

Journal of Dental Research

Spatial patterns of dental disease in patients with low salivary flow

Journal:	<i>Journal of Dental Research</i>
Manuscript ID	Draft
Manuscript Type:	Research Reports
Date Submitted by the Author:	n/a
Complete List of Authors:	Proctor, Diana; Stanford University School of Medicine Seiler, Christof; Maastricht University Burns, Adam; Stanford University School of Medicine Walker, Samuel; UCSF Jung, Tina; UCSF Weng, Jonathan; UCSF Sastiel, Shanne; UCSF Rajendran, Yoga; UCSF Millman, Meredith; UCSF Armitage, Gary; UCSF Loomer, Peter; UT Health San Antonio School of Dentistry Holmes, Susan; Stanford University Ryder, Mark; University of California, San Francisco, Dept of Orofacial Sciences Relman, David; Stanford University School of Medicine; Veterans Affairs Palo Alto Health Care System,
Keywords:	Microbiome, Microbial ecology, Caries, Bacteria, Sjögren's syndrome, Saliva
Abstract:	Low salivary flow, or hyposalivation, is associated with an increased incidence of dental caries and a shift in their location from biting surfaces towards coronal and root surfaces. However, the relationship between salivary flow and periodontal disease is less clear. To identify clinical indicators of low salivary flow -- including the spatial pattern of dental and periodontal disease, features of the supra- and subgingival microbiota, and symptoms of dry mouth -- we enrolled individuals into two cohorts. The low flow cohort (N = 32) consisted of individuals with a presumptive diagnosis of the autoimmune disorder Sjögren's Syndrome (SS) while the control cohort (N = 119) consisted of healthy controls. We constructed a series of tooth-specific linear models to quantify the extent to which patient cohort, age, and unstimulated whole salivary flow rate (UWS-FR), independent of each other, are associated with dental and periodontal disease at each tooth. While age and a diagnosis of SS correlated with the site-specific increment of disease so too did UWS-FR. Not only were lower UWS-FRs associated with a greater number of decayed, missing, or filled surfaces at 21 teeth, but they were also associated with increased recession, as measured by clinical attachment loss (CAL), at 10 teeth (adjusted p < 0.05). In addition, we examined

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

microbiota community structure at different tooth sites using data from 427 subgingival and supragingival samples of 6 individuals and found that microbial dispersal is reduced in patients with low salivary flow, but only at supragingival and not at subgingival sites. Finally, we found that complaints by subjects of a negative impact on overall quality of life were associated with a UWS-FR less than 0.1 mL/min. Overall, our results suggest that novel predictors of hyposalivation can be identified by integrating clinical, microbial, and patient history data.

SCHOLARONE™
Manuscripts

1
2
3 1 Spatial patterns of dental disease in patients with low salivary flow
4
5
6 2 Diana M. Proctor^{1,2}, Christof Seiler^{3,6}, Adam R. Burns¹, Samuel Walker⁴, Tina Jung⁴, Jonathan Weng⁴,
7
8 3 Shanne Sastiel⁴, Yoga Rajendran⁴, Yvonne Kapila⁴, Meredith E. Millman⁴, Gary C. Armitage⁴, Peter M.
9
10 4 Loomer⁵, Susan P. Holmes⁶, Mark I. Ryder⁴, David A. Relman^{1,7,8*}
11
12 5
13
14
15
16 6 **Affiliations**
17
18 7 ¹Division of Infectious Disease & Geographic Medicine, Department of Medicine, Stanford University
19
20 8 School of Medicine, Stanford, CA 94305 USA
21
22 9 ²National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892 USA
23
24 10 ³Department of Data Science and Knowledge Engineering, Mathematics Centre Maastricht, Maastricht
25
26
27 11 University, The Netherlands
28
29 12 ⁴Division of Periodontology, University of California, San Francisco School of Dentistry, San
30
31 13 Francisco, CA 94143 USA
32
33
34 14 ⁵School of Dentistry, University of Texas Health Science Center at San Antonio, San Antonio, TX
35
36 15 78229 USA
37
38 16 ⁶Department of Statistics, Stanford University, Stanford, CA 94305 USA
39
40
41 17 ⁷Infectious Diseases Section, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304
42
43 18 USA
44
45
46 19 ⁸Department of Microbiology & Immunology, Stanford University School of Medicine, Stanford, CA
47
48 20 94305 USA
49
50 21 *Corresponding author: David A. Relman: relman@stanford.edu; Address: Encina E209, 616 Jane
51
52 22 Stanford Way, Stanford, California 94305-6165; Phone: 650-736-6822; Fax: 650-852-3291
53
54
55 23
56
57
58
59
60

