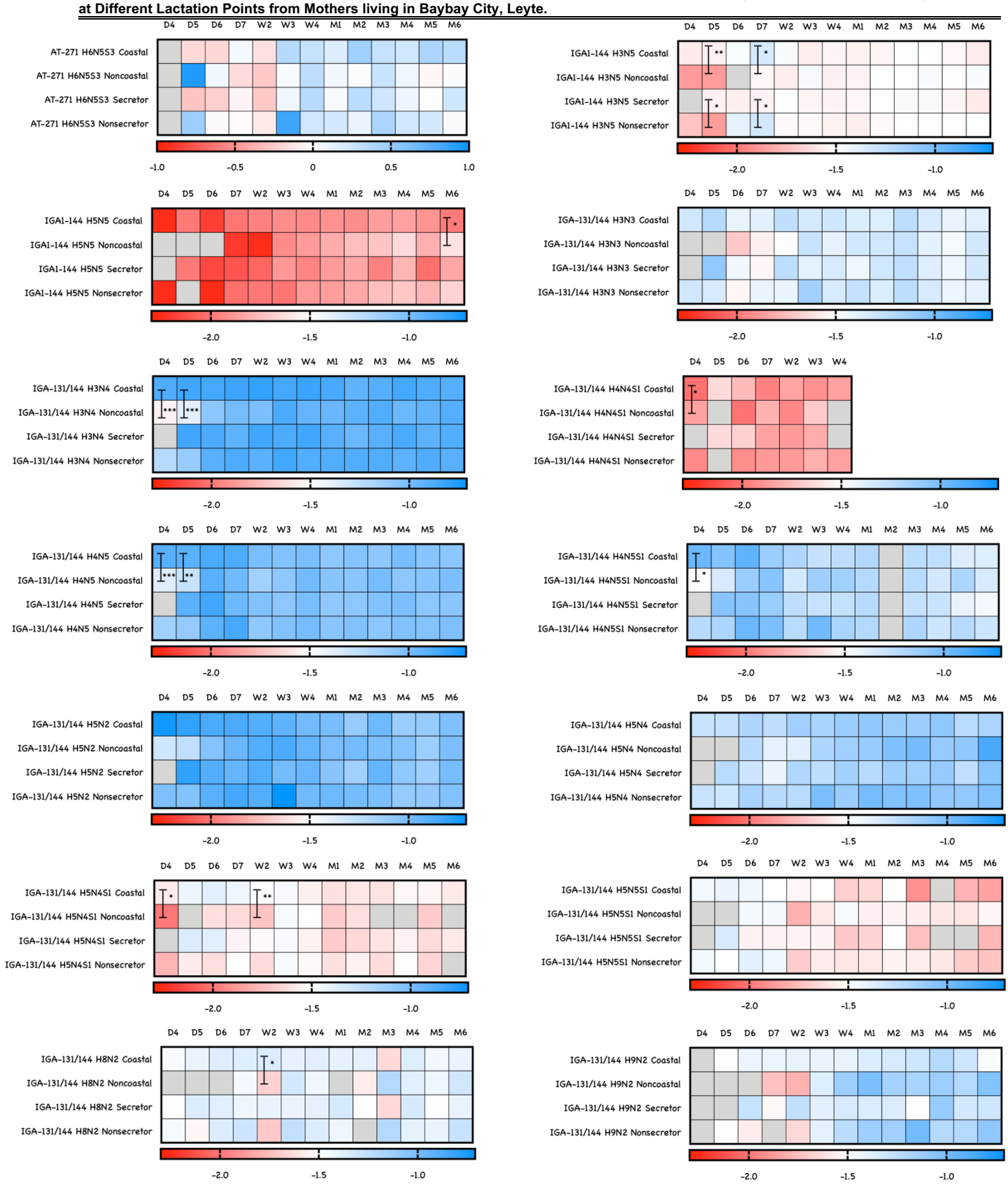

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4F2S2 Coastal
LF-497 H5N4F2S2 Noncoastal
LF-497 H5N4F2S2 Secretor
LF-497 H5N4F2S2 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4F3 Coastal
LF-497 H5N4F3 Noncoastal
LF-497 H5N4F3 Secretor
LF-497 H5N4F3 Nonsecretor
-1.0
-1.5
-2.0

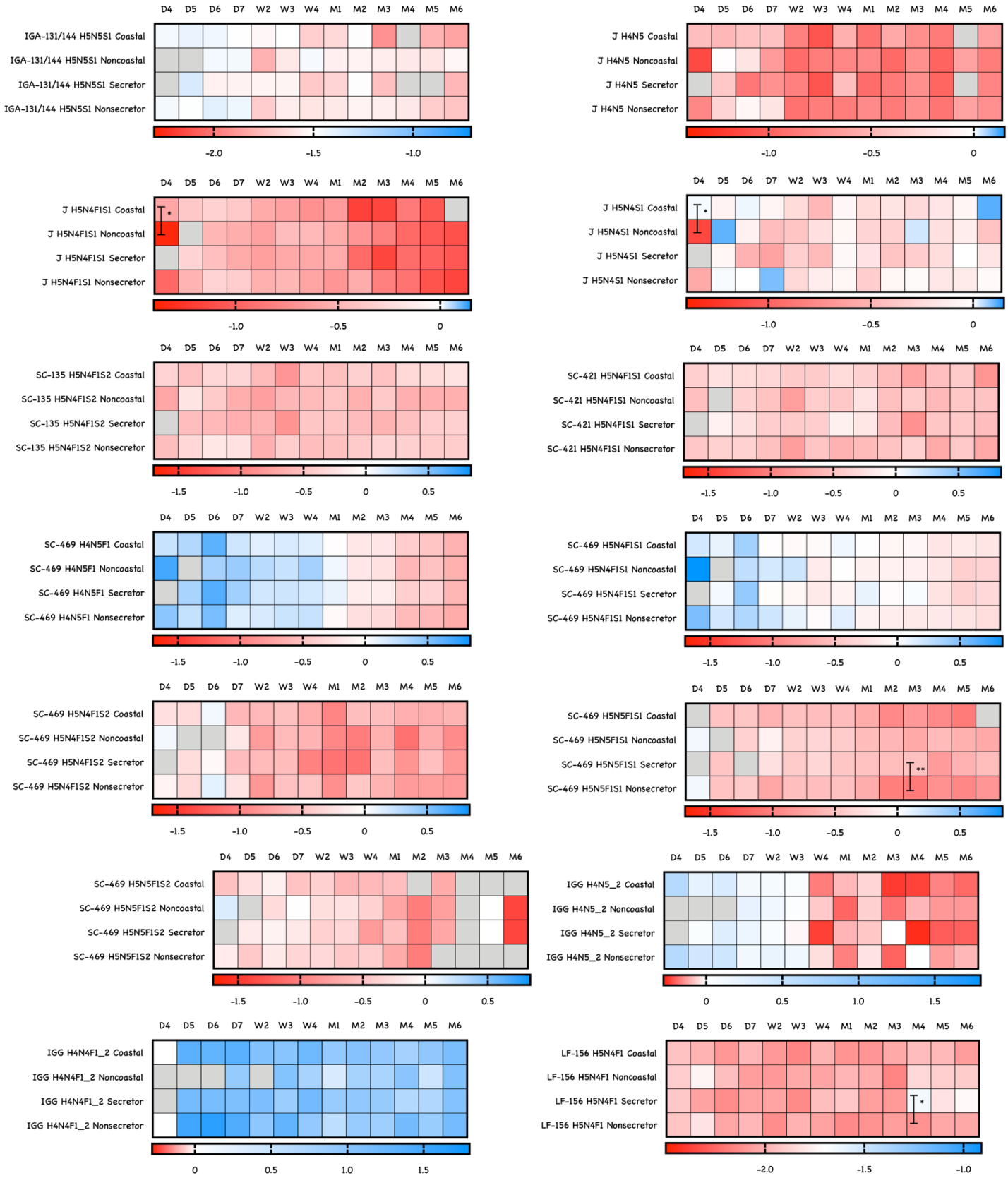

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5
LF-497 H5N4F3S1 Coastal
LF-497 H5N4F3S1 Noncoastal
LF-497 H5N4F3S1 Secretor
LF-497 H5N4F3S1 Nonsecretor
-1.0
-1.5
-2.0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4S1 Coastal
LF-497 H5N4S1 Noncoastal
LF-497 H5N4S1 Secretor
LF-497 H5N4S1 Nonsecretor
-1.0
-1.5
-2.0

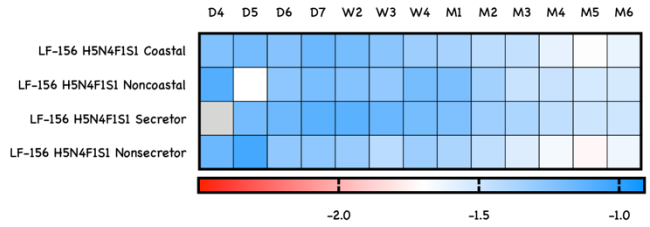


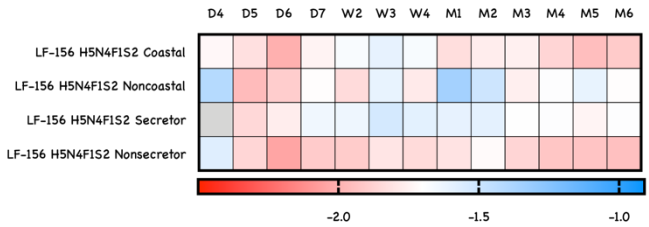
* identifies significant results at P values ≤ 0.05
** identifies highly significant results at P values ≤ 0.01
*** identifies very high significant results at P values ≤ 0.001

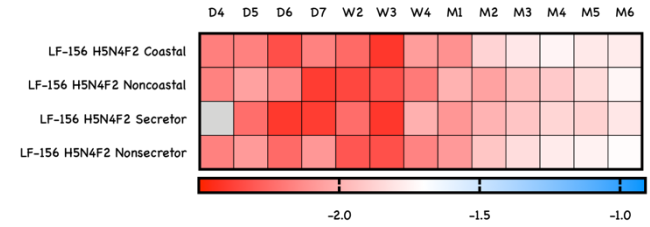
SI Table 10: Sidak's Multiple Comparisons Test on Coastal, Noncoastal, Secretor, and Nonsecretor Glycoforms of Human Milk Glycoproteins at Different Lactation Points from Mothers living in Baybay City, Leyte.

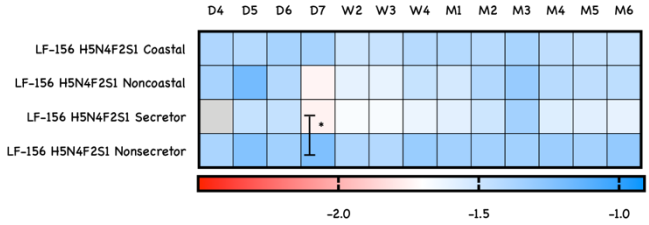


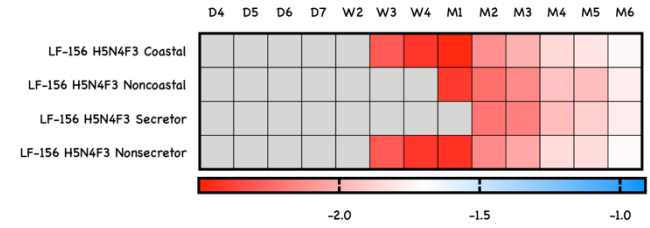

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IGA-131/144 H5N5S1 Coastal
IGA-131/144 H5N5S1 Noncoastal
IGA-131/144 H5N5S1 Secretor
IGA-131/144 H5N5S1 Nonsecretor
-2.0 -1.5 -1.0
J H4N5 Coastal
J H4N5 Noncoastal
J H4N5 Secretor
J H4N5 Nonsecretor
-1.0 -0.5 0
J H5N4F1S1 Coastal
J H5N4F1S1 Noncoastal
J H5N4F1S1 Secretor
J H5N4F1S1 Nonsecretor
-1.0 -0.5 0
J H5N4S1 Coastal
J H5N4S1 Noncoastal
J H5N4S1 Secretor
J H5N4S1 Nonsecretor
-1.0 -0.5 0
SC-135 H5N4F1S2 Coastal
SC-135 H5N4F1S2 Noncoastal
SC-135 H5N4F1S2 Secretor
SC-135 H5N4F1S2 Nonsecretor
-1.5 -1.0 -0.5 0 0.5
SC-421 H5N4F1S1 Coastal
SC-421 H5N4F1S1 Noncoastal
SC-421 H5N4F1S1 Secretor
SC-421 H5N4F1S1 Nonsecretor
-1.5 -1.0 -0.5 0 0.5
SC-469 H4N5F1 Coastal
SC-469 H4N5F1 Noncoastal
SC-469 H4N5F1 Secretor
SC-469 H4N5F1 Nonsecretor
-1.5 -1.0 -0.5 0 0.5
SC-469 H5N4F1S1 Coastal
SC-469 H5N4F1S1 Noncoastal
SC-469 H5N4F1S1 Secretor
SC-469 H5N4F1S1 Nonsecretor
-1.5 -1.0 -0.5 0 0.5
SC-469 H5N4F1S2 Coastal
SC-469 H5N4F1S2 Noncoastal
SC-469 H5N4F1S2 Secretor
SC-469 H5N4F1S2 Nonsecretor
-1.5 -1.0 -0.5 0 0.5
SC-469 H5N5F1S1 Coastal
SC-469 H5N5F1S1 Noncoastal
SC-469 H5N5F1S1 Secretor
SC-469 H5N5F1S1 Nonsecretor
-1.5 -1.0 -0.5 0 0.5
SC-469 H5N5F1S2 Coastal
SC-469 H5N5F1S2 Noncoastal
SC-469 H5N5F1S2 Secretor
SC-469 H5N5F1S2 Nonsecretor
-1.5 -1.0 -0.5 0 0.5
IGG H4N5_2 Coastal
IGG H4N5_2 Noncoastal
IGG H4N5_2 Secretor
IGG H4N5_2 Nonsecretor
0 0.5 1.0 1.5
IGG H4N4F1_2 Coastal
IGG H4N4F1_2 Noncoastal
IGG H4N4F1_2 Secretor
IGG H4N4F1_2 Nonsecretor
0 0.5 1.0 1.5
LF-156 H5N4F1 Coastal
LF-156 H5N4F1 Noncoastal
LF-156 H5N4F1 Secretor
LF-156 H5N4F1 Nonsecretor
-2.0 -1.5 -1.0

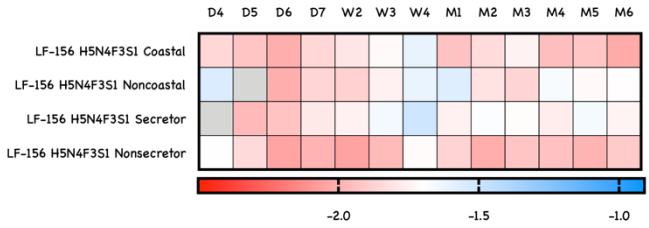

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F1S1 Coastal
LF-156 H5N4F1S1 Noncoastal
LF-156 H5N4F1S1 Secretor
LF-156 H5N4F1S1 Nonsecretor
-2.0 -1.5 -1.0

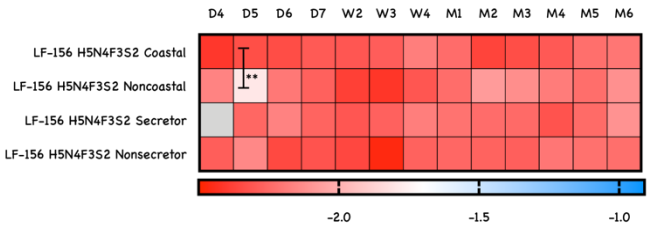

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F1S2 Coastal
LF-156 H5N4F1S2 Noncoastal
LF-156 H5N4F1S2 Secretor
LF-156 H5N4F1S2 Nonsecretor
-2.0 -1.5 -1.0

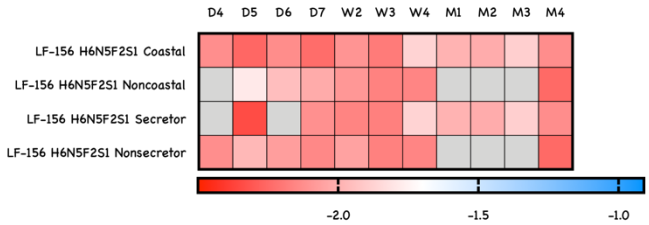

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F2 Coastal
LF-156 H5N4F2 Noncoastal
LF-156 H5N4F2 Secretor
LF-156 H5N4F2 Nonsecretor
-2.0 -1.5 -1.0

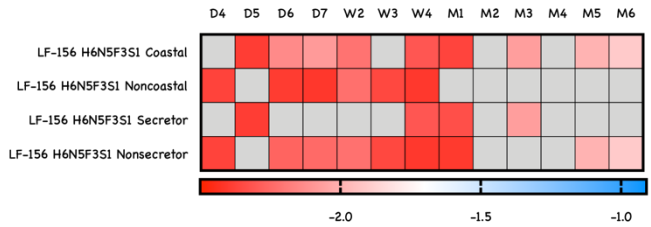

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F2S1 Coastal
LF-156 H5N4F2S1 Noncoastal
LF-156 H5N4F2S1 Secretor
LF-156 H5N4F2S1 Nonsecretor
*
-2.0 -1.5 -1.0

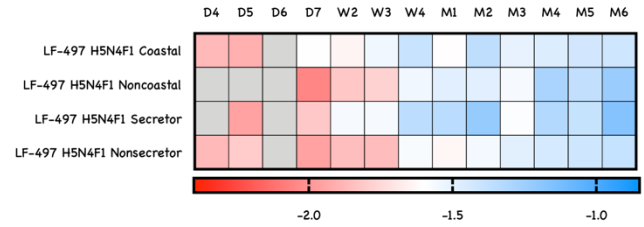

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F3 Coastal
LF-156 H5N4F3 Noncoastal
LF-156 H5N4F3 Secretor
LF-156 H5N4F3 Nonsecretor
-2.0 -1.5 -1.0

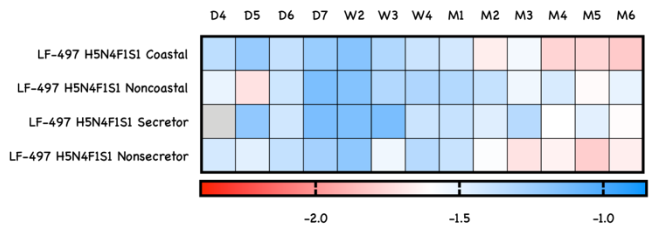

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F3S1 Coastal
LF-156 H5N4F3S1 Noncoastal
LF-156 H5N4F3S1 Secretor
LF-156 H5N4F3S1 Nonsecretor
-2.0 -1.5 -1.0

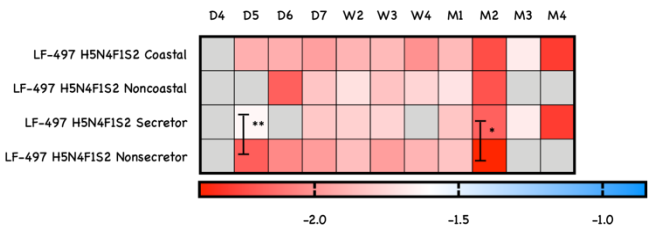

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H5N4F3S2 Coastal
LF-156 H5N4F3S2 Noncoastal
LF-156 H5N4F3S2 Secretor
LF-156 H5N4F3S2 Nonsecretor
**
-2.0 -1.5 -1.0

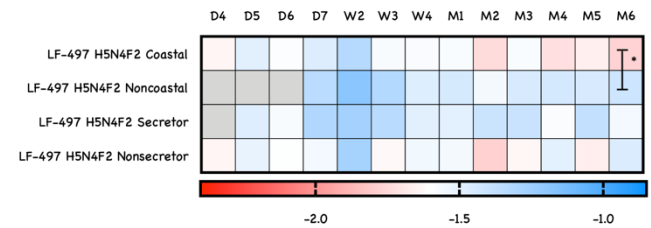

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4
LF-156 H6N5F2S1 Coastal
LF-156 H6N5F2S1 Noncoastal
LF-156 H6N5F2S1 Secretor
LF-156 H6N5F2S1 Nonsecretor
-2.0 -1.5 -1.0

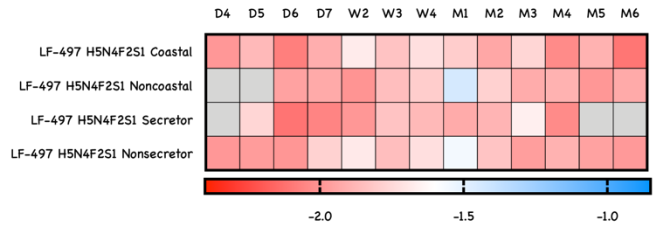

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-156 H6N5F3S1 Coastal
LF-156 H6N5F3S1 Noncoastal
LF-156 H6N5F3S1 Secretor
LF-156 H6N5F3S1 Nonsecretor
-2.0 -1.5 -1.0

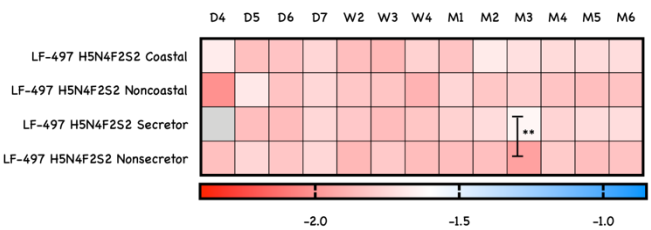

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4F1 Coastal
LF-497 H5N4F1 Noncoastal
LF-497 H5N4F1 Secretor
LF-497 H5N4F1 Nonsecretor
-2.0 -1.5 -1.0

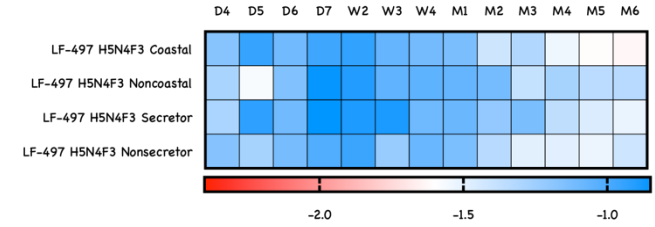

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4F1S1 Coastal
LF-497 H5N4F1S1 Noncoastal
LF-497 H5N4F1S1 Secretor
LF-497 H5N4F1S1 Nonsecretor
-2.0 -1.5 -1.0

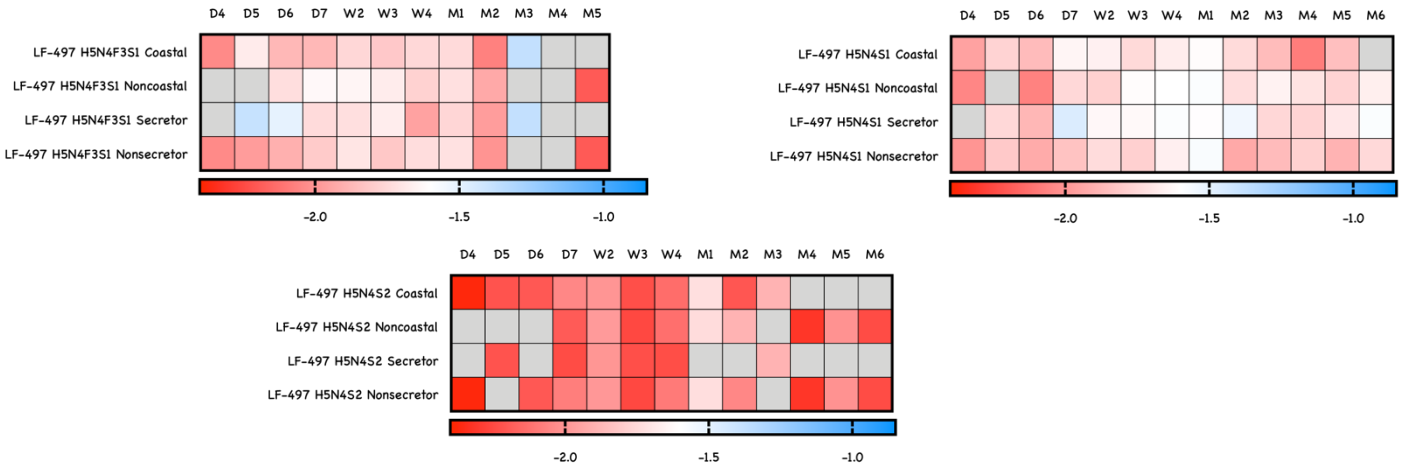

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4
LF-497 H5N4F1S2 Coastal
LF-497 H5N4F1S2 Noncoastal
LF-497 H5N4F1S2 Secretor
LF-497 H5N4F1S2 Nonsecretor
**
*
-2.0 -1.5 -1.0


D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4F2 Coastal
LF-497 H5N4F2 Noncoastal
LF-497 H5N4F2 Secretor
LF-497 H5N4F2 Nonsecretor
*
-2.0 -1.5 -1.0


D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4F2S1 Coastal
LF-497 H5N4F2S1 Noncoastal
LF-497 H5N4F2S1 Secretor
LF-497 H5N4F2S1 Nonsecretor
-2.0 -1.5 -1.0


D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4F2S2 Coastal
LF-497 H5N4F2S2 Noncoastal
LF-497 H5N4F2S2 Secretor
LF-497 H5N4F2S2 Nonsecretor
**
-2.0 -1.5 -1.0


D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LF-497 H5N4F3 Coastal
LF-497 H5N4F3 Noncoastal
LF-497 H5N4F3 Secretor
LF-497 H5N4F3 Nonsecretor
-2.0 -1.5 -1.0



* identifies significant results at P values ≤ 0.05
** identifies highly significant results at P values ≤ 0.01
*** identifies very high significant results at P values ≤ 0.001

SI Table 11: HMO Profile of Filipino Mothers Living in Baybay City, Leyte.