1
2
3 24 Abstract word count: 295
4
5 25 Total word count: 5064
6
7 26 Tables/Figures: 5
8
9 27 References: 31
10
11 28 Keywords: supragingival microbiota, subgingival microbiota, dental caries, Sjögren's Syndrome,
12
13 29 hyposalivation, microbial dispersal, xerostomia
14
15
16 30
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 31 **Abstract**
4
5
6

7 32 Low salivary flow, or hyposalivation, is associated with an increased incidence of dental caries
8 33 and a shift in their location from biting surfaces towards coronal and root surfaces. However, the
9 34 relationship between salivary flow and periodontal disease is less clear. To identify clinical indicators of
10 35 low salivary flow -- including the spatial pattern of dental and periodontal disease, features of the supra-
11 36 and subgingival microbiota, and symptoms of dry mouth -- we enrolled individuals into two cohorts.
12
13 37 The low flow cohort ($N = 32$) consisted of individuals with a presumptive diagnosis of the autoimmune
14 38 disorder Sjögren's Syndrome (SS) while the control cohort ($N = 119$) consisted of healthy controls. We
15 39 constructed a series of tooth-specific linear models to quantify the extent to which patient cohort, age,
16 40 and unstimulated whole salivary flow rate (UWS-FR), independent of each other, are associated with
17 41 dental and periodontal disease at each tooth. While age and a diagnosis of SS correlated with the site-
18 42 specific increment of disease so too did UWS-FR. Not only were lower UWS-FRs associated with a
19 43 greater number of decayed, missing, or filled surfaces at 21 teeth, but they were also associated with
20 44 increased recession, as measured by clinical attachment loss (CAL), at 10 teeth (adjusted $p < 0.05$). In
21 45 addition, we examined microbiota community structure at different tooth sites using data from 427
22 46 subgingival and supragingival samples of 6 individuals and found that microbial dispersal is reduced in
23 47 patients with low salivary flow, but only at supragingival and not at subgingival sites. Finally, we found
24 48 that complaints by subjects of a negative impact on overall quality of life were associated with a UWS-
25 49 FR less than 0.1 mL/min. Overall, our results suggest that novel predictors of hyposalivation can be
26 50 identified by integrating clinical, microbial, and patient history data.

51
52
53
54
55
56
57
58
59
60

1

2

3

52 Introduction

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

On average, the unstimulated whole salivary flow rate (UWS-FR) of healthy adults ranges between 0.3 and 0.4 mL/min (Becks and Wainwright 1943). Clinically, hyposalivation, or low salivary flow, is defined as an UWS-FR < 0.1 mL/min. Medication is the most common cause of hyposalivation and Sjögren's Syndrome (SS), a prevalent chronic autoimmune disorder, is a second major cause (von Bultzingslowen et al. 2007). In contrast to patients who experience low salivary flow due to irradiation of cancers involving the head and neck, these two patient populations experience an insidious onset of hyposalivation with delayed diagnoses. Most Sjogren's patients are diagnosed 6.5 or more years after the onset of xerostomia and nine years after tooth loss has occurred (Baudet-Pommel et al. 1994; Mignogna et al. 2005; Shibuski et al. 2012).

Delays in diagnosing hyposalivation may be due to several reasons. First, the salivary flow rate threshold of 0.1 ml/min is imperfectly correlated with clinical phenotypes. Individuals with flow rates < 0.1 mL/min may not experience signs or symptoms of low salivary flow, while individuals with flow rates as high as 0.3 ml/min may complain of dry mouth (Ben-Aryeh et al. 1985; Dawes 2004). Second, hyposalivation may antedate xerostomia, and patients often suffer from reduced salivary flow for years before it is perceived (Mathews et al. 2008; von Bultzingslowen et al. 2007). Finally, even when patients do report xerostomia to physicians, UWS-FR is not measured in the primary care setting. Collectively these factors lead to diagnostic delays, preventable tooth loss, and an increased burden of caries in medicated patients and in patients with SS (Baudet-Pommel et al. 1994).

Individuals with hyposalivation not only experience more caries, but they also experience a shift in the spatial pattern of dental disease. In the elderly, the risk of root surface caries in the lower jaw increases with the number of prescribed medications with xerostomizing effects (Kitamura et al. 1986).