Formula	Compound	Type	Experimental Mass (Da)	RT	Annotation
C18 H34 O15	2_0_1_0	Fuc	490.188	6.88	2_0_1_0b
C18 H34 O15	2_0_1_0	Fuc	490.188	12.38	2_0_1_0c
C18 H34 O15	2_0_1_0	Fuc	490.189	14.83	2_0_1_0d
C18 H34 O15	2_0_1_0	Fuc	490.189	12.83	2'FL
C18 H34 O15	2_0_1_0	Fuc	490.189	1.55	3'FL
C24 H44 O19	2_0_2_0	Fuc	636.241	15.47	2_0_2_0b
C24 H44 O19	2_0_2_0	Fuc	636.242	15.83	2_0_2_0c
C24 H44 O19	2_0_2_0	Fuc	636.244	17.17	2_0_2_0e
C24 H44 O19	2_0_2_0	Fuc	636.245	13.88	2_0_2_0a
C24 H44 O19	2_0_2_0	Fuc	636.246	16.3	2_0_2_0d
C24 H44 O19	2_0_2_0	Fuc	636.246	19.66	2_0_2_0f
C24 H44 O19	2_0_2_0	Fuc	636.246	14.83	LDFT
C24 H44 O20	3_0_1_0	Fuc	652.242	19.02	3_0_1_0a
C32 H57 N O25	3_1_1_0	Fuc	855.32	17.28	3_1_1_0b
C32 H57 N O25	3_1_1_0	Fuc	855.322	12.03	3_1_1_0a
C32 H57 N O25	3_1_1_0	Fuc	855.323	17.46	3_1_1_0c
C32 H57 N O25	3_1_1_0	Fuc	855.323	12.44	LNFP II
C32 H57 N O25	3_1_1_0	Fuc	855.323	14.67	LNFP III/I
C38 H67 N O29	3_1_2_0	Fuc	1001.362	20.07	3_1_2_0f
C38 H67 N O29	3_1_2_0	Fuc	1001.378	15.73	3_1_2_0d
C38 H67 N O29	3_1_2_0	Fuc	1001.378	15.87	3_1_2_0e
C38 H67 N O29	3_1_2_0	Fuc	1001.379	14.64	3_1_2_0c
C38 H67 N O29	3_1_2_0	Fuc	1001.38	11.95	3_1_2_0a
C38 H67 N O29	3_1_2_0	Fuc	1001.38	12.38	3_1_2_0b
C38 H67 N O29	3_1_2_0	Fuc	1001.381	11.92	LNDFH I
C38 H67 N O30	4_1_1_0	Fuc	1017.375	14.25	4_1_1_0a
C38 H67 N O30	4_1_1_0	Fuc	1017.375	19.44	4_1_1_0b
C40 H70 N2 O30	3_2_1_0	Fuc	1058.363	1.19	3_2_1_0a
C40 H70 N2 O30	3_2_1_0	Fuc	1058.392	1.29	3_2_1_0b
C40 H70 N2 O30	3_2_1_0	Fuc	1058.398	14.18	3_2_1_0d
C40 H70 N2 O30	3_2_1_0	Fuc	1058.401	13.25	3_2_1_0c
C40 H70 N2 O30	3_2_1_0	Fuc	1058.401	15.38	3_2_1_0e
C40 H70 N2 O30	3_2_1_0	Fuc	1058.401	15.67	3_2_1_0f
C40 H70 N2 O30	3_2_1_0	Fuc	1058.402	16.43	3_2_1_0g
C46 H80 N2 O34	3_2_2_0	Fuc	1204.458	14.67	3_2_2_0b
C46 H80 N2 O34	3_2_2_0	Fuc	1204.466	1.18	3_2_2_0a
C46 H80 N2 O35	4_2_1_0	Fuc	1220.452	16.16	4_2_1_0b
C46 H80 N2 O35	4_2_1_0	Fuc	1220.452	18.61	4_2_1_0c
C46 H80 N2 O35	4_2_1_0	Fuc	1220.452	17.37	IFLNH III
C46 H80 N2 O35	4_2_1_0	Fuc	1220.453	19.21	IFLNH I
C46 H80 N2 O35	4_2_1_0	Fuc	1220.454	15.46	4_2_1_0a
C46 H80 N2 O35	4_2_1_0	Fuc	1220.454	19.61	4_2_1_0d
C46 H80 N2 O35	4_2_1_0	Fuc	1220.454	17.12	MFLNH III/I
C46 H80 N2 O35	4_2_1_0	Fuc	1220.455	16.43	MFpLNH IV
C52 H90 N2 O39	4_2_2_0	Fuc	1366.51	18.83	4_2_2_0b
C52 H90 N2 O39	4_2_2_0	Fuc	1366.511	18.25	4_2_2_0a
C52 H90 N2 O39	4_2_2_0	Fuc	1366.511	18.28	DFLNHc
C52 H90 N2 O39	4_2_2_0	Fuc	1366.513	15.72	DFLNHa
C52 H90 N2 O39	4_2_2_0	Fuc	1366.513	14.61	DFLNHb
C52 H90 N2 O39	4_2_2_0	Fuc	1366.513	14	DFpLNH II
C58 H100 N2 O43	4_2_3_0	Fuc	1512.554	19.06	4_2_3_0e
C58 H100 N2 O43	4_2_3_0	Fuc	1512.554	20.98	4_2_3_0g
C58 H100 N2 O43	4_2_3_0	Fuc	1512.555	19.86	4_2_3_0f
C58 H100 N2 O43	4_2_3_0	Fuc	1512.568	14.02	4_2_3_0b
C58 H100 N2 O43	4_2_3_0	Fuc	1512.568	18.47	4_2_3_0d
C58 H100 N2 O43	4_2_3_0	Fuc	1512.57	13.87	4_2_3_0a
C58 H100 N2 O43	4_2_3_0	Fuc	1512.57	14.55	4_2_3_0c
C60 H103 N3 O45	5_3_1_0	Fuc	1585.587	18.46	5_3_1_0b
C60 H103 N3 O45	5_3_1_0	Fuc	1585.587	18.67	5_3_1_0c
C60 H103 N3 O45	5_3_1_0	Fuc	1585.587	19.69	FLNO
C60 H103 N3 O45	5_3_1_0	Fuc	1585.588	18.05	5_3_1_0a
C60 H103 N3 O45	5_3_1_0	Fuc	1585.588	19.11	5_3_1_0d
C60 H103 N3 O45	5_3_1_0	Fuc	1585.588	20.05	5_3_1_0e
C60 H103 N3 O45	5_3_1_0	Fuc	1585.588	20.27	5_3_1_0f
C60 H103 N3 O45	5_3_1_0	Fuc	1585.588	21.03	5_3_1_0g

Formula	Compound	Type	Experimental Mass (Da)	RT	Annotation
C66 H113 N3 O49	5_3_2_0	Fuc	1731.632	21.65	5_3_2_0e
C66 H113 N3 O49	5_3_2_0	Fuc	1731.638	21.35	5_3_2_0d
C66 H113 N3 O49	5_3_2_0	Fuc	1731.646	19.2	DFLNnO I/DFLNO II
C66 H113 N3 O49	5_3_2_0	Fuc	1731.646	18.1	DFLNO I
C66 H113 N3 O49	5_3_2_0	Fuc	1731.647	16.68	5_3_2_0a
C66 H113 N3 O49	5_3_2_0	Fuc	1731.647	17.31	5_3_2_0b
C66 H113 N3 O49	5_3_2_0	Fuc	1731.647	17.46	5_3_2_0c
C66 H113 N3 O49	5_3_2_0	Fuc	1731.647	18.25	DFLNnO II
C66 H113 N3 O50	6_3_1_0	Fuc	1747.64	19.8	6_3_1_0b
C66 H113 N3 O50	6_3_1_0	Fuc	1747.643	18.9	6_3_1_0a
C72 H123 N3 O53	5_3_3_0	Fuc	1877.69	20.8	5_3_3_0d
C72 H123 N3 O53	5_3_3_0	Fuc	1877.69	21.13	5_3_3_0e
C72 H123 N3 O53	5_3_3_0	Fuc	1877.69	21.55	5_3_3_0f
C72 H123 N3 O53	5_3_3_0	Fuc	1877.706	15.86	5_3_3_0a
C72 H123 N3 O53	5_3_3_0	Fuc	1877.706	17.17	5_3_3_0b
C72 H123 N3 O53	5_3_3_0	Fuc	1877.706	18.56	5_3_3_0c
C72 H123 N3 O53	5_3_3_0	Fuc	1877.706	17.95	TFiLNO
C74 H126 N4 O55	6_4_1_0	Fuc	1950.721	20.95	6_4_1_0e
C74 H126 N4 O55	6_4_1_0	Fuc	1950.721	21.12	6_4_1_0f
C74 H126 N4 O55	6_4_1_0	Fuc	1950.723	20.14	6_4_1_0b
C74 H126 N4 O55	6_4_1_0	Fuc	1950.723	20.78	6_4_1_0d
C74 H126 N4 O55	6_4_1_0	Fuc	1950.724	19.73	6_4_1_0a
C74 H126 N4 O55	6_4_1_0	Fuc	1950.725	20.45	6_4_1_0c
C78 H133 N3 O57	5_3_4_0	Fuc	2023.761	17.05	Tetra-iso-LNO
C80 H136 N4 O59	6_4_2_0	Fuc	2096.767	21.76	6_4_2_0f
C80 H136 N4 O59	6_4_2_0	Fuc	2096.781	20.38	6_4_2_0e
C80 H136 N4 O59	6_4_2_0	Fuc	2096.782	19.85	6_4_2_0c
C80 H136 N4 O59	6_4_2_0	Fuc	2096.783	19.56	6_4_2_0b
C80 H136 N4 O59	6_4_2_0	Fuc	2096.783	20.08	6_4_2_0d
C80 H136 N4 O59	6_4_2_0	Fuc	2096.784	19.1	6_4_2_0a
C84 H143 N3 O61	5_3_5_0	Fuc	2169.801	21.3	5_3_5_0b
C84 H143 N3 O61	5_3_5_0	Fuc	2169.802	21.1	5_3_5_0a
C86 H146 N4 O63	6_4_3_0	Fuc	2242.824	21.74	6_4_3_0f
C86 H146 N4 O63	6_4_3_0	Fuc	2242.826	21.35	6_4_3_0g
C86 H146 N4 O63	6_4_3_0	Fuc	2242.84	19.76	6_4_3_0e
C86 H146 N4 O63	6_4_3_0	Fuc	2242.841	18.1	6_4_3_0a
C86 H146 N4 O63	6_4_3_0	Fuc	2242.841	18.66	6_4_3_0b
C86 H146 N4 O63	6_4_3_0	Fuc	2242.841	19.41	6_4_3_0d
C86 H146 N4 O63	6_4_3_0	Fuc	2242.842	18.89	6_4_3_0c
C92 H156 N4 O67	6_4_4_0	Fuc	2388.883	21.3	6_4_4_0c
C92 H156 N4 O67	6_4_4_0	Fuc	2388.885	21.14	6_4_4_0b
C92 H156 N4 O67	6_4_4_0	Fuc	2388.899	18.21	6_4_4_0a
C43 H74 N2 O33	3_1_1_1	Fuc Sia	1146.416	19.26	3_1_1_1a
C51 H87 N3 O38	3_2_1_1	Fuc Sia	1349.494	19.13	3_2_1_1a
C51 H87 N3 O38	3_2_1_1	Fuc Sia	1349.502	19.89	3_2_1_1b
C51 H87 N3 O38	3_2_1_1	Fuc Sia	1349.502	20.4	3_2_1_1c
C57 H97 N3 O43	4_2_1_1	Fuc Sia	1511.55	19.07	FS-LNH III
C57 H97 N3 O43	4_2_1_1	Fuc Sia	1511.551	19.88	FS-LNH
C57 H97 N3 O43	4_2_1_1	Fuc Sia	1511.551	20.98	FS-LNH I
C57 H97 N3 O43	4_2_1_1	Fuc Sia	1511.552	21.92	FS-LNnH I
C63 H107 N3 O47	4_2_2_1	Fuc Sia	1657.592	23.2	DFS-LNnH
C63 H107 N3 O47	4_2_2_1	Fuc Sia	1657.611	21.67	DFS-LNH
C71 H120 N4 O53	5_3_1_1	Fuc Sia	1876.687	21.55	5_3_1_1a
C71 H120 N4 O53	5_3_1_1	Fuc Sia	1876.687	21.12	FS-LNO
C71 H120 N4 O53	5_3_1_1	Fuc Sia	1876.689	21.86	5_3_1_1b
C85 H143 N5 O63	6_4_1_1	Fuc Sia	2241.822	21.35	6_4_1_1a
C85 H143 N5 O63	6_4_1_1	Fuc Sia	2241.822	21.73	6_4_1_1b
C91 H153 N5 O67	6_4_2_1	Fuc Sia	2387.879	21.14	6_4_2_1a
C91 H153 N5 O67	6_4_2_1	Fuc Sia	2387.88	21.3	6_4_2_1b
C91 H153 N5 O67	6_4_2_1	Fuc Sia	2387.88	21.39	6_4_2_1c
C23 H41 N O19	2_0_0_1	Sia	635.226	18.45	3'SL
C23 H41 N O19	2_0_0_1	Sia	635.227	12.78	6'SL

Formula	Compound	Type	Experimental Mass (Da)	RT	Annotation
C37 H64 N2 O29	3_1_0_1	Sia	1000.358	18.11	3_1_0_1a
C37 H64 N2 O29	3_1_0_1	Sia	1000.36	20.08	3_1_0_1b
C51 H87 N3 O39	4_2_0_1	Sia	1365.492	20.69	S-LNH
C51 H87 N3 O39	4_2_0_1	Sia	1365.493	21.19	4_2_0_1c
C51 H87 N3 O39	4_2_0_1	Sia	1365.5	14.62	4_2_0_1a
C51 H87 N3 O39	4_2_0_1	Sia	1365.507	15.7	4_2_0_1b
C62 H104 N4 O47	4_2_0_2	Sia	1656.589	23.21	4_2_0_2a
C65 H110 N4 O49	5_3_0_1	Sia	1730.625	17.31	5_3_0_1a
C65 H110 N4 O49	5_3_0_1	Sia	1730.628	21.65	5_3_0_1b
C79 H133 N5 O59	6_4_0_1	Sia	2095.762	21.76	6_4_0_1a
C79 H133 N5 O59	6_4_0_1	Sia	2095.764	22.43	6_4_0_1b
C18 H34 O16	3_0_0_0	Undec	506.183	11.32	3_0_0_0b
C18 H34 O16	3_0_0_0	Undec	506.183	12.14	3_0_0_0c
C18 H34 O16	3_0_0_0	Undec	506.184	1.18	3_0_0_0a
C20 H37 N O16	2_1_0_0	Undec	547.21	15.02	2_1_0_0a
C26 H47 N O21	3_1_0_0	Undec	709.26	13.41	3_1_0_0a
C26 H47 N O21	3_1_0_0	Undec	709.265	15.02	LNT/LNnT
C32 H57 N O26	4_1_0_0	Undec	871.316	15.79	4_1_0_0a
C32 H57 N O26	4_1_0_0	Undec	871.316	18.75	4_1_0_0b
C34 H60 N2 O26	3_2_0_0	Undec	912.344	16.59	3_2_0_0a
C34 H60 N2 O26	3_2_0_0	Undec	912.344	17.4	3_2_0_0b
C40 H70 N2 O31	4_2_0_0	Undec	1074.389	20.35	4_2_0_0e
C40 H70 N2 O31	4_2_0_0	Undec	1074.392	19.72	4_2_0_0d
C40 H70 N2 O31	4_2_0_0	Undec	1074.393	18.34	4_2_0_0c
C40 H70 N2 O31	4_2_0_0	Undec	1074.394	18.01	4_2_0_0b
C40 H70 N2 O31	4_2_0_0	Undec	1074.394	20	pLNH
C40 H70 N2 O31	4_2_0_0	Undec	1074.397	16.45	4_2_0_0a
C40 H70 N2 O31	4_2_0_0	Undec	1074.397	17.41	LNH
C40 H70 N2 O31	4_2_0_0	Undec	1074.397	17.87	LNnH
C54 H93 N3 O41	5_3_0_0	Undec	1439.525	19.03	5_3_0_0a
C54 H93 N3 O41	5_3_0_0	Undec	1439.528	19.2	5_3_0_0b
C54 H93 N3 O41	5_3_0_0	Undec	1439.529	20.68	5_3_0_0e
C54 H93 N3 O41	5_3_0_0	Undec	1439.53	19.75	5_3_0_0c
C54 H93 N3 O41	5_3_0_0	Undec	1439.53	19.81	5_3_0_0d
C60 H103 N3 O46	6_3_0_0	Undec	1601.583	20.42	6_3_0_0a
C62 H106 N4 O46	5_4_0_0	Undec	1642.595	20.1	5_4_0_0a
C68 H116 N4 O51	6_4_0_0	Undec	1804.663	21.16	6_4_0_0c
C68 H116 N4 O51	6_4_0_0	Undec	1804.664	20.92	6_4_0_0b
C68 H116 N4 O51	6_4_0_0	Undec	1804.665	20.71	6_4_0_0a
C68 H116 N4 O51	6_4_0_0	Undec	1804.666	21.34	6_4_0_0a
C82 H139 N5 O61	7_5_0_0	Undec	2169.799	21.09	7_5_0_0a
C82 H139 N5 O61	7_5_0_0	Undec	2169.8	21.29	7_5_0_0b
C88 H149 N5 O65	7_5_1_0	Undec	2315.859	20.95	7_5_1_0a
C94 H159 N5 O69	7_5_2_0	Undec	2461.917	20.57	7_5_2_0a
C94 H159 N5 O69	7_5_2_0	Undec	2461.917	20.8	7_5_2_0b
C102 H172 N6 O75	8_6_1_0	Undec	2680.991	21.01	8_6_1_0a

SI Table 12: Mixed-Effects Analysis of Variance (ANOVA) on Fucosylated, Fuc-Sialylated, Sialylated, Undecorated, and Total HMOs at Different Lactation Points from Coastal, Noncoastal, Secretor, and Nonsecretor Mothers living in Baybay City, Leyte.

Mixed-effects model (REML)	Matching: Stacked
Assume sphericity?	Yes
Alpha	0.05

Fucosylated HMOs

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 4.051
Coastal vs. Noncoastal	0.032	*	Yes	F (1, 13) = 5.773
Lactation Points x Coastal vs. Noncoastal	0.072	ns	No	F (12, 106) = 1.722

Was the matching effective?	
Chi-square, df	12.64, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 4.346
Secretor Status	0.024	*	Yes	F (1, 13) = 6.483
Lactation Points x Secretor Status	0.145	ns	No	F (12, 106) = 1.474

Was the matching effective?	
Chi-square, df	8.398, 1
P value	0.004
P value summary	**
Is there significant matching (P < 0.05)?	Yes

Fucosylated-Sialylated HMOs

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 14.40
Coastal vs. Noncoastal	.984	ns	No	F (1, 13) = 0.0004277
Lactation Points x Coastal vs. Noncoastal	.370	ns	No	F (12, 106) = 1.098

Was the matching effective?	
Chi-square, df	41.74, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 13.74
Secretor Status	.476	ns	No	F (1, 13) = 0.5380
Lactation Points x Secretor Status	.963	ns	No	F (12, 106) = 0.3943

Was the matching effective?

Chi-square, df	34.66, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Sialylated HMOs

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 15.93
Coastal vs. Noncoastal	.957	ns	No	F (1, 13) = 0.003034
Lactation Points x Coastal vs. Noncoastal	.239	ns	No	F (12, 106) = 1.284

Was the matching effective?

Chi-square, df	23.30, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 16.08
Secretor Status	.808	ns	No	F (1, 13) = 0.06125
Lactation Points x Secretor Status	.821	ns	No	F (12, 106) = 0.6204

Was the matching effective?

Chi-square, df	19.82, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Undecorated HMOs

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 6.516
Coastal vs. Noncoastal	.816	ns	No	F (1, 13) = 0.05643
Lactation Points x Coastal vs. Noncoastal	.113	ns	No	F (12, 106) = 1.565

Was the matching effective?