1
2
3 74 Likewise, surgical removal of the salivary glands in rodents leads to an increase in root surface caries
4
5 75 affecting mandibular surfaces (Bowen et al. 1988). Compared with healthy individuals, patients with SS
6
7 76 are at an increased risk of developing not only root surface, but also coronal caries (Ravald and List
8
9 77 1998). Irradiation of salivary glands in the course of cancer therapy leads to caries on the incisal edges
10
11 78 of anterior teeth, cusp tips of posterior teeth, and lingual surfaces (Dreizen et al. 1977). Shifts in the site-
12
13 79 specific occurrence of disease in 3 distinct patient populations suggests salivary flow normally functions
14
15 80 to protect mandibular root and coronal sites from caries. Moreover, these population level data imply
16
17 81 that salivary flow normally plays a role – either directly or indirectly -- in maintaining a beneficial
18
19 82 microbiota at these sites since the metabolic activity of the microbial consortia at the tooth surface must
20
21 83 change in order for cavitation to occur. When it occurs chronically, the loss of salivary flow leads to a
22
23 84 persistent shift in the spatial patterning of the oral microbiota (Proctor et al. 2018) .
24
25
26
27
28
29
30 85 Uncertainties surrounding the flow rate threshold predictive of increased disease risk highlight
31
32 86 the need for new diagnostic indicators, which may be used to predict low flow, before caries occur.
33
34 87 Towards that end, we sought to identify features of xerostomia and the microbiome that correlate with
35
36 88 flow rate and which may predict hyposalivation, since such features would permit early intervention.
37
38 89 Our results suggest that chronic low salivary flow is associated with an increase in DMFS and CAL at a
39
40 90 wider array of tooth sites than previously appreciated. In addition, we identify features of the microbiota,
41
42 91 including reduced rates of microbial dispersal, and symptoms of dry mouth that may serve as indicators
43
44 92 of hyposalivation. These observations are significant since they indicate new avenues for diagnosing
45
46 93 hyposalivation, a condition for which UWS-FR is imperfectly sensitive and specific.
47
48
49
50
51 94
52
53
54 95
55
56
57
58
59
60

1
2
3 96 **Results**
4
5
6
7 97 **Clinical and demographic features of the patient population**
8
9
10 98 The complete set of inclusion and exclusion criteria for each cohort is provided as supplementary
11
12 99 data (**Supplementary Methods**). Data were collected from 151 participants in 2 cohorts: the low flow
13
14 100 cohort (N = 32) consisted of individuals with a presumptive diagnosis of SS while the control cohort (N
15
16 101 = 119) consisted of healthy controls. Gender identity, race, and ethnicity did not significantly differ
17
18 102 between the two groups (Chi-square tests, p > 0.1) (**Table S1**). The majority of subjects in both cohorts
19
20
21 103 were White or Asian and did not identify as Hispanic or Latino. The proportion of females in the low
22
23
24 104 flow group did not significantly differ from that of the controls (Chi-square test, p = 0.2), though
25
26 105 substantially more females than males were enrolled in both cohorts. Roughly 93% of the low flow
27
28 106 cohort identified as female, consistent with the known sex bias in the occurrence of SS. Likewise, SS
29
30 107 tends to be diagnosed in middle to late life, and the average age of individuals in the low flow cohort
31
32
33 108 (mean, 60.8 years) was higher than the average age of controls (mean, 32.6 years). Despite a mean
34
35 109 difference of 28 years, there was considerable overlap between the two groups (**Figure 1a**; 95%
36
37 110 confidence intervals (CIs) surrounding the unpaired mean difference, 23.8-32 years). Indeed, the controls
38
39
40 111 appeared to segregate into 2 groups, the largest group consisting of subjects under the age of 40 and the
41
42 112 second group reflecting our push to enroll controls similar in age to the SS patients. Despite our best
43
44 113 efforts, just 20% of the controls sampled in this study were over the age of 40.
45
46
47
48 114 As expected, based on inclusion and exclusion criteria, UWS-FRs varied significantly between
49
50 115 the two groups (**Figure 1b**). The mean UWS-FR of patients in the control cohort (0.470 mL/min) was
51
52 116 on average 0.335 mL/min higher than the mean rate of the low flow cohort (0.129 mL/min) (95% CI,
53
54 117 0.253--0.410 mL/min). Given that we excluded individuals with active disease, all measures of disease
55
56
57
58
59
60

1
2
3 118 reflect a past history of dental disease.
4
5

6
7 119 Further, the incidence of past dental disease was higher in the low flow cohort compared to the
8
9 120 controls. The mean difference in decayed missing filled surfaces (DMFS) between cohorts differed
10
11 121 significantly from 0 (**Figure 1c**) (95% CI, 30.4-52.4) with a mean DMFS of 27.5 in controls and mean
12
13 122 DMFS of 68.9 in the low flow cohort. Similarly, mean clinical attachment loss (CAL) was significantly
14
15 123 higher in the low flow cohort (2.25) compared to the controls (0.891) with a mean difference of 1.38
16
17 124 (**Figure 1d**) (95% CI, 1.01-1.74). The gingival margin cemento-enamel junction (GM-CEJ) was also
18
19 125 higher in the low flow cohort (mean, 5.23) compared to controls (mean, 2.93) with a substantial mean
20
21 126 difference of 2.3 (**Figure 1e**) (95% CI, 1.68-2.88). Despite higher measures of DMFS, CAL, GM-CEJ
22
23 127 in the low flow cohort, bleeding on probing (BOP) and probing depth (PD) did not significantly differ
24
25 128 between the two groups suggesting differences in GM-CEJ and CAL reflected past or chronic
26
27 129 periodontal disease rather than active disease (**Figures 1f, 1g**).
30
31
32
33 130
34
35
36 131 **Age and cohort exert independent effects on the spatial pattern of dental disease**
37
38
39 132 Given that age is known to be correlated with the prevalence of dental disease (Algarni et al.
40
41 133 2018; Kassebaum et al. 2017) we sought to evaluate the relationship between salivary flow and site-
42
43 specific prevalence of dental disease while controlling for age. We used regression coefficients to
44
45 134 quantify the extent to which the site-specific increment of disease (DMFS, CAL, GM-CEJ, BOP, PD)
46
47 135 varied depending on a one-unit change in each of several standardized predictors (age, UWS-FR, cohort,
48
49 136 and the interaction between cohort and UWS-FR) while holding the other 3 predictors constant.
50
51 137
52
53
54 138 A site specific DMFS increment was directly correlated with age across virtually all teeth
55
56
57
58
59
60

1
2
3 139 (adjusted p < 0.05) excluding 3 anterior teeth (teeth 6, 24, 27). The site-specific effects of age can be
4
5 140 discerned by comparing the regression coefficients for each tooth to each other. At tooth 8, DMFS
6
7 141 increased by 0.076 Ordered Quantile (ORQ) transformed surfaces for each year of life while at tooth 15
8
9 142 DMFS increased by 0.289 QRQ surfaces, a rate approximately 3.8 times higher (**Figure 2a**). Indeed,
10
11 143 DMFS coefficients for age were about 3-20 times higher at posterior compared to anterior sites,
12
13 144 consistent with a higher rate of attack for posterior compared to anterior teeth (**Figure 2a**). Similarly,
14
15 145 age was a significant and direct predictor of CAL (**Figure 2b**), and GM-CEJ (**Figure 2c**) for all tooth
16
17 146 sites (adjusted p < 0.05). While coefficients for periodontal measures tended to be higher at posterior
18
19 147 versus anterior teeth, in both jaws, coefficients did not clearly segregate into anterior and posterior
20
21 148 groups, as they did for DMFS. In contrast to CAL and GM-CEJ, BOP and PD were correlated with age
22
23 149 at a limited number of tooth sites. BOP (**Figure 2d**) was correlated with age at 4 mandibular sites (teeth
24
25 150 19, 23, 24, 30) and 1 maxillary site (tooth 2) while PD (**Figure 2e**) was significantly correlated with age
26
27 151 at 3 maxillary sites (teeth 2, 5, 15) and 1 mandibular site (tooth 24).

32
33
34 152 Next, we sought to examine the independent contribution of cohort, or having a presumptive
35
36 153 diagnosis of SS, on the site-specific increment of dental disease. Across all disease metrics, the
37
38 154 regression coefficients for cohort were smaller than those for age, suggesting that cohort has a smaller
39
40 155 effect size than age, though it still explains variation in the occurrence of dental disease. With a
41
42 156 categorical variable as the predictor, the regression coefficients represent the difference in the average
43
44 157 disease increment between the control and low flow cohorts. In the low flow cohort, 16 teeth had
45
46 158 significant coefficients that could be distinguished from zero (**Figure 2f**). Negative coefficients were
47
48 159 observed at several anterior teeth in the maxilla (teeth 6, 8, 11) and mandible (teeth 22, 23, 24, 25). In
50
51 160 addition, while both groups tended to have more caries at posterior sites, low flow subjects had between
52
53 161 0.09 and 0.23 more QRQ DMFS per tooth at maxillary teeth 3, 5, 6, and 14, and mandibular teeth 18,

1
2
3 162 19, 30, and 31, as compared to the reference group.
4
5
6 163 After adjusting for multiple testing, CAL differed significantly at 21 sites between patient cohorts
7
8
9 164 (**Figure 2g**). Compared to controls, recession was on average 0.48 QRQ mm (range, 0.41-0.56 QRQ
10 mm) greater in the low flow cohort at anterior maxillary teeth (teeth 6, 7, 8, 9, 10, 11) compared to
11 controls, again holding all other predictors constant. Similarly, CAL was greater by an average of 0.53
12 mm at anterior mandibular (teeth 22, 23, 24, 25, 26, 27) sites, 0.34 mm at posterior maxillary (teeth
13 166 3, 4, 5, 12, 13, 14) and 0.39 QRQ mm at posterior mandibular (teeth 20, 21, 30) sites. In contrast to CAL,
14
15
16 168 GM-CEJ tended to be greater in the low flow cohort compared to controls at a more limited number of
17 teeth (**Figure 2h**), including just 9 teeth in the mandible (teeth 19, 21, 22, 23, 24, 25, 26, 27, 30) and 3
18 in the maxilla (teeth 7, 10, 12). BOP was not significantly correlated with cohort at any site (**Figure 2i**)
19
20 while patients with low flow had higher PD at predominantly maxillary sites (teeth 3, 4, 6, 8) though one
21
22 mandibular site (tooth 24) also reached significance (**Figure 2j**).
23
24
25
26
27
28
29
30
31
32