Chi-square, df	12.33, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 6.908
Secretor Status	.386	ns	No	F (1, 13) = 0.8056
Lactation Points x Secretor Status	.816	ns	No	F (12, 106) = 0.6265

Was the matching effective?

Chi-square, df	9.530, 1
P value	.002

P value summary	**
Is there significant matching (P < 0.05)?	Yes

Total HMOs

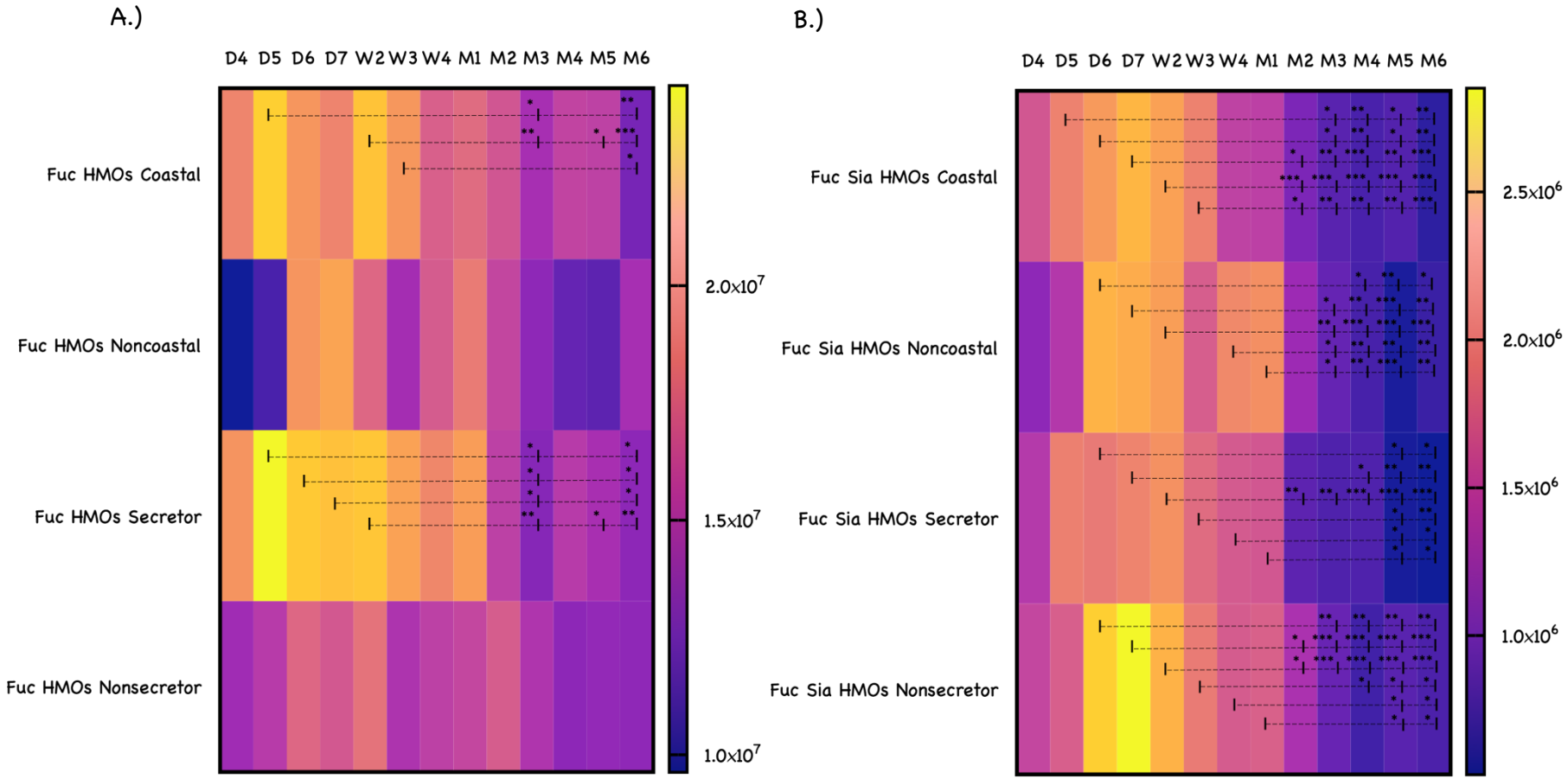
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.702	ns	No	F (12, 106) = 0.7474
Coastal vs. Noncoastal	.821	ns	No	F (1, 13) = 0.05301
Lactation Points x Coastal vs. Noncoastal	.492	ns	No	F (12, 106) = 0.9593

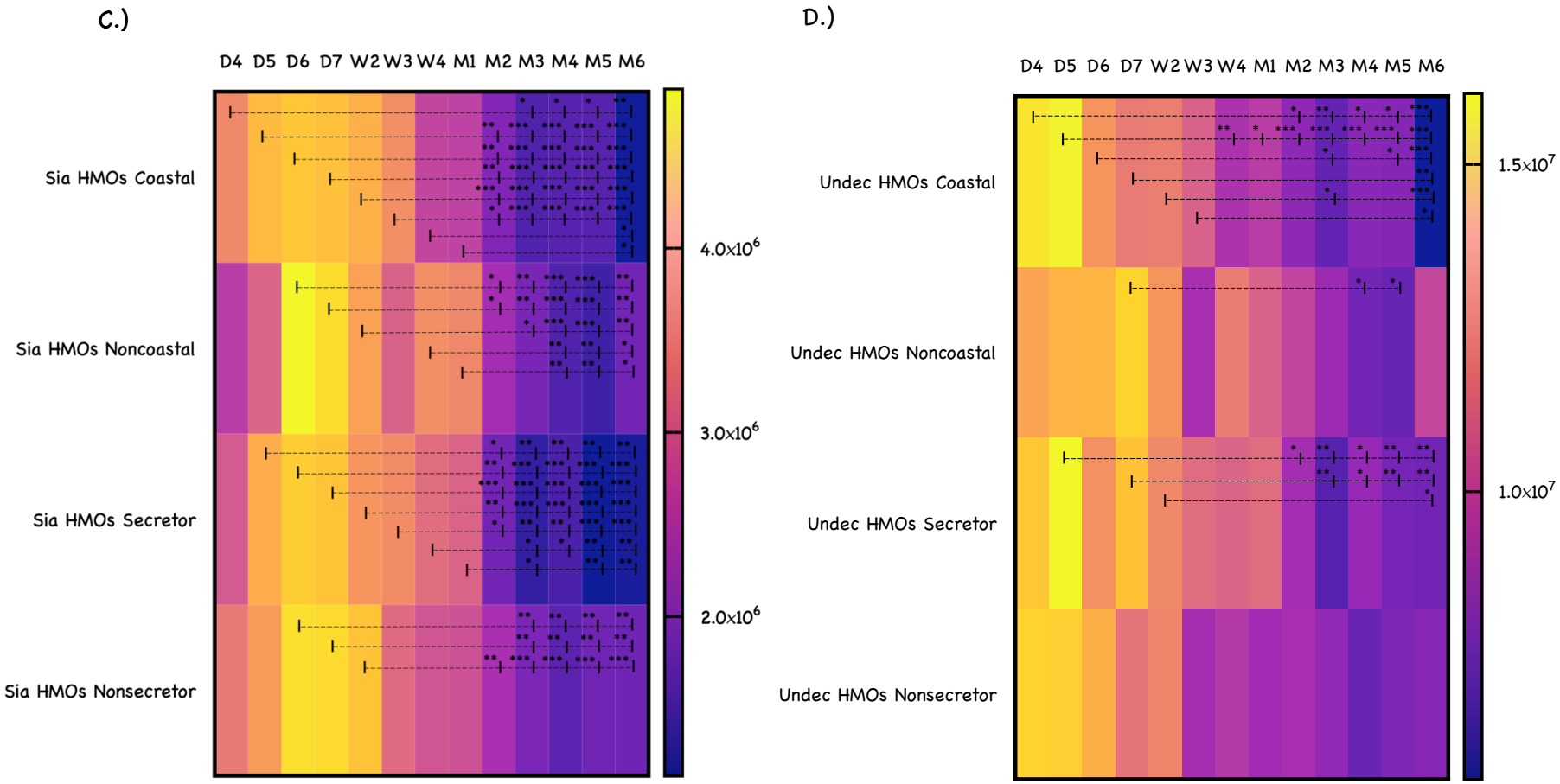
Was the matching effective?	
Chi-square, df	0.5351, 1
P value	.464
P value summary	ns
Is there significant matching (P < 0.05)?	No

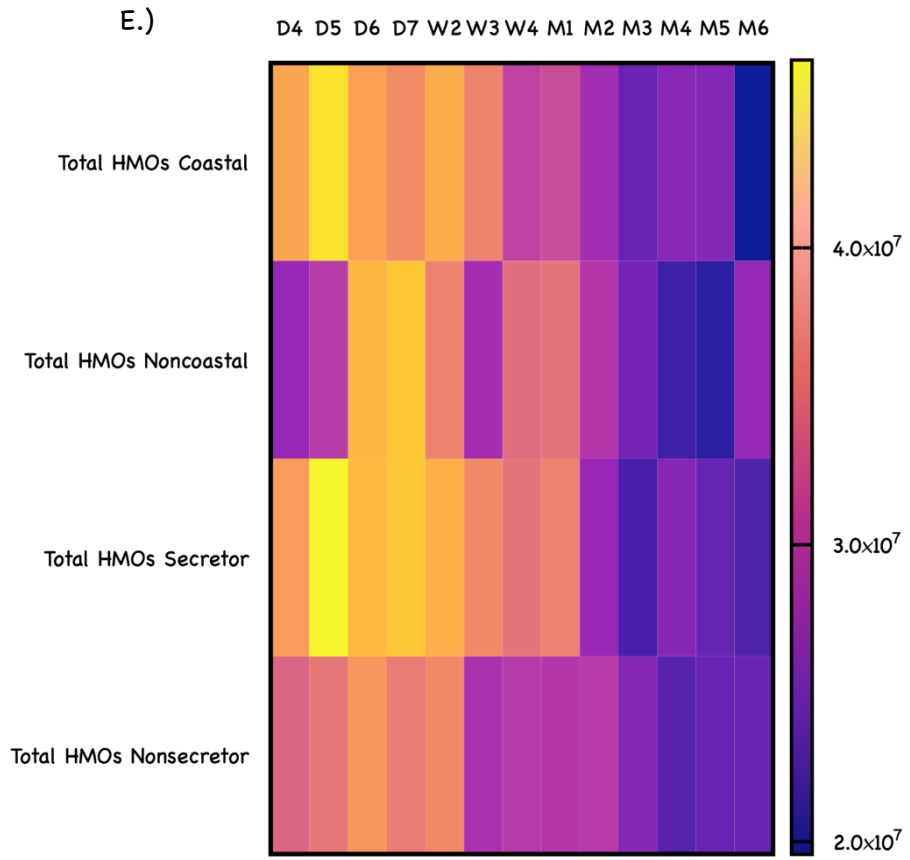
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.648	ns	No	F (12, 106) = 0.8012
Secretor Status	.673	ns	No	F (1, 13) = 0.1862
Lactation Points x Secretor Status	.130	ns	No	F (12, 106) = 1.516

Was the matching effective?	
Chi-square, df	0.4678, 1
P value	.494
P value summary	ns
Is there significant matching (P < 0.05)?	No

SI Table 13: Tukey's Multiple Comparisons Test on Fucosylated, Fuc-Sialylated, Sialylated, Undecorated, and Total HMOs at Different Lactation Points from Breastmilk of Coastal, Noncoastal, Secretor, and Nonsecretor Mothers living in Baybay City, Leyte.

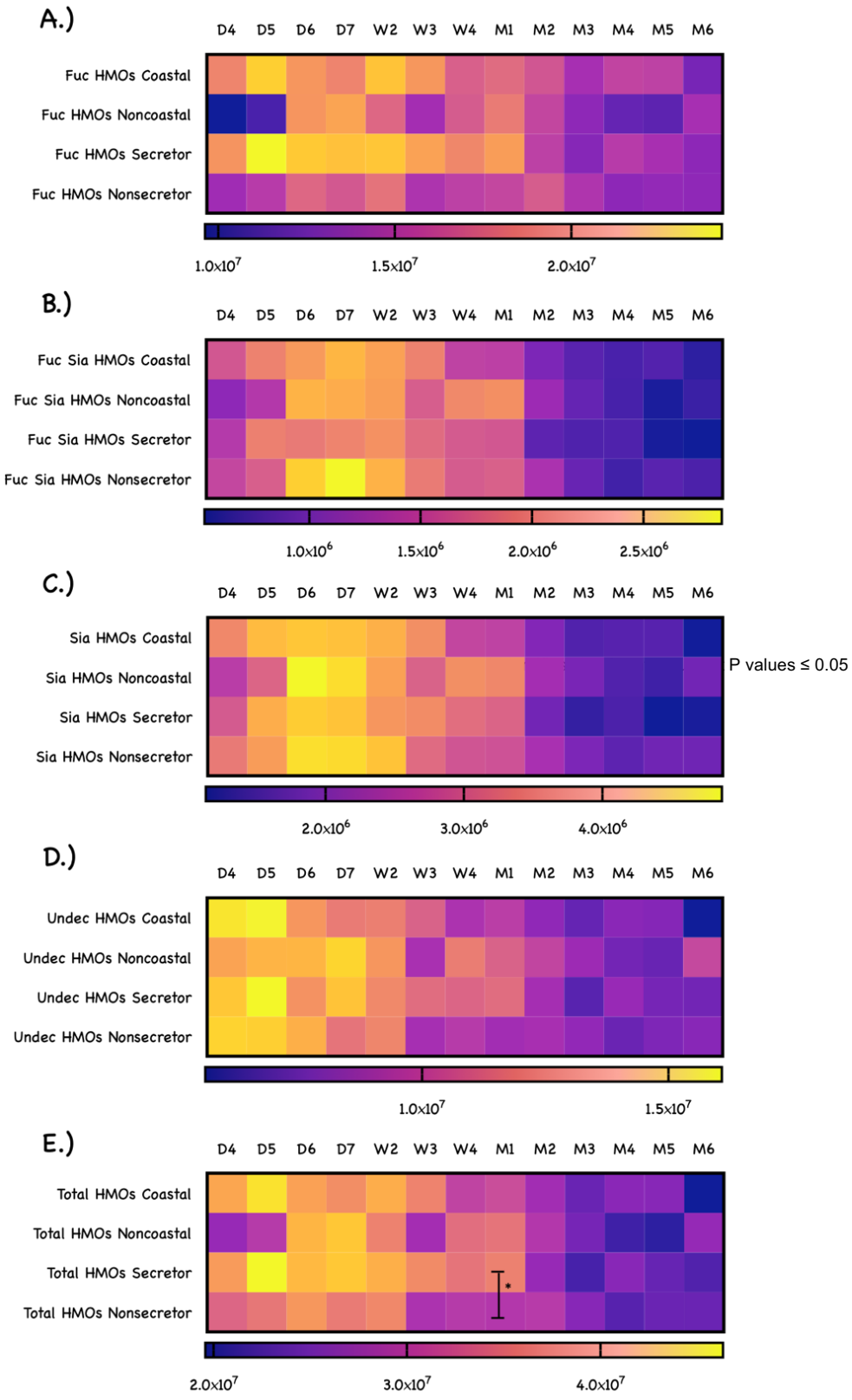



C.)
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
Sia HMOs Coastal
Sia HMOs Noncoastal
Sia HMOs Secretor
Sia HMOs Nonsecretor
4.0x10^6
3.0x10^6
2.0x10^6
D.)
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
Undec HMOs Coastal
Undec HMOs Noncoastal
Undec HMOs Secretor
Undec HMOs Nonsecretor
1.5x10^7
1.0x10^7



* identifies significant results at P values ≤ 0.05
** identifies highly significant results at P values ≤ 0.01
*** identifies very high significant results at P values ≤ 0.00

SI Table 14: Sidak's Multiple Comparisons Test on Coastal, Noncoastal, Secretor, and Nonsecretor HMOs at Different Lactation Points from Mothers living in Baybay City, Leyte.



P values ≤ 0.05

SI Table 15: Mixed-Effects Analysis of Variance (ANOVA) on Annotated Fucosylated HMOs at Different Lactation Points from Coastal, Noncoastal, Secretor and Nonsecretor Mothers living in Baybay City, Leyte.

	Matching:
Mixed-effects model (REML)	Stacked
Assume sphericity?	Yes
Alpha	0.05

2'FL

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.905	ns	No	F (12, 106) = 0.5085
Coastal vs. Noncoastal	.132	ns	No	F (1, 13) = 2.576
Lactation Points x Coastal vs. Noncoastal	.816	ns	No	F (12, 106) = 0.6258

Was the matching effective?	
Chi-square, df	78.75, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	.865	ns	No	F (12, 106) = 0.5659
Secretor Status	.003	**	Yes	F (1, 13) = 13.27
Lactation Points x Secretor Status	.739	ns	No	F (12, 106) = 0.7092

Was the matching effective?	
Chi-square, df	35.12, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

3'FL

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 7.901
Coastal vs. Noncoastal	.608	ns	No	F (1, 13) = 0.2760
Lactation Points x Coastal vs. Noncoastal	.635	ns	No	F (12, 106) = 0.8147

Was the matching effective?	
Chi-square, df	68.03, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 9.189
Secretor Status	.005	**	Yes	F (1, 13) = 11.46
Lactation Points x Secretor Status	.172	ns	No	F (12, 106) = 1.411

Was the matching effective?	
Chi-square, df	29.71, 1

P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LDFT

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.310	ns	No	F (12, 106) = 1.176
Coastal vs. Noncoastal	.015	*	Yes	F (1, 13) = 7.764
Lactation Points x Coastal vs. Noncoastal	.859	ns	No	F (12, 106) = 0.5732

Was the matching effective?	
Chi-square, df	44.50, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.092	ns	No	F (12, 106) = 1.639
Secretor Status	.137	ns	No	F (1, 13) = 2.506
Lactation Points x Secretor Status	.568	ns	No	F (12, 106) = 0.8815

Was the matching effective?	
Chi-square, df	50.27, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LNFP II

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.004	**	Yes	F (12, 106) = 2.610
Coastal vs. Noncoastal	.754	ns	No	F (1, 13) = 0.1020
Lactation Points x Coastal vs. Noncoastal	.004	**	Yes	F (12, 106) = 2.602

Was the matching effective?	
Chi-square, df	138.2, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.213	ns	No	F (12, 106) = 1.329
Secretor Status	<.001	***	Yes	F (1, 13) = 18.77
Lactation Points x Secretor Status	.965	ns	No	F (12, 106) = 0.3885

Was the matching effective?

Chi-square, df	49.01, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LNFP III/I

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 5.119
Coastal vs. Noncoastal	.569	ns	No	F (1, 13) = 0.3421
Lactation Points x Coastal vs. Noncoastal	.193	ns	No	F (12, 106) = 1.368

Was the matching effective?	
Chi-square, df	66.83, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 7.887
Secretor Status	<.001	***	Yes	F (1, 13) = 34.75
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 6.034

Was the matching effective?	
Chi-square, df	35.03, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

LNDFH I

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.969	ns	No	F (12, 106) = 0.3764
Coastal vs. Noncoastal	.043	*	Yes	F (1, 13) = 5.014
Lactation Points x Coastal vs. Noncoastal	.630	ns	No	F (12, 106) = 0.8197

Was the matching effective?	
Chi-square, df	87.21, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	.980	ns	No	F (12, 106) = 0.3373
Secretor Status	.078	ns	No	F (1, 13) = 3.654
Lactation Points x Secretor Status	.075	ns	No	F (12, 106) = 1.707

Was the matching effective?	
Chi-square, df	86.19, 1

P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

IFLNH I

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 5.258
Coastal vs. Noncoastal	0.527	ns	No	F (1, 13) = 0.4218
Lactation Points x Coastal vs. Noncoastal	0.101	ns	No	F (12, 106) = 1.605

Was the matching effective?

Chi-square, df	105.0, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 6.396
Secretor Status	0.004	**	Yes	F (1, 13) = 12.47
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 5.260

Was the matching effective?

Chi-square, df	102.4, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

IFLNH III

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 8.941
Coastal vs. Noncoastal	0.551	ns	No	F (1, 13) = 0.3755
Lactation Points x Coastal vs. Noncoastal	0.259	ns	No	F (12, 106) = 1.250

Was the matching effective?

Chi-square, df	38.72, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 9.320
Secretor Status	0.061	ns	No	F (1, 13) = 4.222
Lactation Points x Secretor Status	0.671	ns	No	F (12, 106) = 0.7785

Was the matching effective?

Chi-square, df	27.03, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

MFLNH III/I

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	0.074	ns	No	F (12, 106) = 1.713
Coastal vs. Noncoastal	0.26	ns	No	F (1, 13) = 1.386
Lactation Points x Coastal vs. Noncoastal	0.643	ns	No	F (12, 106) = 0.8064

Was the matching effective?

Chi-square, df	66.61, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	0.059	ns	No	F (12, 106) = 1.790
Secretor Status	0.614	ns	No	F (1, 13) = 0.2664
Lactation Points x Secretor Status	0.667	ns	No	F (12, 106) = 0.7823

Was the matching effective?

Chi-square, df	67.10, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

MFpLNH IV

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 13.61
Coastal vs. Noncoastal	0.601	ns	No	F (1, 13) = 0.2866
Lactation Points x Coastal vs. Noncoastal	0.031	*	Yes	F (12, 106) = 2.002

Was the matching effective?

Chi-square, df	10.36, 1
P value	0.001
P value summary	**
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 12.96
Secretor Status	0.687	ns	No	F (1, 13) = 0.1700
Lactation Points x Secretor Status	0.962	ns	No	F (12, 106) = 0.3978

Was the matching effective?

Chi-square, df	4.563, 1

P value	0.033
P value summary	*
Is there significant matching (P < 0.05)?	Yes

DFLNHa

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 5.892
Coastal vs. Noncoastal	0.043	*	Yes	F (1, 13) = 5.030
Lactation Points x Coastal vs. Noncoastal	0.053	ns	No	F (12, 106) = 1.826

Was the matching effective?	
Chi-square, df	54.27, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 9.896
Secretor Status	<.001	***	Yes	F (1, 13) = 19.75
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 5.391

Was the matching effective?	
Chi-square, df	29.94, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

DFLNHb

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 3.102
Coastal vs. Noncoastal	0.944	ns	No	F (1, 13) = 0.005179
Lactation Points x Coastal vs. Noncoastal	0.106	ns	No	F (12, 106) = 1.589

Was the matching effective?	
Chi-square, df	142.2, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	0.009	**	Yes	F (12, 106) = 2.393
Secretor Status	0.007	**	Yes	F (1, 13) = 10.23
Lactation Points x Secretor Status	0.286	ns	No	F (12, 106) = 1.210

Was the matching effective?

Chi-square, df	85.69, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

DFLNHc

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 6.507
Coastal vs. Noncoastal	0.062	ns	No	F (1, 13) = 4.157
Lactation Points x Coastal vs. Noncoastal	0.189	ns	No	F (12, 106) = 1.376

Was the matching effective?