33 174 Since we enrolled individuals with a presumptive diagnosis of Sjogren's Syndrome in our low
34 flow cohort, effects related to cohort could be attributable to a reduction in salivary flow as well as
35 changes in salivary composition or immune function. To assess the explicit effect of flow rate on dental
36 disease, we also included UWS-FR in our models, allowing us to see its contribution independent of
37 either age or cohort. Compared to age and cohort, coefficient sizes for UWS-FR were universally smaller
38 across all disease metrics, indicating an overall smaller effect of flow rate on disease outcomes. Despite
39 a relative difference in the magnitude of effect sizes, UWS-FR was significantly correlated with DMFS,
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 185 increased the number of DMFS decreased at these sites. In contrast to the wide variety of sites exhibiting
4
5 186 a correlation between DMFS and UWS-FR, CAL was correlated with UWS-FR at a smaller number of
6
7 187 sites (**Figure 2l**) including 3 sites in the upper right jaw (teeth 2, 5, 6) and 7 sites in the lower jaw (teeth
8
9 188 20, 21, 22, 23, 27, 27, 31). Even fewer sites exhibited a correlation between GM-CEJ and UWS-FR
10
11 189 (**Figure 2m**), including 2 molars (teeth 2, 19) and 1 incisor (tooth 26). BOP was correlated with UWS-
12
13 190 FR at just one tooth site (tooth 9; (**Figure 2n**) while PD was not correlated with UWS-FR at any site
14
15 191 (**Figure 2o**). Taken together, these data suggest that in patients with a presumptive diagnosis of Sjogren
16
17 192 Syndrome there is a detectable increase in the site-specific increment of caries and periodontal disease
18
19 193 that is attributable to salivary flow rate.
20
21
22
23
24

25 194 Next, we explored the interaction between cohort and UWS-FR. In particular, we were interested
26
27 195 in knowing whether the direction or magnitude of the effect of UWS-FR differed between the reference
28
29 196 cohort (i.e, controls) and the low flow cohort. Out of 25 significant coefficients for the interaction
30
31 197 between cohort and UWSFR as a predictor of DMFS, 22 were negative. The mandibular molars were
32
33 198 the exception (tooth 18, 19, 30) with coefficients that ranged between 0.10-0.17 (**Figure 2p**). Consistent
34
35 199 with this finding, we observed in a less sophisticated model that as UWS-FR decreased in the low flow
36
37 200 cohort the DMFS increment increased at a greater rate than the DMFS increment increased with
38
39 201 reductions in UWS-FR in the control cohort (**Supplementary Figure 1**). Several coefficients for the
40
41 202 interaction between cohort and CAL were also significant in the maxilla (teeth 4, 5, 6, 10, 11, 12, 13,
42
43 203 15) and mandible (teeth 19, 20, 28, 31) (**Figure 2q**) with similar patterns of disease observed when
44
45 204 considering GM-CEJ (**Figure 2r**). The interaction between cohort and UWS-FR was not significantly
46
47 205 correlated with BOP (**Figure 2s**) or PD (**Figure 2t**) at any site.
48
49
50
51
52
53
54 206
55
56
57
58
59
60

1
2
3 207 **Impact of age and low salivary flow on the microbiota**
4
5

6
7 208 To examine whether the differences in past dental disease were associated with shifts in the oral
8
9 209 microbiota we next analyzed the relationship of age, cohort, and UWS-FR to structure of the subgingival
10
11 210 microbiota from each of 3 control and 3 low flow patients. Subgingival microbiota analysis from the
12
13 211 first molars, canines, and central incisors was integrated with previously-published data from the
14
15 212 supragingival microbiota at these same teeth in these same 6 patients (Proctor et al. 2018).
16
17

18
19 213 Differences in structure of the supragingival and subgingival communities, based on Bray Curtis
20
21 214 dissimilarity, explained segregation of samples along axis 1, which accounted for 17.6% of the variation
22
23 215 (**Figure 3**). UWS-FR explained differences among subgingival and supragingival communities along
24
25 216 axis 2, which accounted for 12.8% of the variation in the data (**Figure 3a**). On the other hand, age co-
26
27 217 segregates with UWS-FR at subgingival but not at supragingival sites (**Figure 3b**). Supragingival sites
28
29 218 scored as 1 or 2 DMFS clustered together in the low flow cohort segregating from supragingival sites
30
31 219 with 0 DMFS (**Figure 3c**). At subgingival sites, samples from younger control subjects tended towards
32
33 219 positive scores along axis 2 while samples from the older low flow subjects tended towards neutral to
34
35 220 negative scores along axis 2. At supragingival sites, the one older control subject's (55.2 years)
36
37 221 supragingival samples clustered with the two young control subject's communities (23 and 28 years).
38
39
40 222 Similar results were obtained when using different distance metrics (**Supplementary Figure 2**).
41
42
43 223 Examining axis 1 scores as a function of tooth class revealed that subgingival communities, unlike
44
45 224 supragingival communities, do not differ by tooth class in either the low flow or control cohorts
46
47 225 (**Supplementary Figure 3**).
48
49
50 226
51
52

53 227 Constrained correspondence analysis revealed that age, GM-CEJ, PD and CAL were correlated
54
55 228 with each other and explained the segregation of low flow samples with positive scores along axis 1,
56
57
58

1
2
3 229 consistent with the site-specific regression models (**Figure 2**). Subgingival and supragingival sites from
4 subject 3-303 had higher CAL and lower DMFS values than sites from subject 3-301. At the same time,
5 subgingival and supragingival sites from subject 3-301 were enriched in *Streptococcus* and *Veillonella*
6 species (ASV12, ASV4, ASV7, and ASV8) and distinct from sites that were enriched in *Prevotella*
7 species (*denticola* (ASV11) along axis 2. Samples from control subjects with higher UWS-FR had negative
8 scores on axis 1 along with samples from one low flow subject, 3-302 who had the highest DMFS scores
9 among these subjects (**Supplementary Figure 4**). Taken together, these pilot data suggest that the
10 composition of the microbiota is correlated with age, cohort, and flow rate, potentially reflecting
11 correlations between the incidence of disease at different sites related to these predictors.
12
13
14
15
16
17
18
19
20
21
22
23
24
25 238 **Low salivary flow is associated with an increase in rates of microbial migration**
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

In our prior work, we observed that communities inhabiting exposed tooth surfaces of different tooth classes could be differentiated from one another in controls, but not low flow subjects. Here, we report that subgingival communities do not differ based on tooth class in either cohort, in these 6 individuals. Based on these observations, we hypothesized that reductions in salivary flow result in a net increase in microbial dispersal, defined here as an increase in the movement or retention of microbes between sites, at supragingival sites. To test this hypothesis, we used two models of microbial dispersal, investigating whether rates of microbial migration differ among tooth habitats (subgingival, supragingival) within an individual as well as between individuals based on cohort (i.e., in 3 low flow patients vs. 3 controls).

Regardless of cohort, migration rates were higher at supragingival compared to subgingival sites (**Figure 4, Table S2**). In addition, migration rates were higher in patients with low salivary flow compared to controls, suggesting that salivary flow moderates the rate of migration in the healthy human

1
2
3 251 oral cavity. Further, we observed a significant interaction between participant cohort and the gingival
4 habitat, with a substantial difference in migration between the two cohorts at supragingival sites with a
5 less pronounced difference at subgingival sites. These findings were robust to the method used to infer
6 migration (**Supplementary Figure 5; Supplementary Results**) and suggest an increased rate of
7 migration across the supragingival area of the mouth in the low flow cohort.
8
9
10 254
11
12 255
13
14
15
16 256
17
18
19 257 **Subjective predictors of low salivary flow**

20
21
22 258 Next, we investigated the extent to which complaints of dry mouth are predictive of low salivary
23 flow using a previously developed Visual Analog Scale (VAS) (Pai et al. 2001) containing six questions
24
25 259 on quality of life and feelings of dry mouth, which we adapted by the addition of a question concerning
26
27 260 the extent to which patients experienced dryness of the hard palate. Members of the low flow cohort
28 complained of impaired ability to swallow due to dry mouth, impaired overall quality of life, impaired
29
30 261 ability to speak, as well as feelings of dryness impacting the throat, tongue, lips and hard palate ($p < .01$,
31
32 262
33
34 263
35
36 264 **Supplementary Figure 6).**

37
38
39 265 Individuals rated their responses to questions, on the VAS, based on the perceived severity of
40 their symptoms. A principal component analysis of these ratings was performed to identify questions
41
42 266 whose responses could segregate patients based on the severity of their complaints of dry mouth (**Figure**
43
44 267 **5a**). The first axis explained 86% of the variation in the data and principally segregated patients into the
45
46 268 low and “not low” cohorts with low flow subjects sharing positive scores along axis 1. The 12 control
47
48 269 subjects who grouped with the low flow screening cohort also tended to have a lower UWS-FR than the
49
50 270 61 control subjects who clustered together, though the difference in means was not significant. A
51
52
53 271 conditional inference tree was used to identify the symptomatic complaints that were most effective at
54
55 272
56
57
58
59
60