Chi-square, df	44.73, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 11.19
Secretor Status	<.001	***	Yes	F (1, 13) = 30.08
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 5.692

Was the matching effective?

Chi-square, df	22.93, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

DFpLNH II

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	0.563	ns	No	F (12, 106) = 0.8865
Coastal vs. Noncoastal	0.801	ns	No	F (1, 13) = 0.06602
Lactation Points x Coastal vs. Noncoastal	0.296	ns	No	F (12, 106) = 1.196

Was the matching effective?

Chi-square, df	87.43, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	0.277	ns	No	F (12, 106) = 1.223
Secretor Status	0.005	**	Yes	F (1, 13) = 11.75
Lactation Points x Secretor Status	0.098	ns	No	F (12, 106) = 1.617

Was the matching effective?

Chi-square, df	51.14, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

FLNO

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 7.061
Coastal vs. Noncoastal	0.488	ns	No	F (1, 13) = 0.5084
Lactation Points x Coastal vs. Noncoastal	0.211	ns	No	F (12, 106) = 1.334

Was the matching effective?

Chi-square, df	39.47, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 8.124
Secretor Status	0.001	**	Yes	F (1, 13) = 16.55
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 4.561

Was the matching effective?

Chi-square, df	34.20, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

DFLNnO I/DFLNO II

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 6.653
Coastal vs. Noncoastal	0.405	ns	No	F (1, 13) = 0.7395
Lactation Points x Coastal vs. Noncoastal	0.11	ns	No	F (12, 106) = 1.573

Was the matching effective?

Chi-square, df	33.10, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 7.110
Secretor Status	<.001	***	Yes	F (1, 13) = 18.21
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 4.742

Was the matching effective?	
Chi-square, df	27.72, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

DFLNnO II

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 6.851
Coastal vs. Noncoastal	0.089	ns	No	F (1, 13) = 3.371
Lactation Points x Coastal vs. Noncoastal	0.283	ns	No	F (12, 106) = 1.215

Was the matching effective?	
Chi-square, df	38.94, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 11.57
Secretor Status	0.003	**	Yes	F (1, 13) = 12.99
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 5.871

Was the matching effective?	
Chi-square, df	42.08, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

DFLNO I

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 5.434
Coastal vs. Noncoastal	0.071	ns	No	F (1, 13) = 3.875
Lactation Points x Coastal vs. Noncoastal	0.285	ns	No	F (12, 106) = 1.212

Was the matching effective?	
Chi-square, df	35.12, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 8.434
Secretor Status	0.014	*	Yes	F (1, 13) = 7.973
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 4.313

Was the matching effective?

Chi-square, df	43.63, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

TFiLNO

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 6.204
Coastal vs. Noncoastal	0.55	ns	No	F (1, 13) = 0.3773
Lactation Points x Coastal vs. Noncoastal	0.594	ns	No	F (12, 106) = 0.8556

Was the matching effective?

Chi-square, df	46.19, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 11.31
Secretor Status	<.001	***	Yes	F (1, 13) = 20.58
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 8.282

Was the matching effective?

Chi-square, df	45.89, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Tetra-iso-LNO

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 4.837
Coastal vs. Noncoastal	0.193	ns	No	F (1, 13) = 1.882
Lactation Points x Coastal vs. Noncoastal	0.1	ns	No	F (12, 106) = 1.610

Was the matching effective?

Chi-square, df	66.35, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 8.086
Secretor Status	0.002	**	Yes	F (1, 13) = 16.00
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 5.760

Was the matching effective?	
Chi-square, df	49.75, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

SI Table 16: Mixed-Effects Analysis of Variance (ANOVA) on Annotated Fucose-Sialylated HMOs at Different Lactation Points from Coastal, Noncoastal, Secretor and Nonsecretor Mothers living in Baybay City, Leyte.

Mixed-effects model (REML)	Matching: Stacked
Assume sphericity?	Yes
Alpha	0.05

FS-LNH I

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 9.221
Coastal vs. Noncoastal	0.653	ns	No	F (1, 13) = 0.2112
Lactation Points x Coastal vs. Noncoastal	0.248	ns	No	F (12, 106) = 1.268

Was the matching effective?	
Chi-square, df	73.47, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 9.681
Secretor Status	0.172	ns	No	F (1, 13) = 2.085
Lactation Points x Secretor Status	0.394	ns	No	F (12, 106) = 1.069

Was the matching effective?	
Chi-square, df	61.01, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

FS-LNH III

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 4.853
Coastal vs. Noncoastal	0.683	ns	No	F (1, 13) = 0.1741
Lactation Points x Coastal vs. Noncoastal	0.265	ns	No	F (12, 106) = 1.241

Was the matching effective?	
Chi-square, df	49.83, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 6.209
Secretor Status	0.012	*	Yes	F (1, 13) = 8.577
Lactation Points x Secretor Status	0.004	**	Yes	F (12, 106) = 2.657

Was the matching effective?	
Chi-square, df	34.90, 1
P value	<.001

P value summary	***
Is there significant matching (P < 0.05)?	Yes

FS-LNH

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 6.775
Coastal vs. Noncoastal	0.404	ns	No	F (1, 13) = 0.7445
Lactation Points x Coastal vs. Noncoastal	0.573	ns	No	F (12, 106) = 0.8761

Was the matching effective?	
Chi-square, df	30.02, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 8.924
Secretor Status	<.001	***	Yes	F (1, 13) = 19.21
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 5.650

Was the matching effective?	
Chi-square, df	23.50, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

FS-LNnH I

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 13.25
Coastal vs. Noncoastal	0.44	ns	No	F (1, 13) = 0.6336
Lactation Points x Coastal vs. Noncoastal	0.5	ns	No	F (12, 106) = 0.9515

Was the matching effective?	
Chi-square, df	18.34, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 15.15
Secretor Status	0.962	ns	No	F (1, 13) = 0.002323
Lactation Points x Secretor Status	0.787	ns	No	F (12, 106) = 0.6589

Was the matching effective?	
Chi-square, df	15.24, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

DFS-LNH

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 3.466
Coastal vs. Noncoastal	0.129	ns	No	F (1, 13) = 2.635
Lactation Points x Coastal vs. Noncoastal	0.25	ns	No	F (12, 106) = 1.266

Was the matching effective?	
Chi-square, df	49.19, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 5.772
Secretor Status	0.001	**	Yes	F (1, 13) = 16.49
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 3.743

Was the matching effective?	
Chi-square, df	31.23, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

DFS-LNnH

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 12.39
Coastal vs. Noncoastal	0.855	ns	No	F (1, 13) = 0.03496
Lactation Points x Coastal vs. Noncoastal	0.771	ns	No	F (12, 106) = 0.6764

Was the matching effective?	
Chi-square, df	30.25, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 12.55
Secretor Status	0.962	ns	No	F (1, 13) = 0.002402
Lactation Points x Secretor Status	0.992	ns	No	F (12, 106) = 0.2727

Was the matching effective?	
Chi-square, df	29.22, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

FS-LNO

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 17.01

Coastal vs. Noncoastal	0.122	ns	No	F (1, 13) = 2.733
Lactation Points x Coastal vs. Noncoastal	0.03	*	Yes	F (12, 106) = 2.012
Was the matching effective?				
Chi-square, df	15.11, 1			
P value	<.001			
P value summary	***			
Is there significant matching (P < 0.05)?	Yes			
Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 14.21
Secretor Status	0.07	ns	No	F (1, 13) = 3.904
Lactation Points x Secretor Status	0.612	ns	No	F (12, 106) = 0.8374
Was the matching effective?				
Chi-square, df	14.92, 1			
P value	<.001			
P value summary	***			
Is there significant matching (P < 0.05)?	Yes			

SI Table 17: Mixed-Effects Analysis of Variance (ANOVA) on Annotated Sialylated HMOs at Different Lactation Points from Coastal, Noncoastal, Secretor and Nonsecretor Mothers living in Baybay City, Leyte.

Mixed-effects model (REML)	Matching: Stacked
Assume sphericity?	Yes
Alpha	0.05

3'SL

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	0.229	ns	No	F (12, 106) = 1.301
Coastal vs. Noncoastal	0.493	ns	No	F (1, 13) = 0.4981
Lactation Points x Coastal vs. Noncoastal	0.288	ns	No	F (12, 106) = 1.208

Was the matching effective?	
Chi-square, df	27.25, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	0.172	ns	No	F (12, 106) = 1.412
Secretor Status	0.433	ns	No	F (1, 13) = 0.6557
Lactation Points x Secretor Status	0.729	ns	No	F (12, 106) = 0.7199

Was the matching effective?	
Chi-square, df	31.47, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

6'SL

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 17.41
Coastal vs. Noncoastal	0.091	ns	No	F (1, 13) = 3.343
Lactation Points x Coastal vs. Noncoastal	0.004	**	Yes	F (12, 106) = 2.619

Was the matching effective?	
Chi-square, df	14.95, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 13.92
Secretor Status	0.619	ns	No	F (1, 13) = 0.2595
Lactation Points x Secretor Status	0.613	ns	No	F (12, 106) = 0.8364

Was the matching effective?	
Chi-square, df	15.61, 1

P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

S-LNH

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 16.94
Coastal vs. Noncoastal	0.622	ns	No	F (1, 13) = 0.2548
Lactation Points x Coastal vs. Noncoastal	0.125	ns	No	F (12, 106) = 1.529

Was the matching effective?	
Chi-square, df	17.74, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 16.44
Secretor Status	0.076	ns	No	F (1, 13) = 3.729
Lactation Points x Secretor Status	0.798	ns	No	F (12, 106) = 0.6464

Was the matching effective?	
Chi-square, df	10.50, 1
P value	0.001
P value summary	**
Is there significant matching (P < 0.05)?	Yes

SI Table 18: Mixed-Effects Analysis of Variance (ANOVA) on Annotated Undecorated HMOs at Different Lactation Points from Coastal, Noncoastal, Secretor and Nonsecretor Mothers living in Baybay City, Leyte.

Mixed-effects model (REML)	Matching: Stacked
Assume sphericity?	Yes
Alpha	0.05

LNT/LNnT

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 4.107
Coastal vs. Noncoastal	0.891	ns	No	F (1, 13) = 0.01960
Lactation Points x Coastal vs. Noncoastal	0.124	ns	No	F (12, 106) = 1.533

Was the matching effective?	
Chi-square, df	14.81, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 4.608
Secretor Status	0.472	ns	No	F (1, 13) = 0.5498
Lactation Points x Secretor Status	0.735	ns	No	F (12, 106) = 0.7135

Was the matching effective?	
Chi-square, df	10.21, 1
P value	0.001
P value summary	**
Is there significant matching (P < 0.05)?	Yes

LNH

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 12.13
Coastal vs. Noncoastal	0.887	ns	No	F (1, 13) = 0.02099
Lactation Points x Coastal vs. Noncoastal	0.549	ns	No	F (12, 106) = 0.9008

Was the matching effective?	
Chi-square, df	33.33, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	**P value**	**P value summary**	**Statistically significant (P < 0.05)?**	**F (DFn, DFd)**
Lactation Points	<.001	***	Yes	F (12, 106) = 13.53
Secretor Status	0.034	*	Yes	F (1, 13) = 5.588
Lactation Points x Secretor Status	0.117	ns	No	F (12, 106) = 1.552

Was the matching effective?	
Chi-square, df	22.59, 1
P value	<.001

P value summary	***
Is there significant matching (P < 0.05)?	Yes

LNnH

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	0.162	ns	No	F (12, 106) = 1.435
Coastal vs. Noncoastal	0.993	ns	No	F (1, 13) = 7.362e-005
Lactation Points x Coastal vs. Noncoastal	0.116	ns	No	F (12, 106) = 1.556

Was the matching effective?	
Chi-square, df	131.9, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	0.021	*	Yes	F (12, 106) = 2.118
Secretor Status	0.019	*	Yes	F (1, 13) = 7.163
Lactation Points x Secretor Status	0.372	ns	No	F (12, 106) = 1.095

Was the matching effective?	
Chi-square, df	97.48, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

pLNH

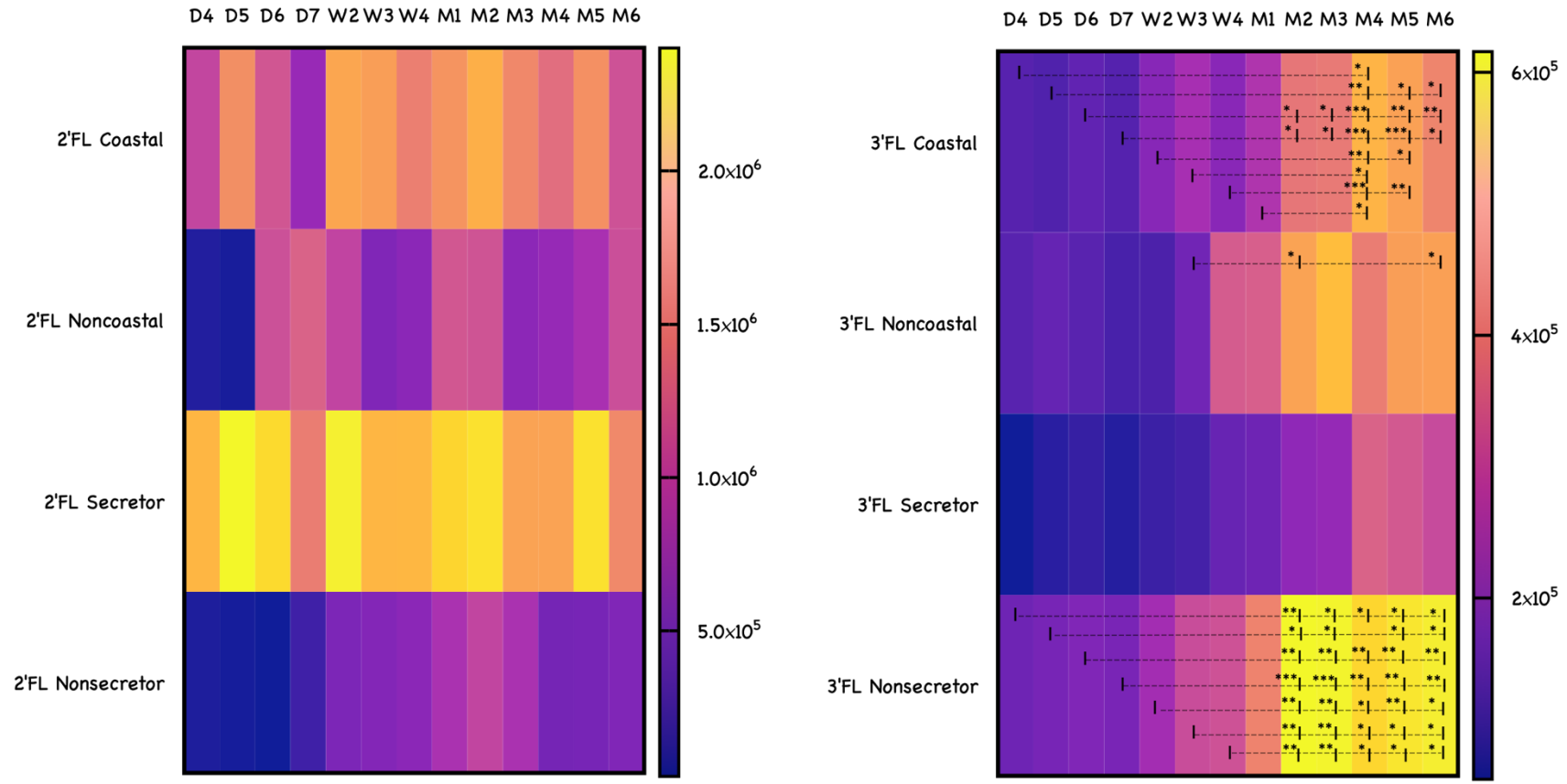
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 4.197
Coastal vs. Noncoastal	0.859	ns	No	F (1, 13) = 0.03306
Lactation Points x Coastal vs. Noncoastal	0.151	ns	No	F (12, 106) = 1.459

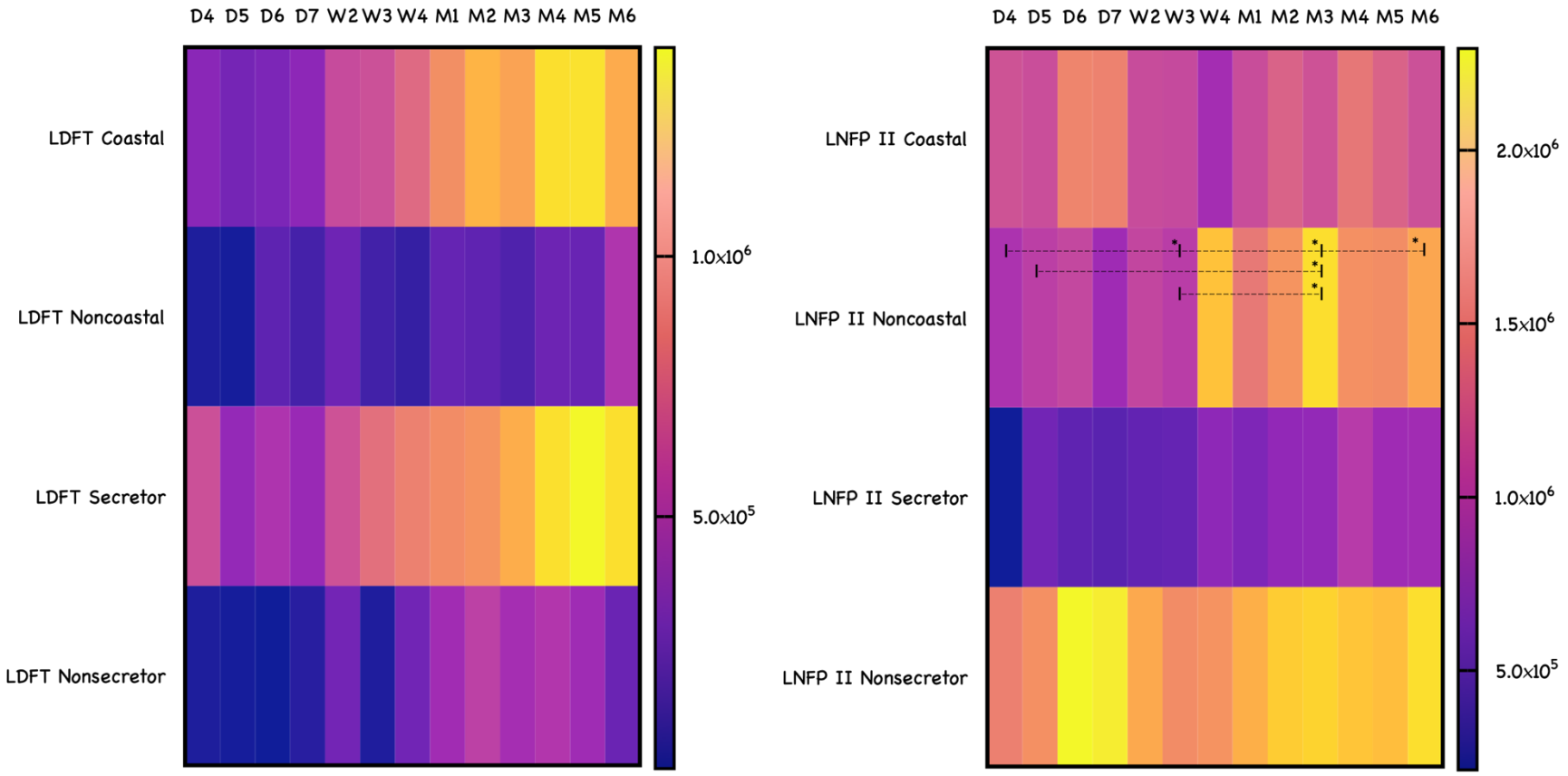
Was the matching effective?	
Chi-square, df	88.98, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

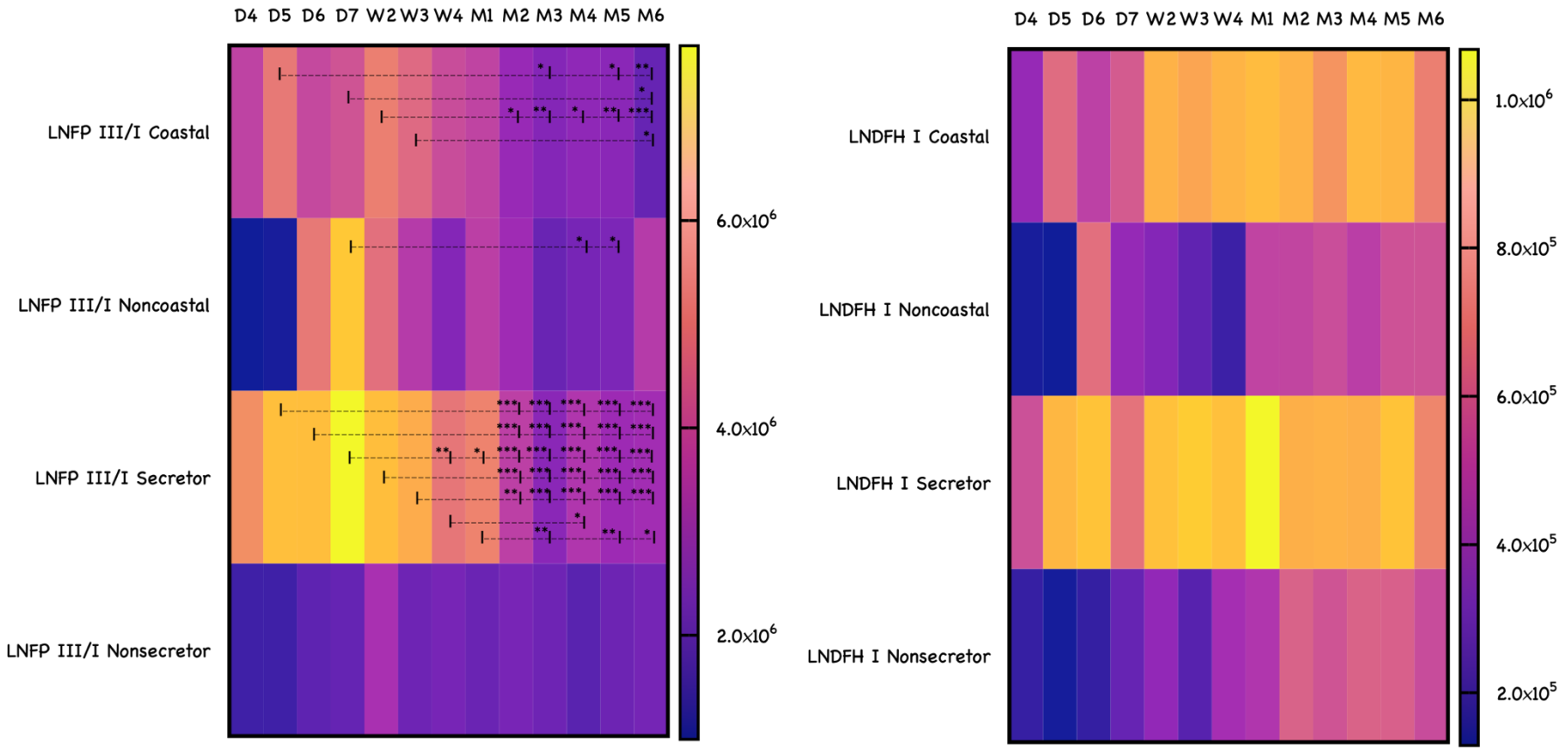
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Lactation Points	<.001	***	Yes	F (12, 106) = 6.667
Secretor Status	0.003	**	Yes	F (1, 13) = 13.08
Lactation Points x Secretor Status	<.001	***	Yes	F (12, 106) = 3.802