1
2
3 273 separating patients into low flow and not low flow cohorts (**Figure 5b**). While these results should be
4 validated on a larger dataset, 84.6% of subjects (11/13) in this study who indicated that low salivary flow
5
6 274 negatively impacted their overall quality of life (VAS ≥ 56) had flow rates of less than 0.1 ml/min. On
7
8 275 the other hand, most subjects who rated a negligible impact of low flow on their quality of life as well
9
10 276 as an absence of dryness on their lips had flow rates exceeding 0.1 ml/min. The AUC of our model
11
12 277 exceeded 84% with 10-fold cross validation. These same significant predictors were also identified using
13
14 278 the random forest algorithm as the most discriminative of patients in the low flow versus the control
15
16 279 cohort (**Supplementary Figure 7**).
17
18
19 280
20
21 281
22
23
24 282 **Discussion**
25
26
27 283 Hyposalivation is associated with reduced quality of life, including reduced oral health quality
28
284 of life, even in patients whose dental health is well managed subsequent to diagnosis. Given that patients
29
30 with hyposalivation are typically not diagnosed until they have lost a first tooth to disease, a critical need
31
32 285 in this patient population is the development of methods to identify low salivary flow before the onset
33
34 286 of dental caries, so that interventions can be implemented to prevent deterioration of oral health quality
35
36 287 of life. Our goal was to identify microbial and symptomatic predictors of salivary flow, analyzing multi-
37
38 288 faceted data on the same patient population.
39
40
41 289
42
43
44 290 Aging is associated with spatial patterns in the incidence of dental disease, impacting surfaces
45
46 above and below the gumline (Algarni et al. 2018; Kassebaum et al. 2017). Gingival recession increases
47
48 as a function of age, exposing root surfaces which become vulnerable to caries. UWS-FR has been shown
49
50 292 to decrease as a function of age in individuals taking medications as well as in the otherwise healthy
51
52 293 elderly (Fure and Zickert 1990). Prior work suggests that the function of the submandibular glands
53
54 294 decreases with age, with reductions in UWS and SWS secretions averaging between 22% to 39%,
55
56 295
57
58
59
60

1
2
3 296 respectively (Baum 1981; Pedersen et al. 1985). Reductions in salivary flow may explain the increase in
4
5 297 the incidence of root surface caries subsequent to the sixth decade of life (Sumney et al. 1973). Our data
6
7 298 support the hypothesis that aging and the effects of chronic low salivary flow exert cumulative effects
8
9 299 on the shifting spatial pattern of dental disease in patients with hyposalivation.
10
11
12

13 300 Dental diseases can be considered ecological catastrophes that leave longstanding impressions
14
15 301 on the oral ecosystem (Marsh 2006). Since restoration of dental caries fails to eliminate cariogenic
16
17 302 bacteria from smooth surface margins, restored surfaces may serve as reservoirs for reinfection of sites
18
19 303 adjacent to or distant from the restoration (Featherstone 2000). Current evidence indicates patients with
20
21 304 low salivary flow have a microbiota enriched in cariogenic bacteria (Almstah et al. 2003; Almstahl et al.
22
23 305 2001; Eliasson et al. 2006; Proctor et al. 2018). Here, we report that patients with hyposalivation
24
25 306 experience a higher number of decayed, missing and filled surfaces, and thus harbor more reservoirs for
26
27 307 cariogenic bacteria, and that they also experience an increase in detectable rates of microbial migration.
28
29
30

31
32
33 308 We hypothesize that the increased rate of migration at supragingival surfaces may underlie the
34
35 309 increased risk of dental caries patients with hyposalivation experience post-restoration and despite
36
37 310 fastidious compliance with dental care regimens (Segal et al. 2009). Consistent with *in vitro* studies of
38
39 311 shear force (Fernandez et al. 2017), we propose that our preliminary data suggest shear associated with
40
41 312 salivary flow may control the diversity and composition of the supragingival biofilm *in vivo* by limiting
42
43 313 dispersal. Under normal circumstances, microbes which detach from supragingival surfaces are
44
45 314 marshalled out of the oral cavity through the combined effects of abrasion from the tongue, salivary
46
47 315 flow, and deglutition. We propose that following detachment, where there is chronic suppression of flow
48
49 316 rates, microbial species are able to successfully attach and grow as part of the tooth-associated
50
51 317 supragingival biofilm at sites distal to their origin. Thus, salivary flow may hinder the process of
52
53 318 attachment and growth at supragingival sites. In contrast to supragingival sites, our preliminary data
54
55
56 319
57
58
59
60

1
2
3 319 suggest that salivary flow does not exert top-down control over the rate of dispersal between subgingival
4
5 320 sites.
6
7
8