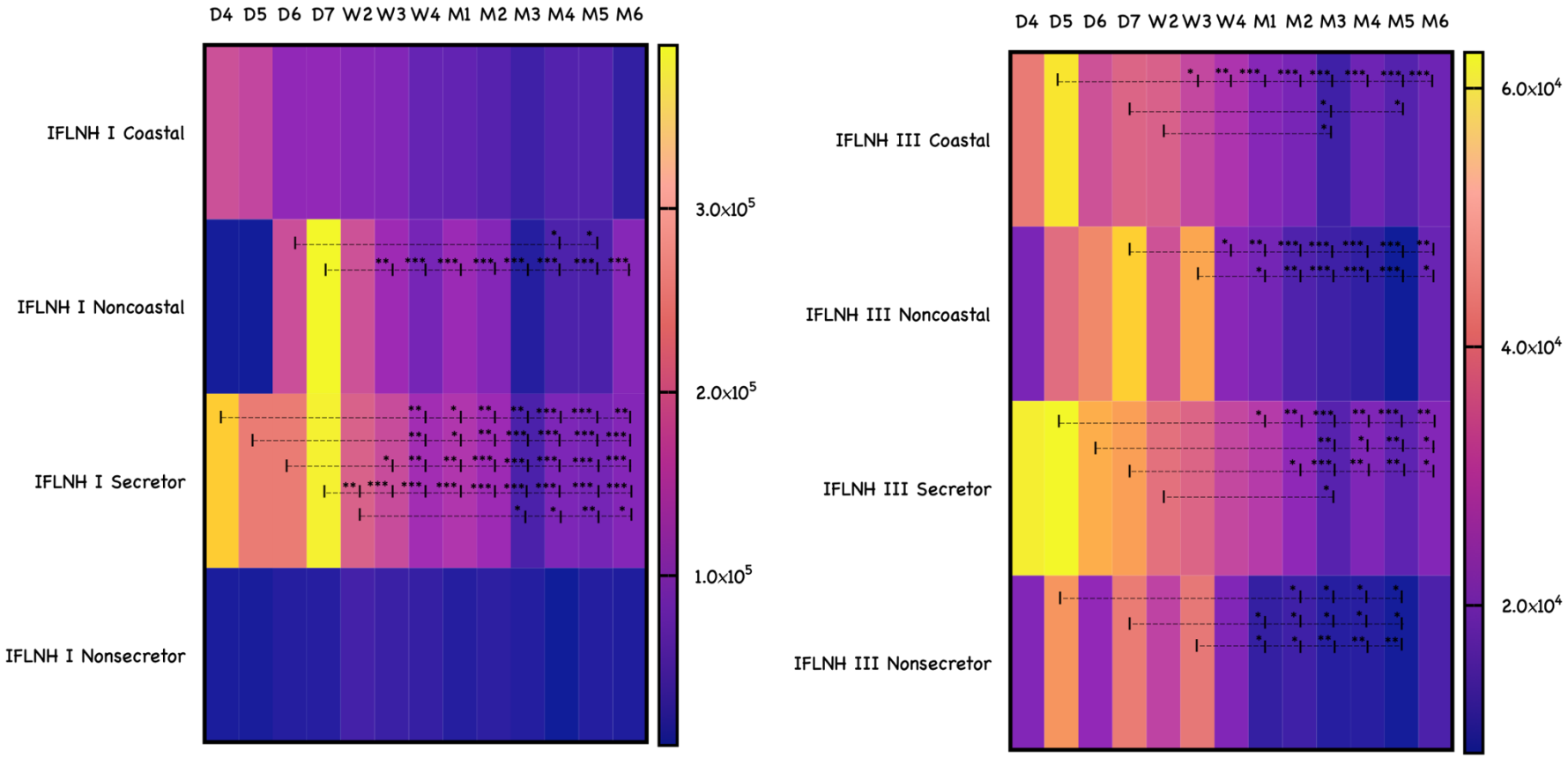
Was the matching effective?	
Chi-square, df	78.00, 1
P value	<.001
P value summary	***
Is there significant matching (P < 0.05)?	Yes

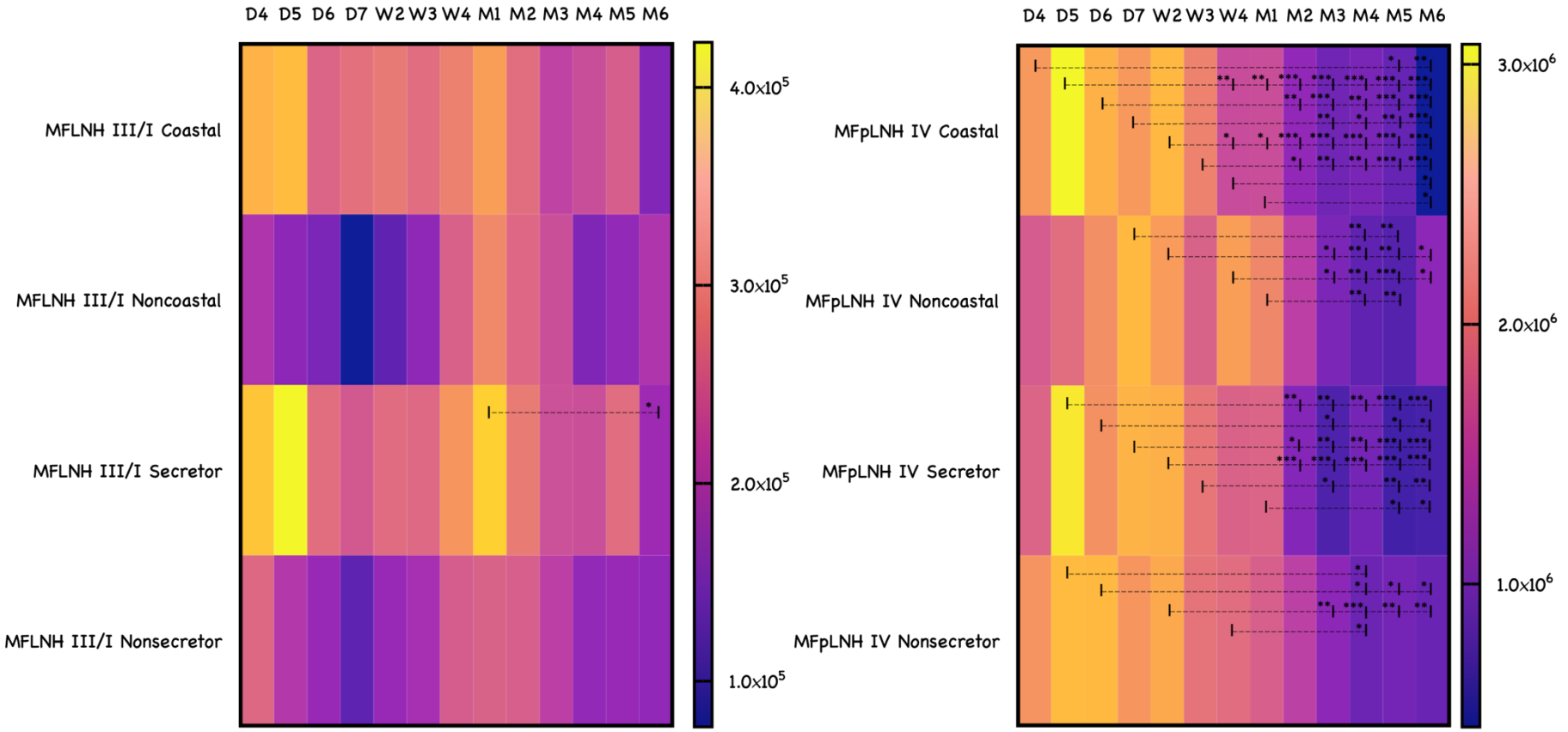
SI Table 19: Tukey's Multiple Comparisons Test on Annotated Fucosylated HMOs at Different Lactation Points from Breastmilk of Coastal, Noncoastal, Secretor, and Nonsecretor Mothers living in Baybay City, Leyte.

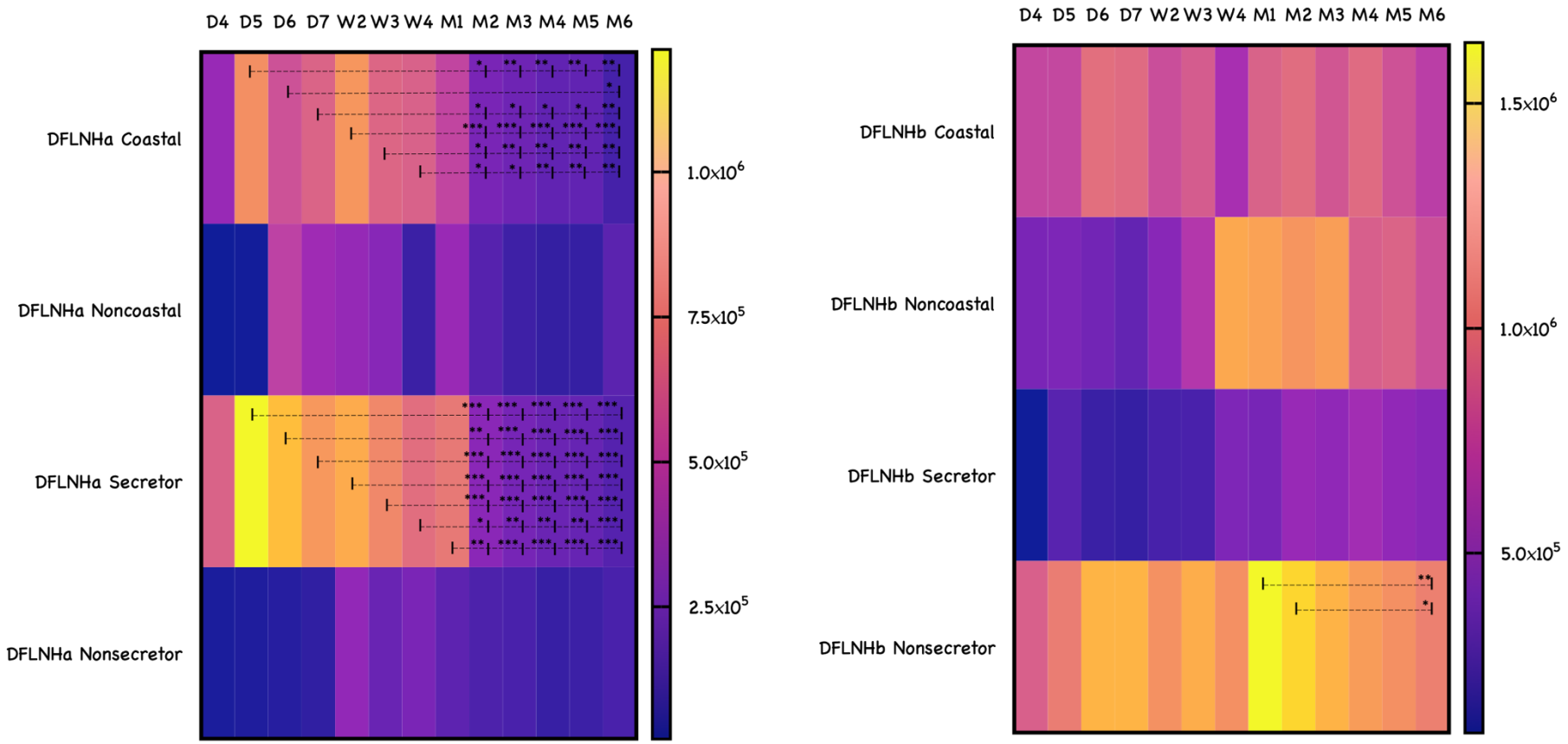


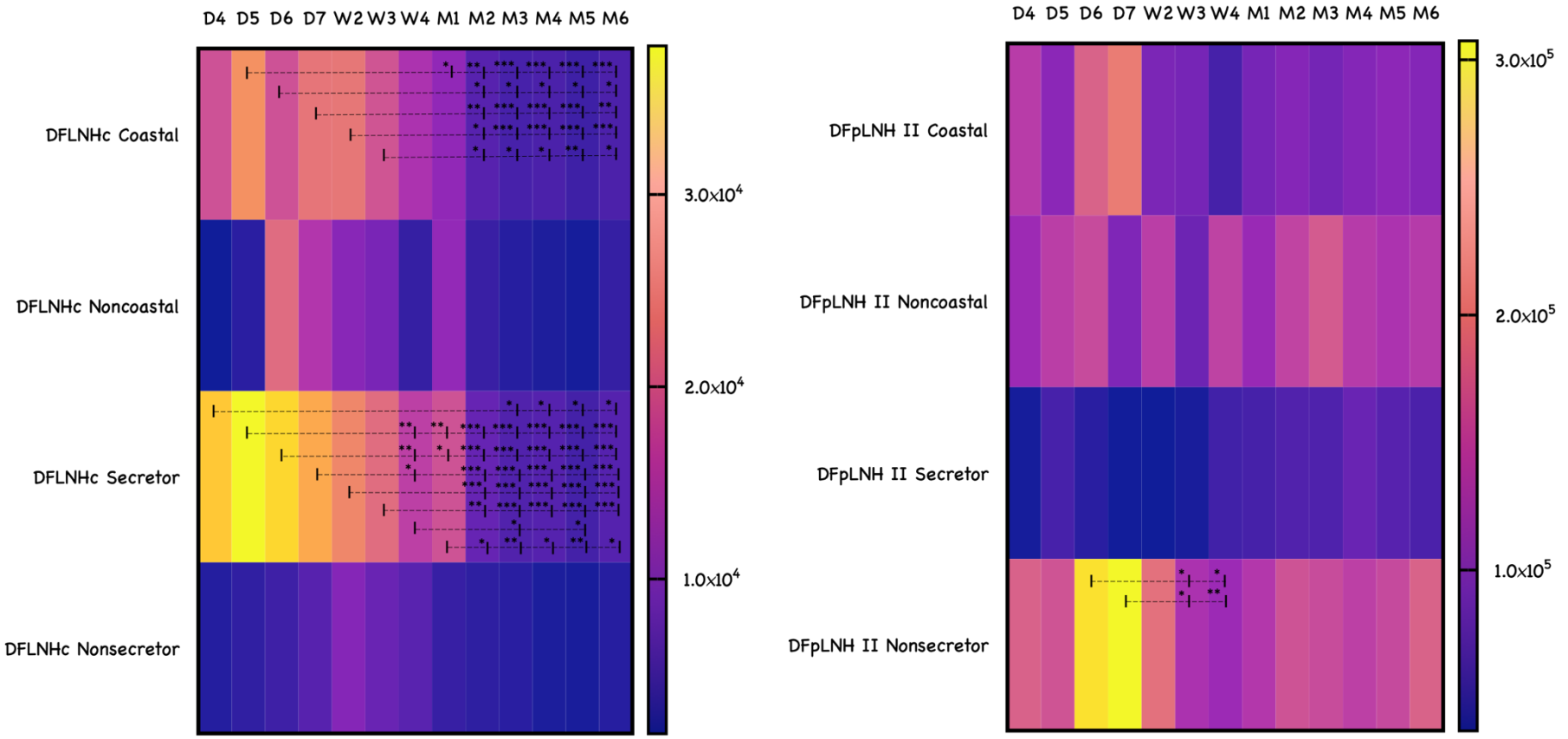

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LDFT Coastal
LDFT Noncoastal
LDFT Secretor
LDFT Nonsecretor
1.0x10^6
5.0x10^5
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LNFP II Coastal
LNFP II Noncoastal
LNFP II Secretor
LNFP II Nonsecretor
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5

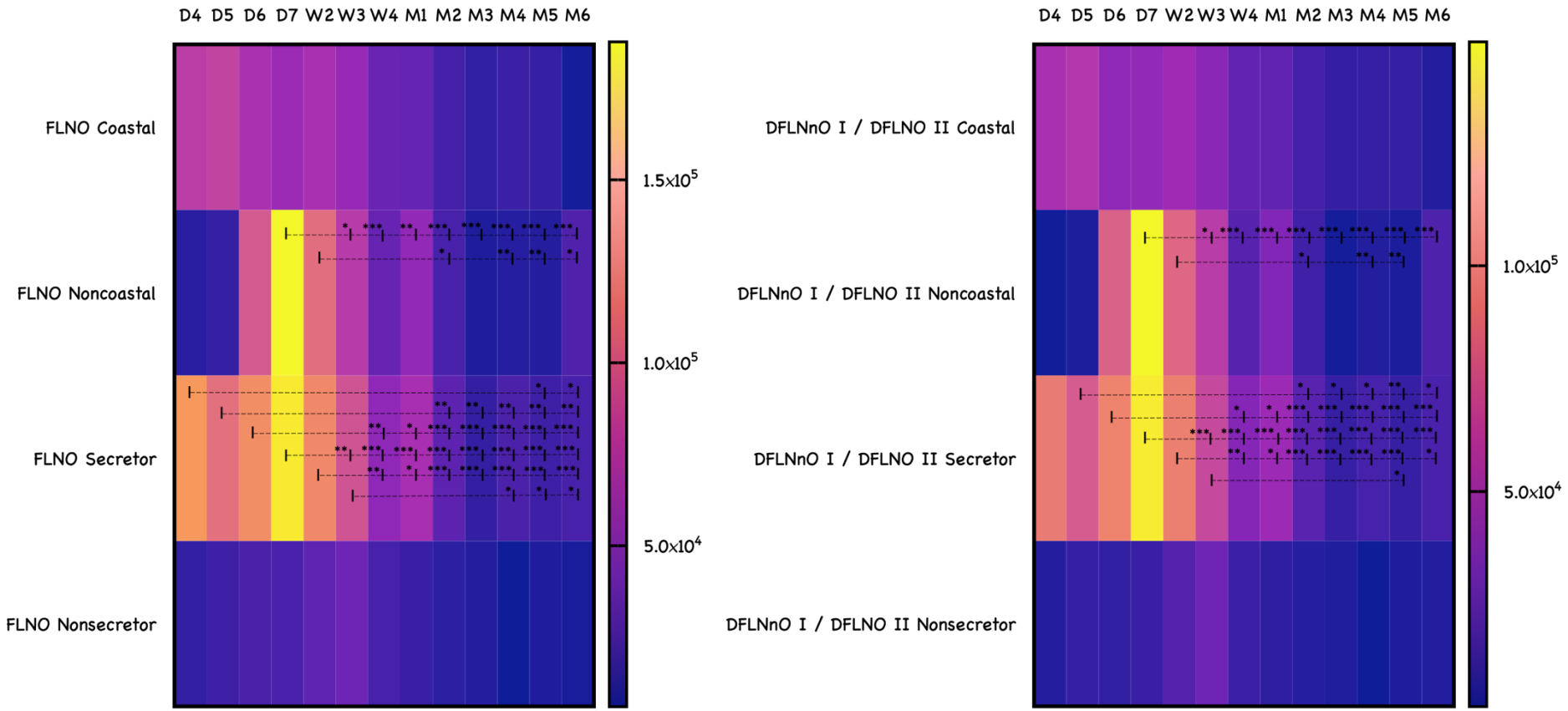

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LNFP III/I Coastal
LNFP III/I Noncoastal
LNFP III/I Secretor
LNFP III/I Nonsecretor
6.0x10^6
4.0x10^6
2.0x10^6
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
LNDFH I Coastal
LNDFH I Noncoastal
LNDFH I Secretor
LNDFH I Nonsecretor
1.0x10^6
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5

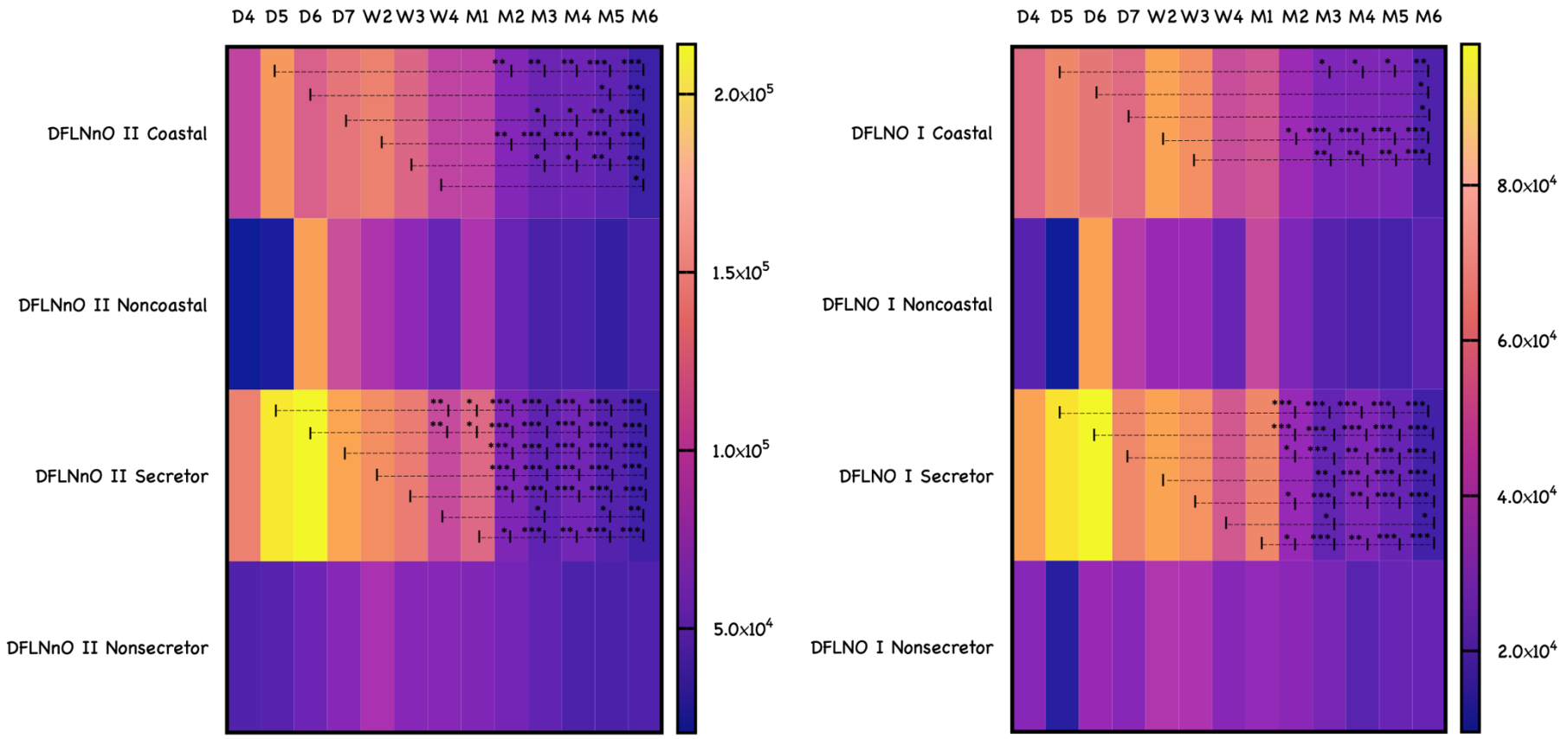

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IFLNH I Coastal
IFLNH I Noncoastal
IFLNH I Secretor
IFLNH I Nonsecretor
3.0x10^5
2.0x10^5
1.0x10^5
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
IFLNH III Coastal
IFLNH III Noncoastal
IFLNH III Secretor
IFLNH III Nonsecretor
6.0x10^4
4.0x10^4
2.0x10^4

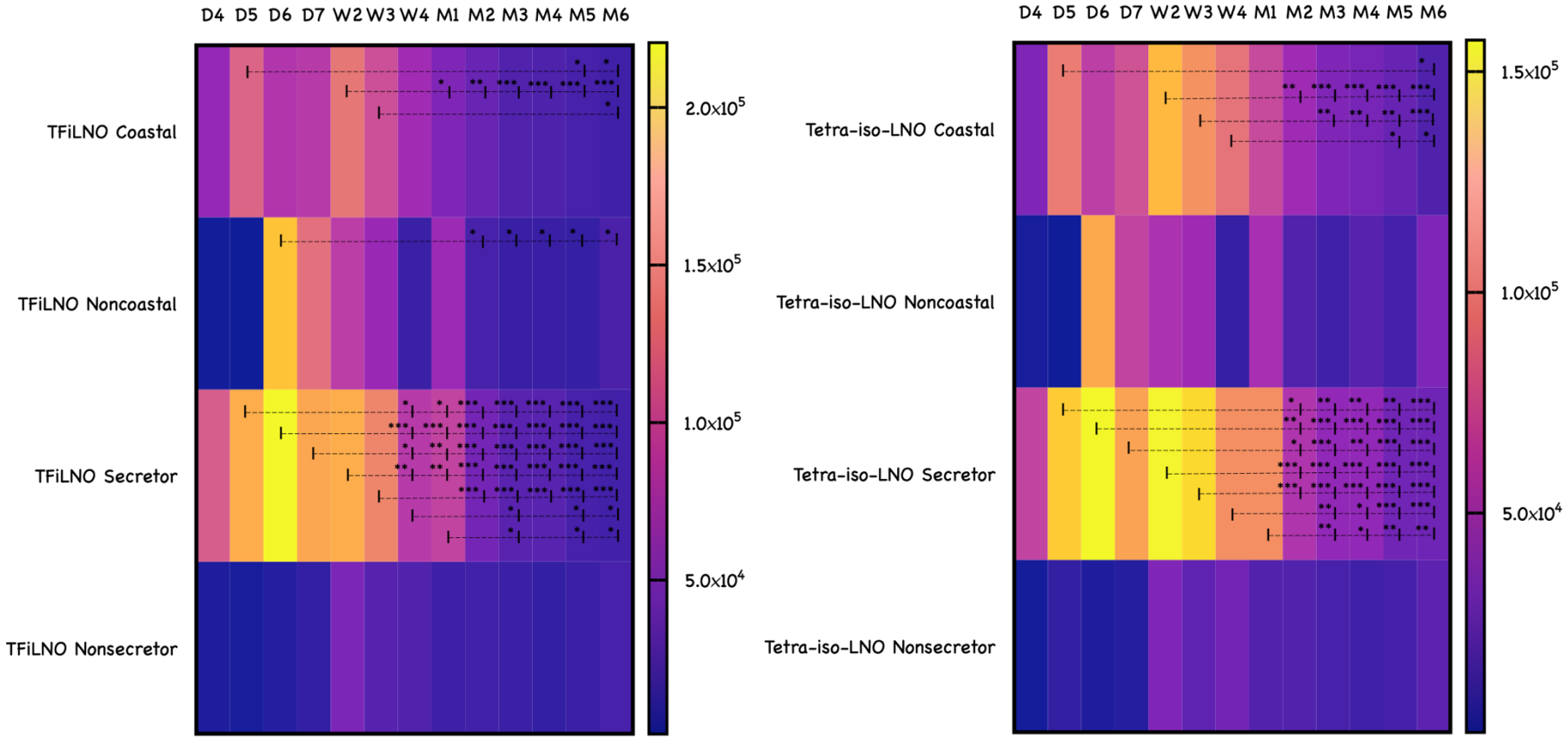

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
MFLNH III/I Coastal
MFLNH III/I Noncoastal
MFLNH III/I Secretor
MFLNH III/I Nonsecretor
4.0x10^5
3.0x10^5
2.0x10^5
1.0x10^5
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
MFpLNH IV Coastal
MFpLNH IV Noncoastal
MFpLNH IV Secretor
MFpLNH IV Nonsecretor
3.0x10^6
2.0x10^6
1.0x10^6


D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
DFLNHa Coastal
DFLNHa Noncoastal
DFLNHa Secretor
DFLNHa Nonsecretor
1.0x10^6
7.5x10^5
5.0x10^5
2.5x10^5
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
DFLNHb Coastal
DFLNHb Noncoastal
DFLNHb Secretor
DFLNHb Nonsecretor
1.5x10^6
1.0x10^6
5.0x10^5


D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
DFLNHc Coastal
DFLNHc Noncoastal
DFLNHc Secretor
DFLNHc Nonsecretor
3.0x10^4
2.0x10^4
1.0x10^4
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
DFpLNH II Coastal
DFpLNH II Noncoastal
DFpLNH II Secretor
DFpLNH II Nonsecretor
3.0x10^5
2.0x10^5
1.0x10^5

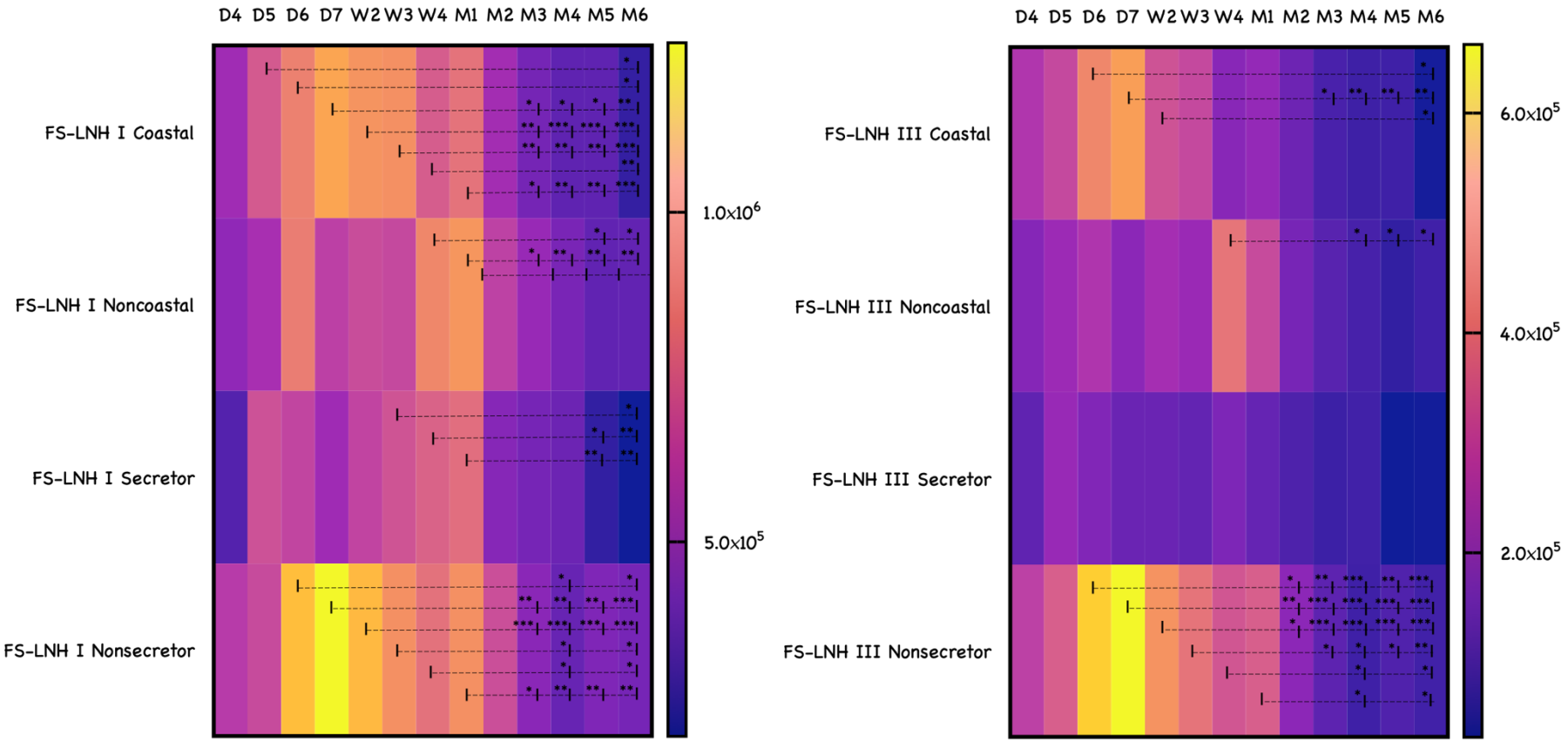

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
FLNO Coastal
FLNO Noncoastal
FLNO Secretor
FLNO Nonsecretor
1.5x10^5
1.0x10^5
5.0x10^4
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
DFLNnO I / DFLNO II Coastal
DFLNnO I / DFLNO II Noncoastal
DFLNnO I / DFLNO II Secretor
DFLNnO I / DFLNO II Nonsecretor
1.0x10^5
5.0x10^4

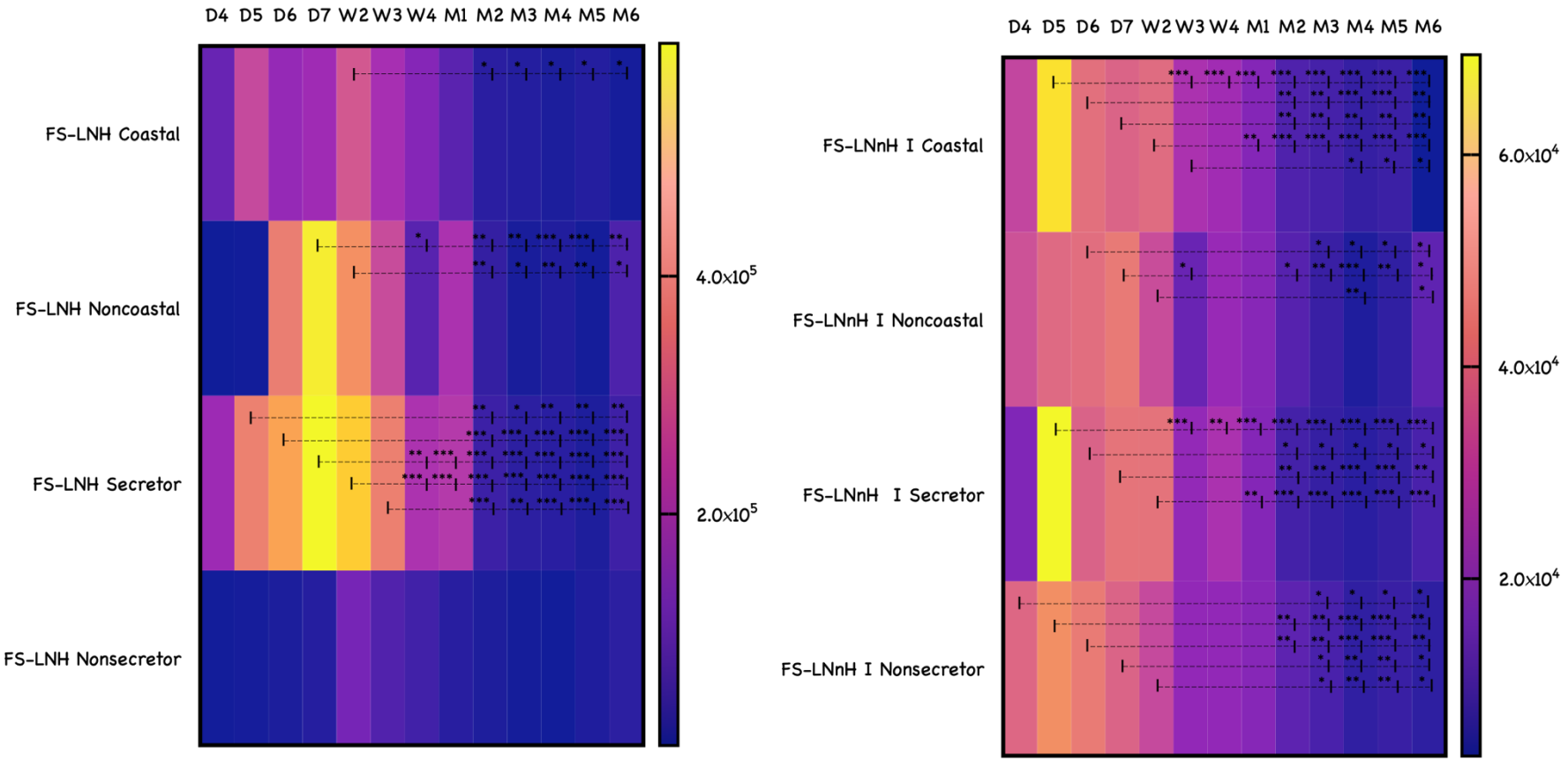

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
DFLNnO II Coastal
DFLNnO II Noncoastal
DFLNnO II Secretor
DFLNnO II Nonsecretor
2.0x10^5
1.5x10^5
1.0x10^5
5.0x10^4
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
DFLNO I Coastal
DFLNO I Noncoastal
DFLNO I Secretor
DFLNO I Nonsecretor
8.0x10^4
6.0x10^4
4.0x10^4
2.0x10^4

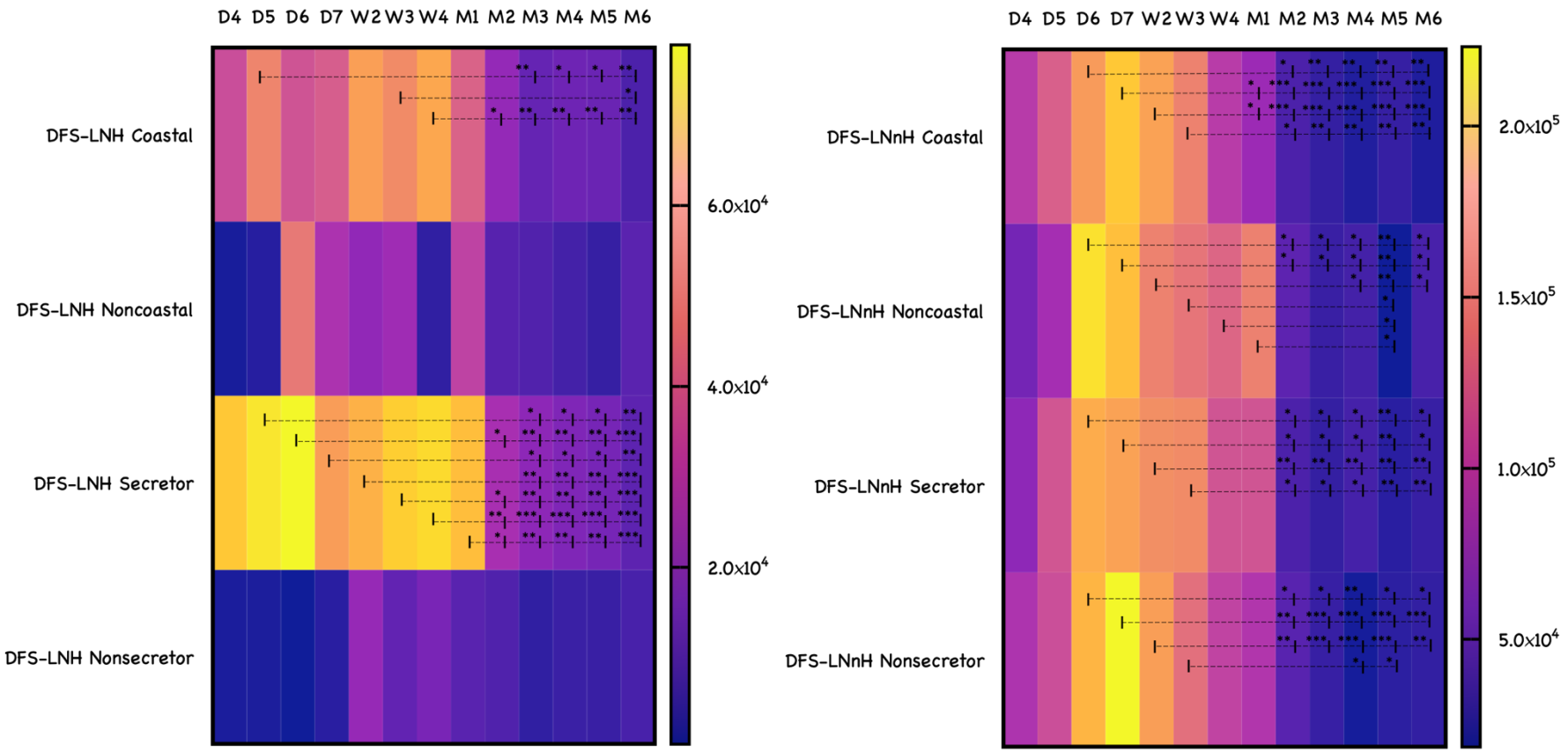


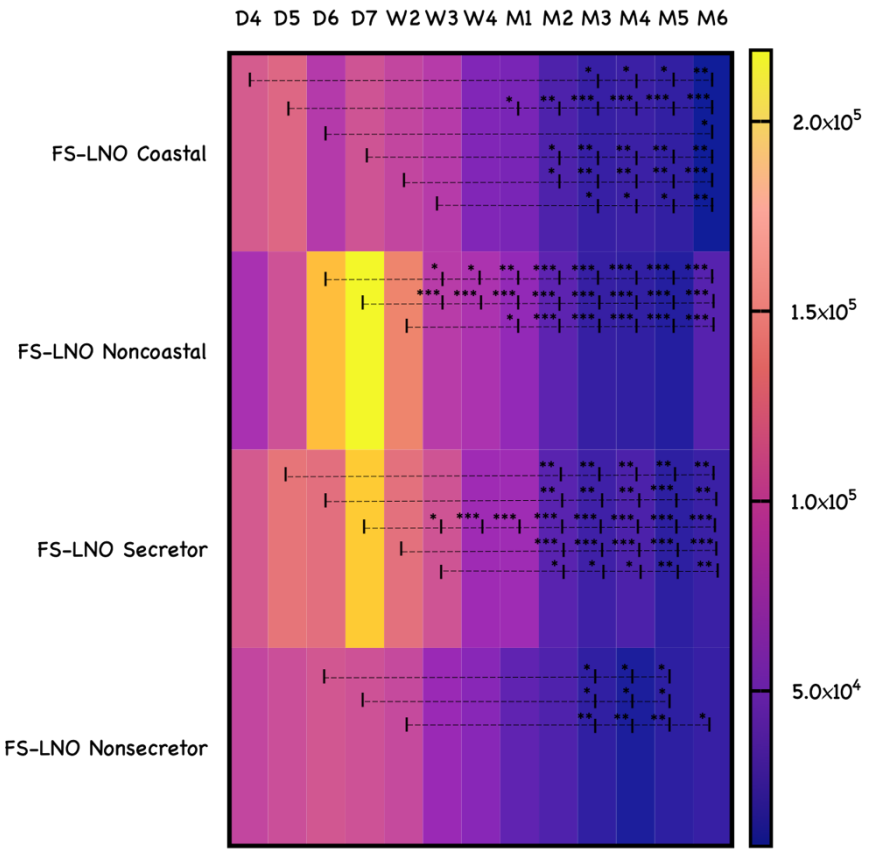
* identifies significant results at P values ≤ 0.05
** identifies highly significant results at P values ≤ 0.01
*** identifies very high significant results at P values ≤ 0.00

SI Table 20: Tukey's Multiple Comparisons Test on Annotated Fucose, -Sialylated HMOs at Different Lactation Points from Breastmilk of Coastal, Noncoastal, Secretor, and Nonsecretor Mothers living in Baybay City, Leyte.



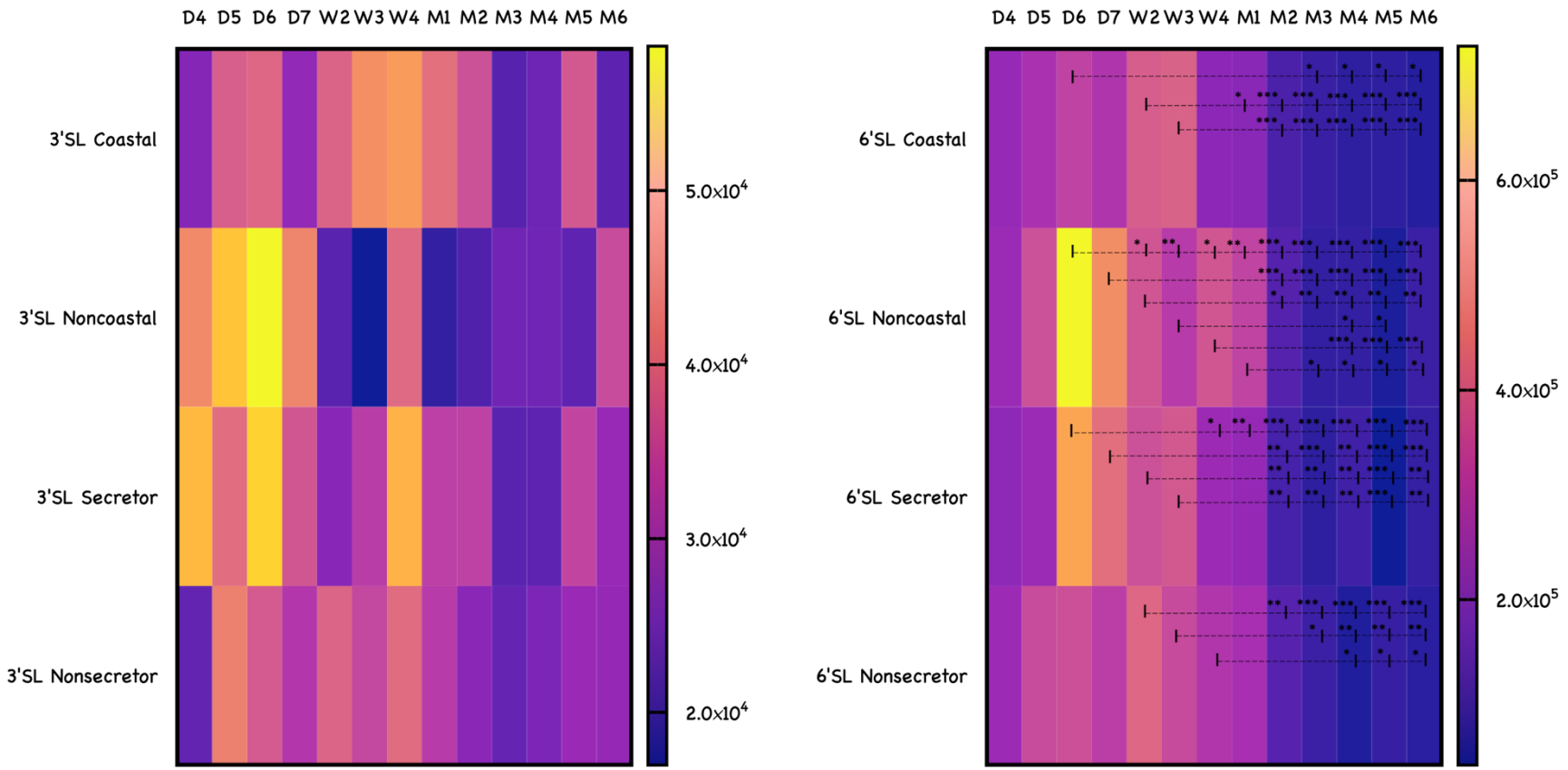

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
FS-LNH Coastal
FS-LNH Noncoastal
FS-LNH Secretor
FS-LNH Nonsecretor
4.0x10^5
2.0x10^5
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
FS-LNnH I Coastal
FS-LNnH I Noncoastal
FS-LNnH I Secretor
FS-LNnH I Nonsecretor
6.0x10^4
4.0x10^4
2.0x10^4

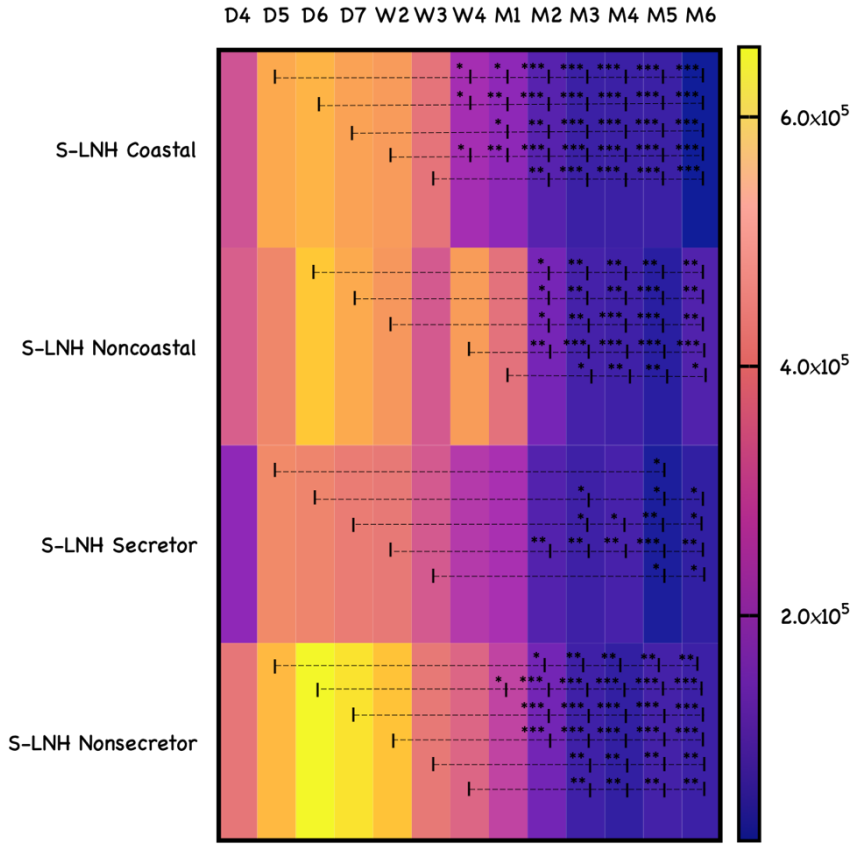

D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
DFS-LNH Coastal
DFS-LNH Noncoastal
DFS-LNH Secretor
DFS-LNH Nonsecretor
6.0x10^4
4.0x10^4
2.0x10^4
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
DFS-LNnH Coastal
DFS-LNnH Noncoastal
DFS-LNnH Secretor
DFS-LNnH Nonsecretor
2.0x10^5
1.5x10^5
1.0x10^5
5.0x10^4



* identifies significant results at P values ≤ 0.05
** identifies highly significant results at P values ≤ 0.01
*** identifies very high significant results at P values ≤ 0.001

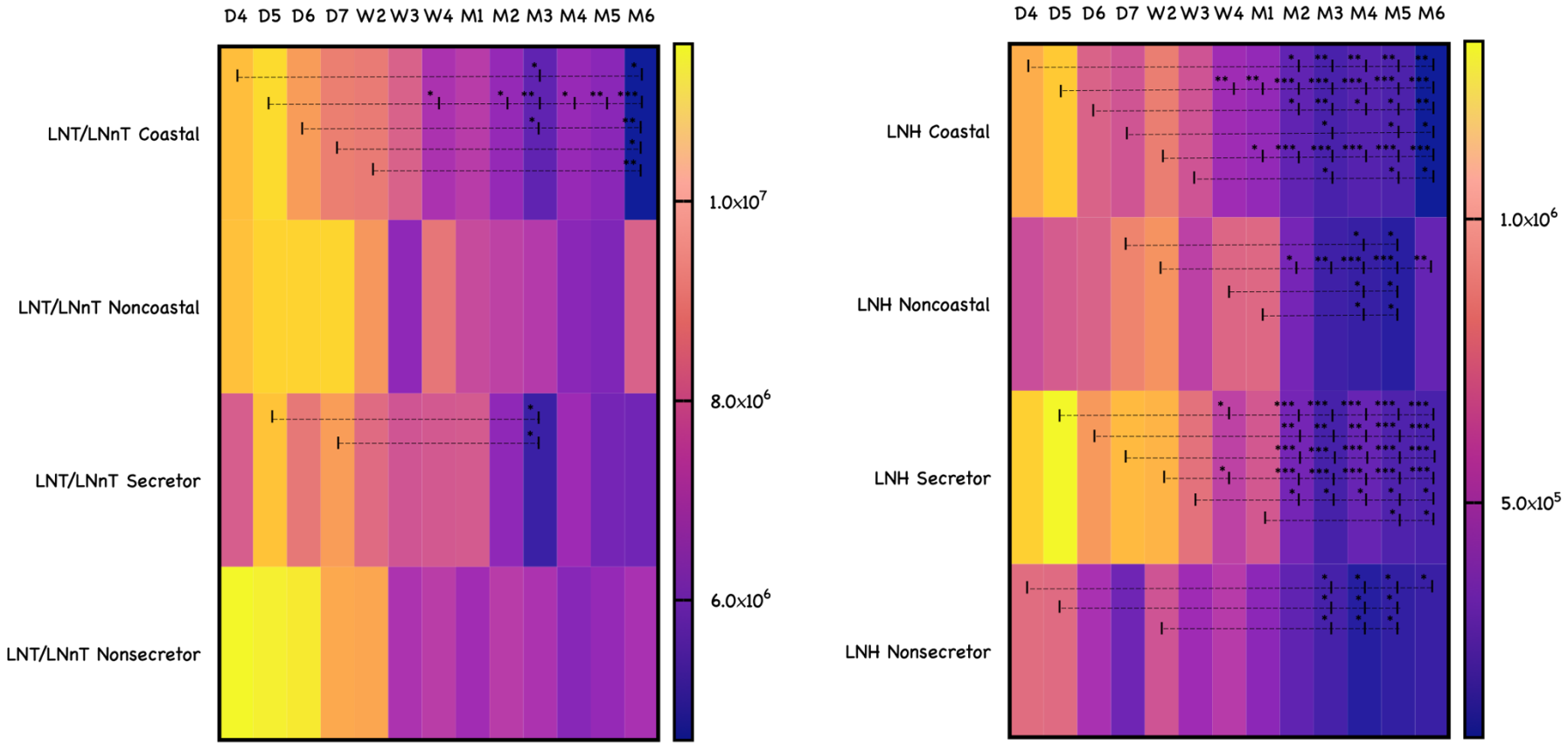
SI Table 21: Tukey's Multiple Comparisons Test on Annotated Sialylated HMOs at Different Lactation Points from Breastmilk of Coastal, Noncoastal, Secretor, and Nonsecretor Mothers living in Baybay City, Leyte.

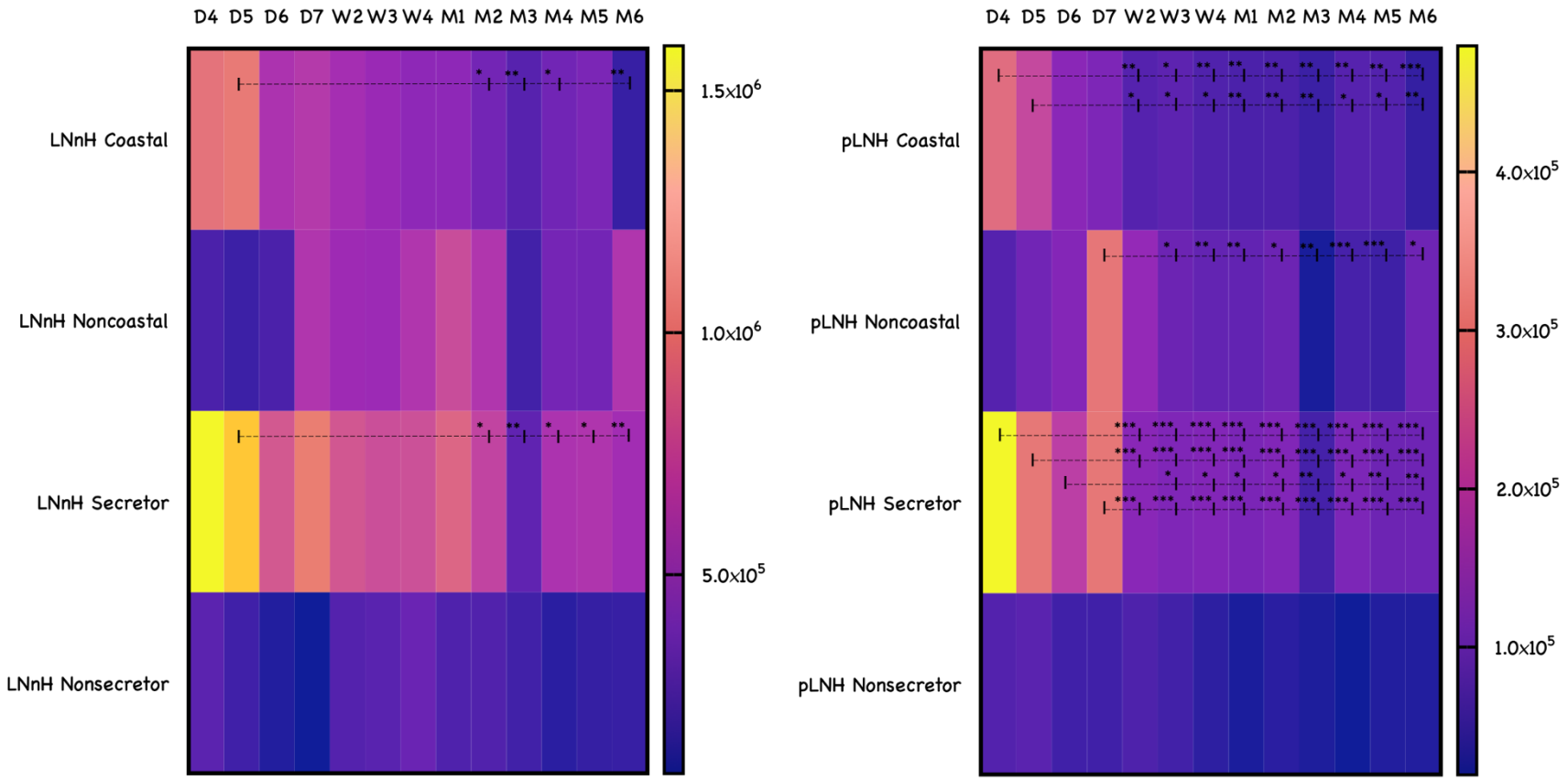




* identifies significant results at P values ≤ 0.05
** identifies highly significant results at P values ≤ 0.01
*** identifies very high significant results at P values ≤ 0.001

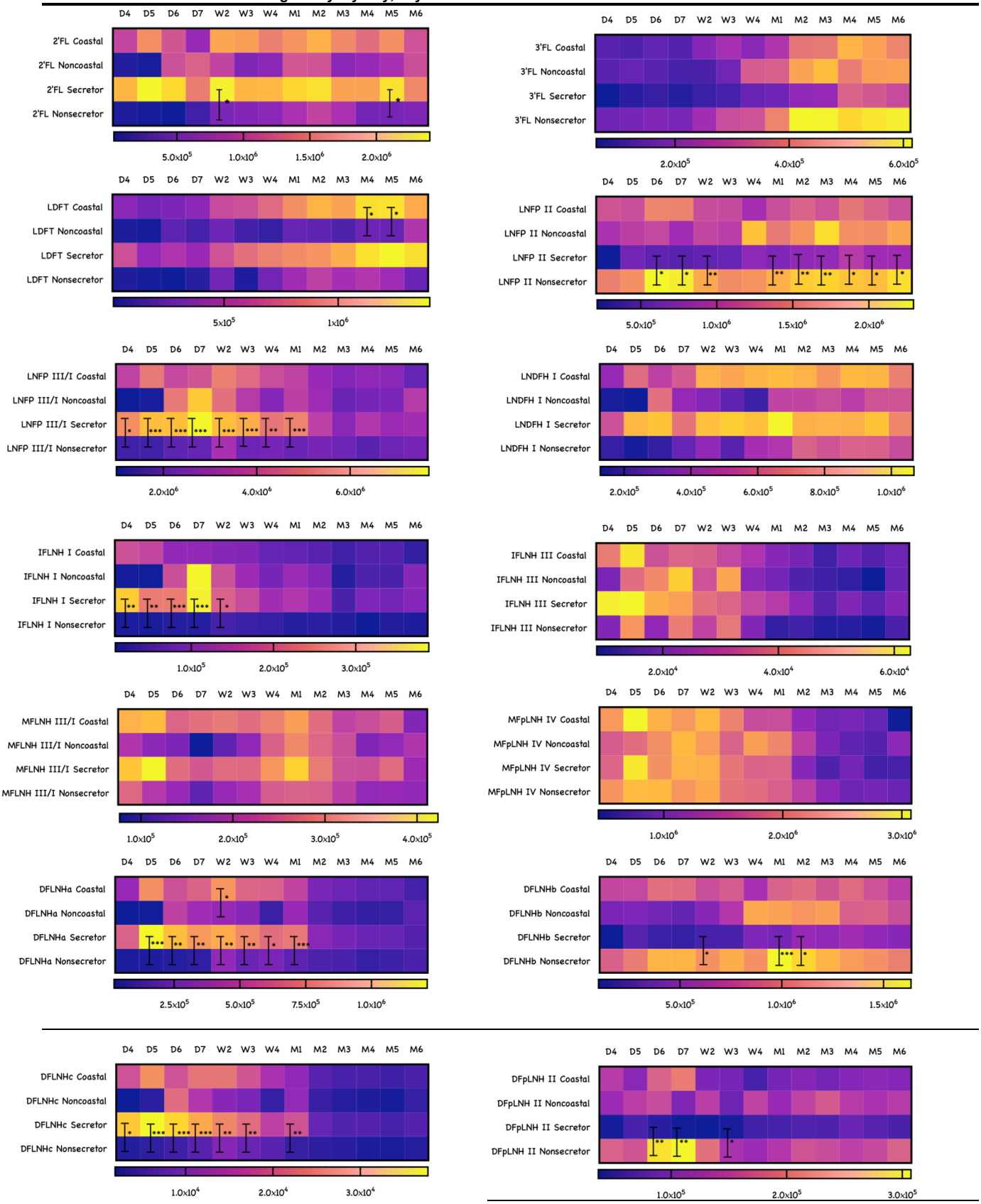
SI Table 22: Tukey's Multiple Comparisons Test on Annotated Undecorated HMOs at Different Lactation Points from Breastmilk of Coastal, Noncoastal, Secretor, and Nonsecretor Mothers living in Baybay City, Leyte.

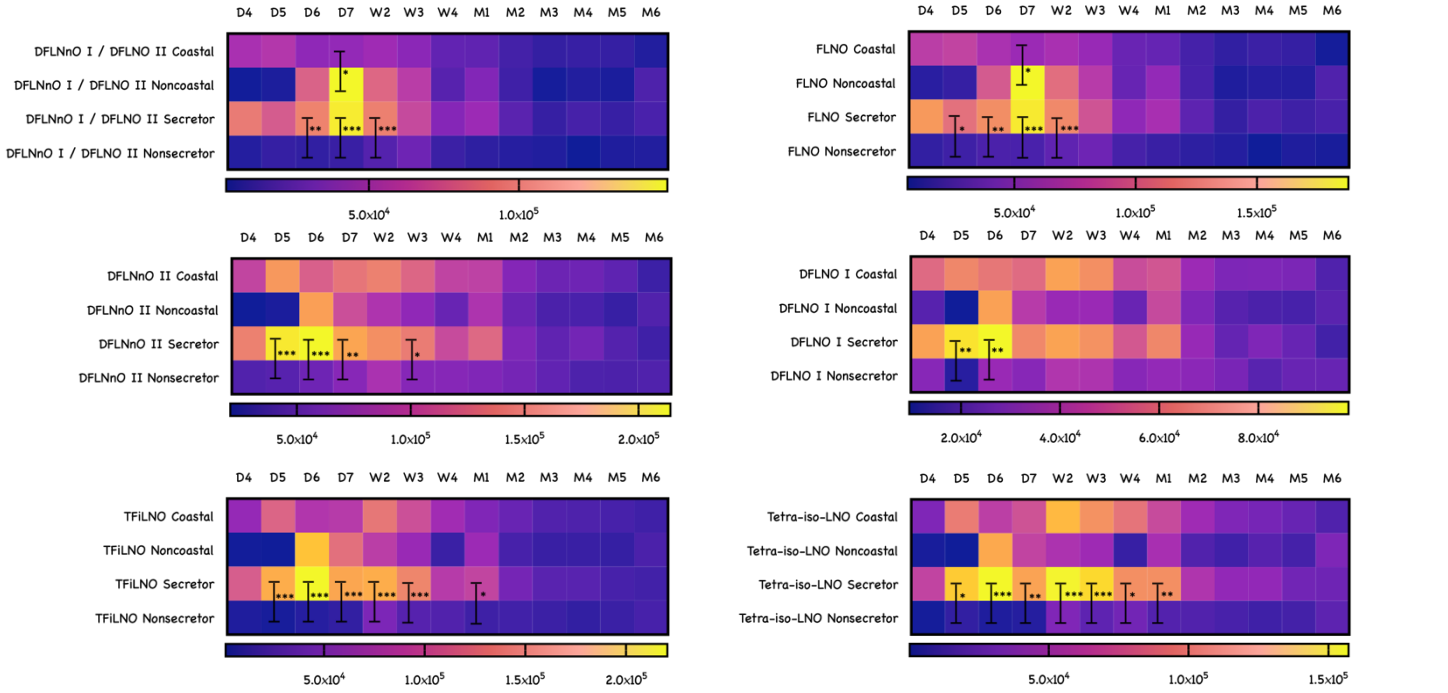




* identifies significant results at P values ≤ 0.05
** identifies highly significant results at P values ≤ 0.01
*** identifies very high significant results at P values ≤ 0.001

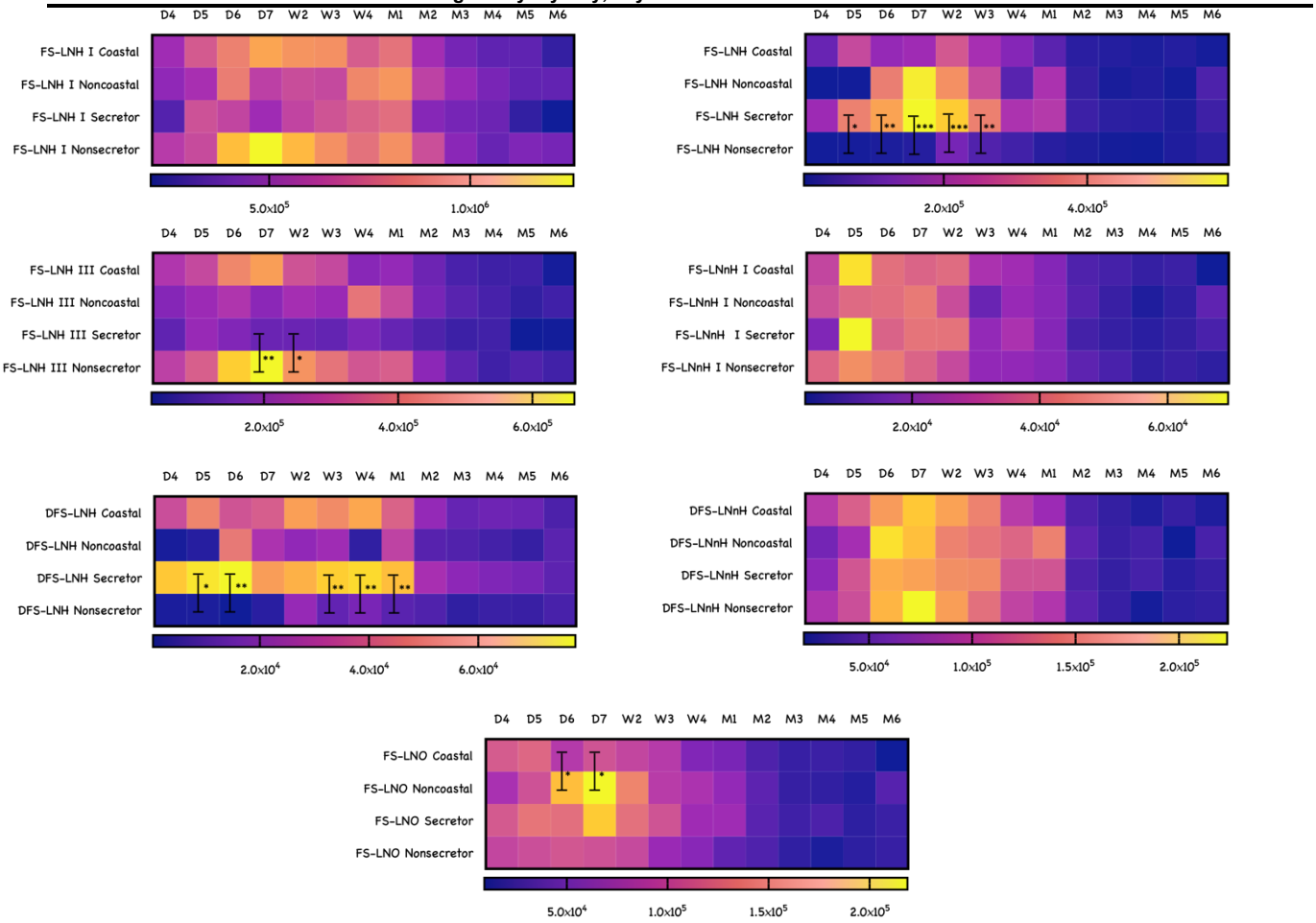
SI Table 23: Sidak's Multiple Comparisons Test on Coastal, Noncoastal, Secretor, and Nonsecretor Annotated Fucosylated HMOs at Different Lactation Points from Mothers living in Baybay City, Leyte.



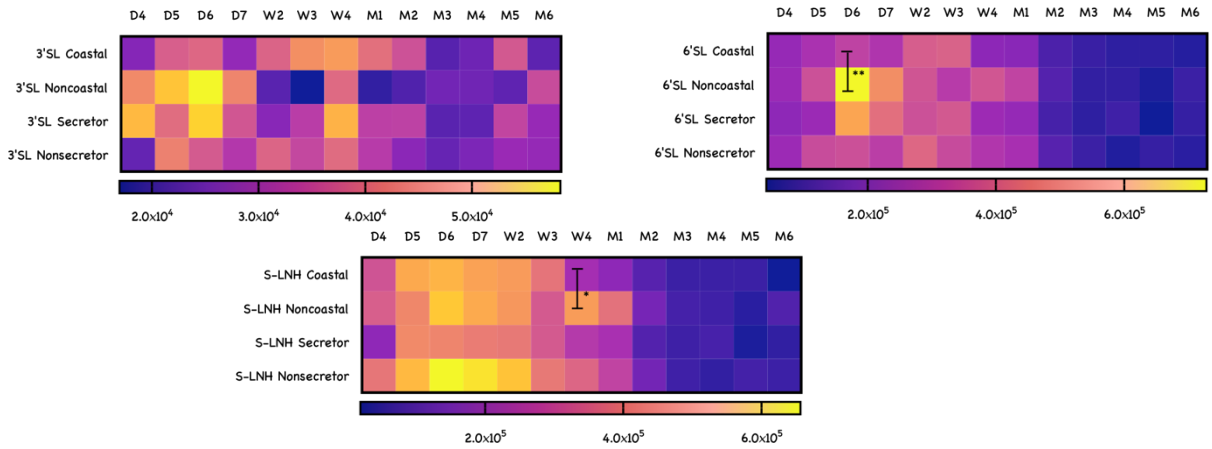


* identifies significant results at P values ≤ 0.05
** identifies highly significant results at P values ≤ 0.01
*** identifies very high significant results at P values ≤ 0.001

SI Table 24: Sidak's Multiple Comparisons Test on Coastal, Noncoastal, Secretor, and Nonsecretor Annotated Fuc-Sialylated HMOs at Different Lactation Points from Mothers living in Baybay City, Leyte.

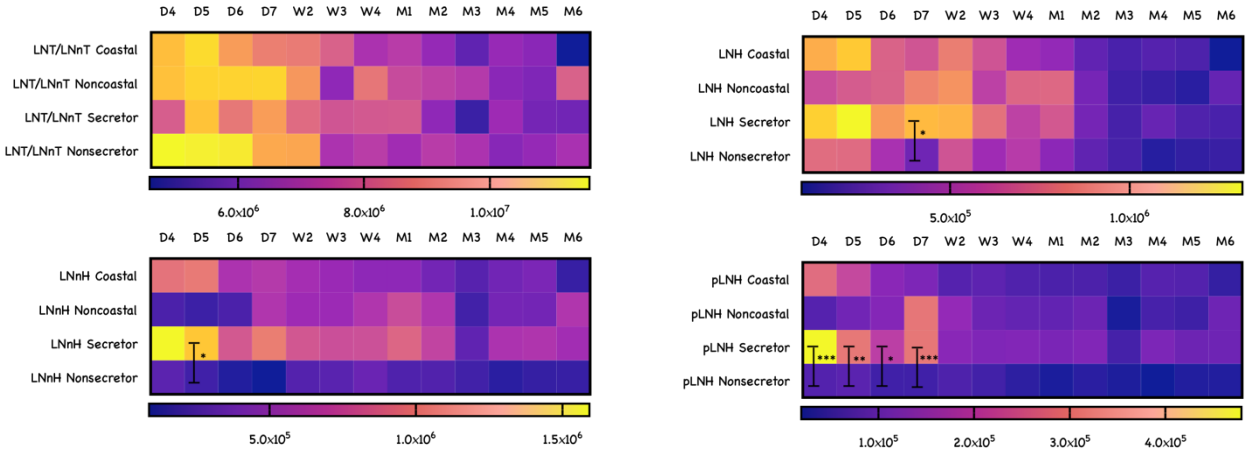


SI Table 25: Sidak's Multiple Comparisons Test on Coastal, Noncoastal, Secretor, and Nonsecretor Annotated Sialylated HMOs at Different Lactation Points from Mothers living in Baybay City, Leyte.

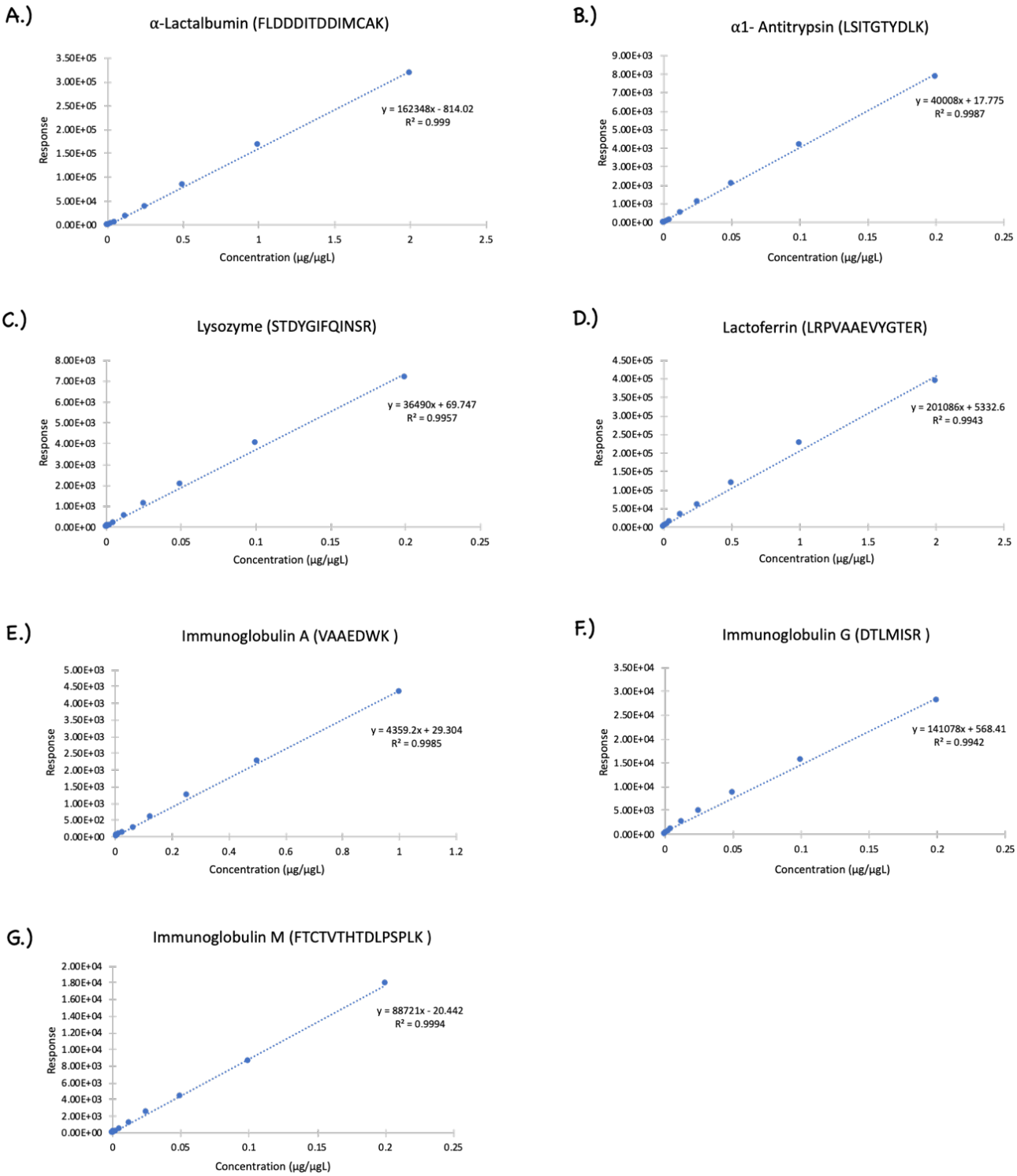


* identifies significant results at P values ≤ 0.05
** identifies highly significant results at P values ≤ 0.01
*** identifies very high significant results at P values ≤ 0.001

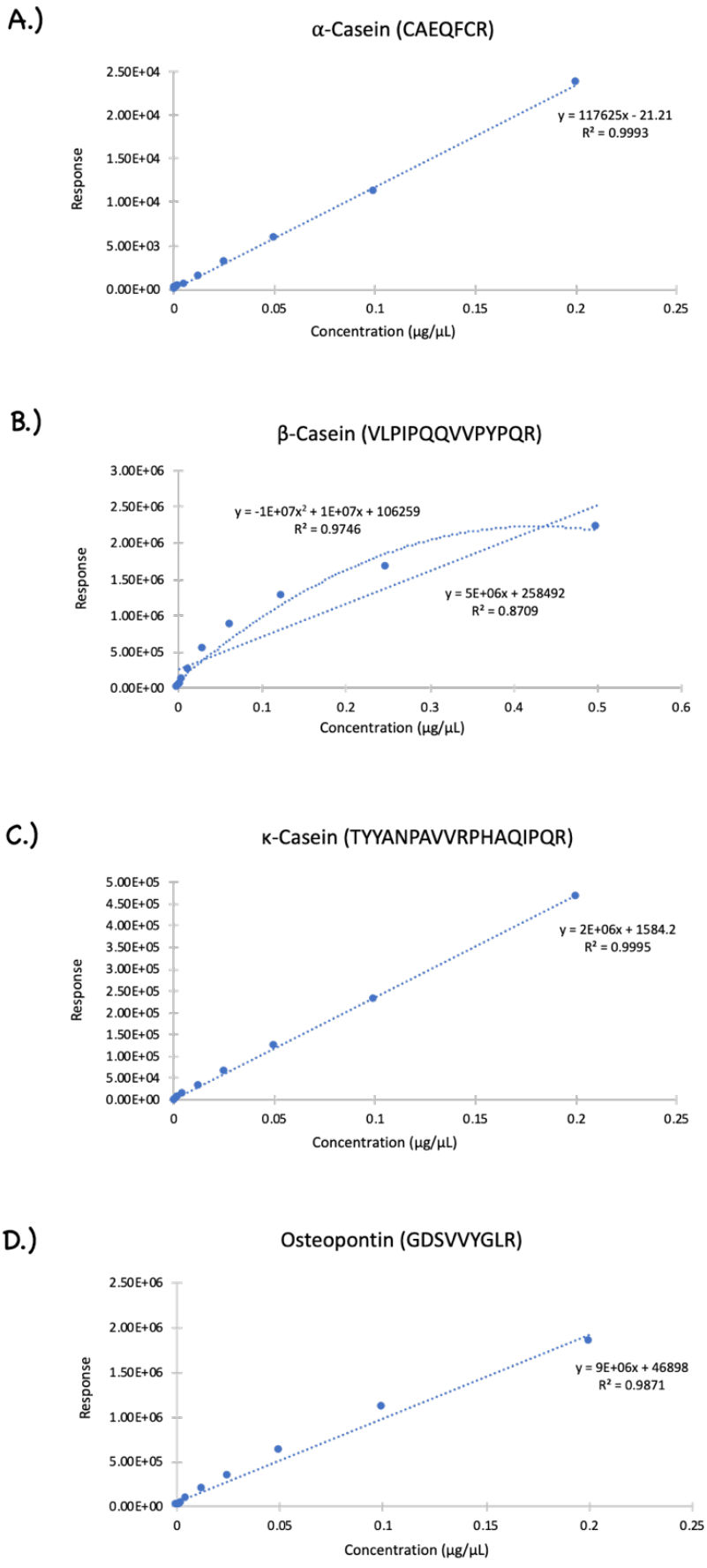
SI Table 26: Sidak's Multiple Comparisons Test on Coastal, Noncoastal, Secretor, and Nonsecretor Annotated Undecorated HMOs at Different Lactation Points from Mothers living in Baybay City, Leyte.



* identifies significant results at P values ≤ 0.05
** identifies highly significant results at P values ≤ 0.01
*** identifies very high significant results at P values ≤ 0.001

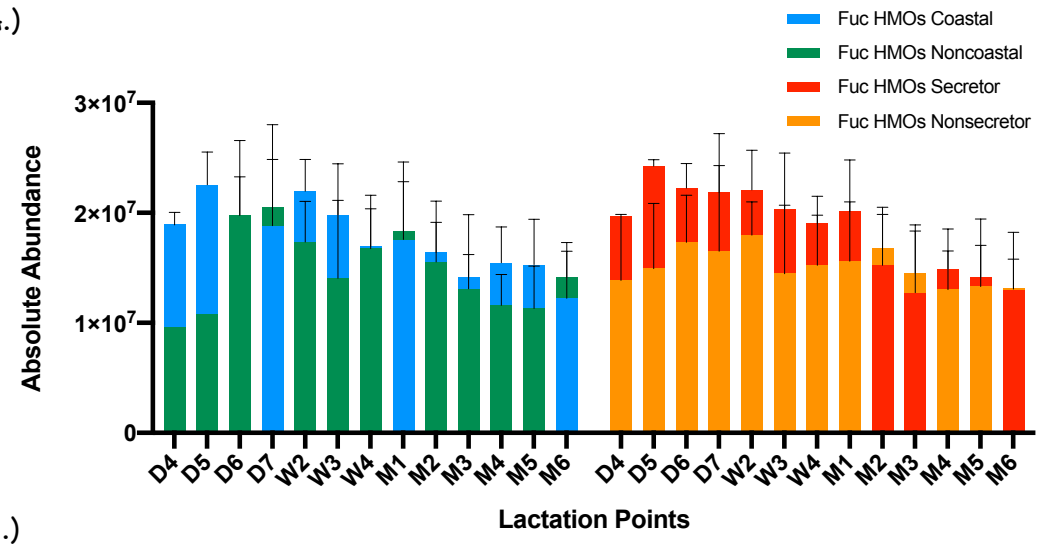


SI Figure 1: Peptide Standard Calibration Curves of Tryptic Digest Protein Standards: A.) α-Lactalbumin, B.) α1-Antitrypsin, C.) Lysozyme, D.) Lactoferrin, E.) Immunoglobulin A, F.) Immunoglobulin G, and G.) Immunoglobulin M using QqQ-LC/MS MRM Analysis.

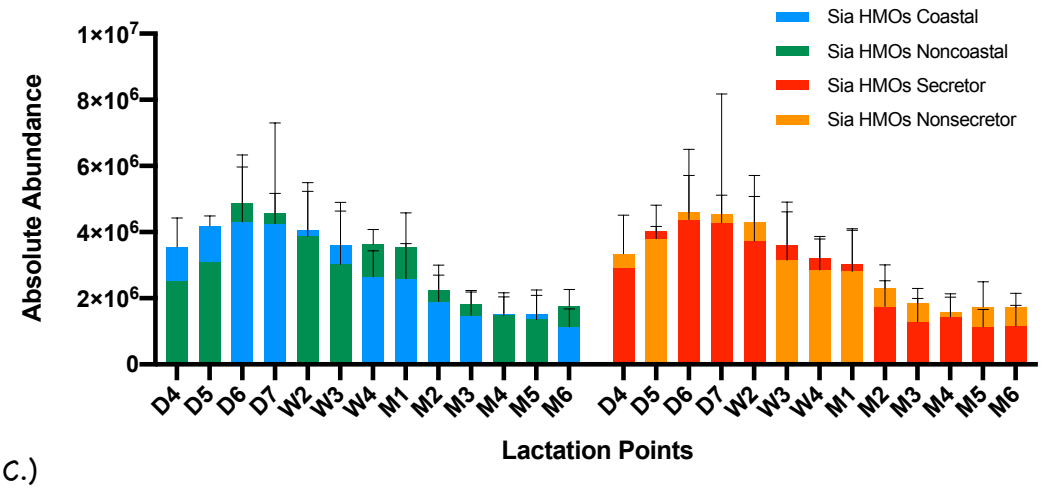


SI Figure 2: Peptide Standard Calibration Curves of Synthesized Tryptic Peptide Standards: A.) α-Casein, B.) β-Casein, C.) κ-Casein, and D.) Osteopontin using QqQ-LC/MS MRM Analysis.

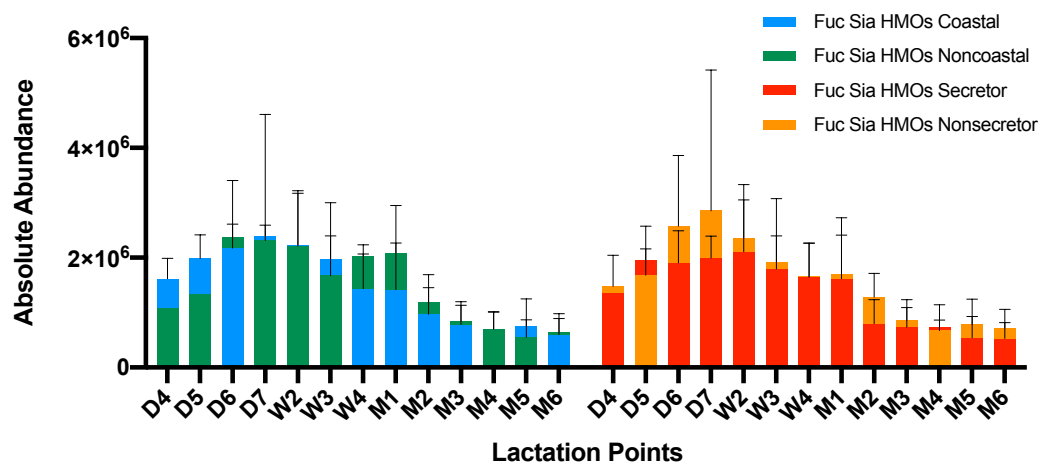
A.)


Fuc HMOs Coastal
Fuc HMOs Noncoastal
Fuc HMOs Secretor
Fuc HMOs Nonsecretor
Absolute Abundance
3×10^7
2×10^7
1×10^7
0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6 D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
Lactation Points

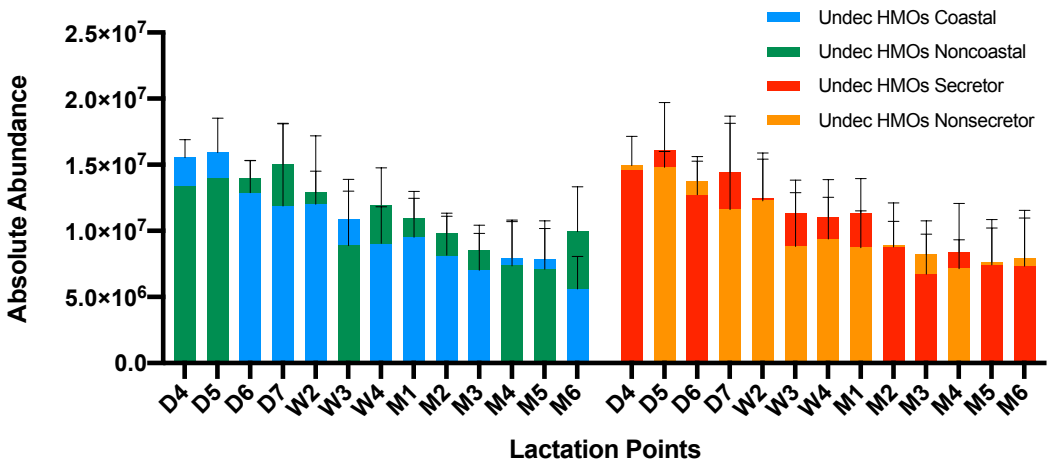
B.)


Sia HMOs Coastal
Sia HMOs Noncoastal
Sia HMOs Secretor
Sia HMOs Nonsecretor
Absolute Abundance
1×10^7
8×10^6
6×10^6
4×10^6
2×10^6
0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6 D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
Lactation Points

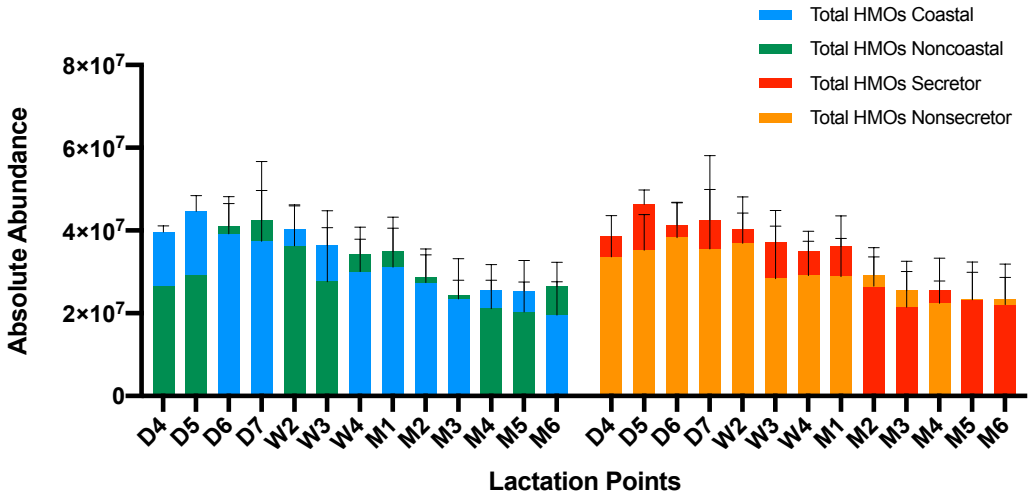
C.)


Fuc Sia HMOs Coastal
Fuc Sia HMOs Noncoastal
Fuc Sia HMOs Secretor
Fuc Sia HMOs Nonsecretor
Absolute Abundance
6×10^6
4×10^6
2×10^6
0
D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6 D4 D5 D6 D7 W2 W3 W4 M1 M2 M3 M4 M5 M6
Lactation Points

D.)



E.)



SI Figure 3: Mean Absolute Abundances of A.) Fucosylated, B.) Sialylated, C.) Fuc-Sialylated, D.) Undecorated and E.) Total HMOs throughout the First Six Months of Lactation from Coastal, Noncoastal, Secretor and Nonsecretor Mothers living in Baybay City, Leyte.