9 321 Dispersal between subgingival sites may be controlled more by the rate of exudation of gingival
10
11 322 crevicular fluid from the gingival sulcus to the gingival margin than by the rate of salivary flow.
12
13 323 Moreover, desquamation of the epithelium may also limit dispersal into the subgingival crevice.
14
15 324 Alternatively, out of 12 teeth that we sampled only 4 teeth (tooth 8 and 9 or 24 and 25) had sites
16 immediately adjacent to each other. As a result, if dispersal between subgingival sites occurs at a greater
17 frequency between adjacent teeth than between distal teeth our power to detect differences in rates of
18 migration between subgingival sites in controls vs. low flow subjects may have been limited, particularly
19 when considering our overall small sample size. Future studies that survey a larger number of teeth at
20 sites above and below the gumline are needed to validate these observations.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

330 Diagnostics that identify patients who may be suffering from occult low salivary flow should
331 mitigate risk of recurrent dental disease in these patients. We identified two symptomatic complaints as
332 the most discriminative, identifying salivary flow rates < 0.1 mL/min with ~85% accuracy. These
333 symptomatic complaints – the extent to which dry mouth negatively impacted overall quality of life, and
334 the extent to which dryness was felt on the tongue – were as effective at distinguishing different microbial
335 communities as UWS-FR, suggesting that, in our cohort, flow rate and xerostomic complaints are highly
336 correlated. Our findings are consistent with prior work suggesting that functional impairment is the most
337 discriminative xerostomic complaint for separating patients based on salivary hypofunction (Sreebny
338 and Valdini 1988). Age related xerostomic complaints are known to markedly increase in the 6th decade
339 of life with women more frequently reporting symptoms of dryness compared to men (Niklander et al.
340 2017). In response to anticholinergic suppression of salivary flow, older adults experience more
341 prolonged functional impairment, as measured by both flow rate and symptomatic complaints of

1
2
3 342 xerostomia, with a longer duration of difficulties speaking and swallowing due to dryness, as well as
4
5 343 longer periods of dryness reported on the lips. Future large-scale prospective studies should endeavor to
6
7 344 examine the relative onset of xerostomic complaints and hyposalivation over the decades of human life.
8
9
10

11 345 Our study was limited by a design that enrolled only patients having low salivary flow due to a
12
13 346 presumptive diagnosis of SS, rather than including patients who experienced low salivary flow due to a
14
15 347 wide variety of conditions. This study design resulted in an observational study with a relatively small
16
17 348 sample size and an uneven distribution of ages with a younger control cohort and an older low flow
18
19 349 cohort. While these challenges can be addressed statistically an ideal design would include age matching
20
21 350 between participant cohorts. Despite these limitations our work provides a framework, including a script
22
23 351 for reproducibility, that can be used by others to integrate the analysis of microbiome data with clinical
24
25 352 data and patient histories.
26
27
28
29
30
31 353
32
33
34 354 **Methods**
35
36
37 355
38
39 356 **Human Subjects**
40
41
42 357 Subjects were enrolled into a larger study of hyposalivation. Written, informed consent was
43
44 358 obtained from all participants prior to dental examination or sample collection in compliance with human
45
46 359 subjects protocols approved by the University of California, San Francisco (UCSF) Human Research
47
48 360 Protection Program and Institutional Review Board (Protocol 11-06273), and the Stanford University
49
50 361 Administrative Panels on Human Subjects in Medical Research (Protocol 21586). Subjects were
51
52 362 recruited into 2 cohorts: (1) 152 healthy adults were recruited into a “control cohort”; and (2) 32
53
54 363 individuals who experienced low salivary flow due to the autoimmune disorder, SS, were recruited into
55
56
57
58
59
60

1
2
3 364 a “low-flow cohort”. A complete description of clinical data measurements and sample processing
4
5 365 workflows are included in **Supplementary Methods**.
6
7
8
9 366 **Statistical analysis of clinical and demographic data**
10
11
12 367 The `dabestr` package in R (Ho et al. 2019) was used to assess between-group differences in
13
14 368 clinical and demographic features. A bootstrap confidence interval was constructed surrounding
15
16 369 estimates of the mean difference between the groups. Bootstrap resampling was used to compute
17
18 370 assumption-free confidence intervals. Bias correction and acceleration were used in cases of skewness.
19
20
21 371 Between-group differences in ethnicity, race and gender were evaluated using chi-square tests.
22
23
24 372 **Evaluation of hyposalivation effects on spatial distribution of oral disease**
25
26
27 373 A series of site-specific generalized linear models were evaluated to determine the spatial
28
29 374 distribution of oral disease (DMFS, CAL, PD, GM-CEJ, and BOP) with respect to the following
30
31 375 predictors: age, cohort, UWS-FR, and cohort:UWS-FR (interaction between cohort and UWS-FR).
32
33
34 376 Response variables were transformed using the Ordered Quantile (ORQ) normalization transformation
35
36 377 with the `orderNorm` function within the package `bestNormalize`. This model was applied to each
37
38 378 tooth (excluding third molars) using the whole tooth average measurement of each clinical variable. The
39
40 379 confidence intervals of each model were plotted to assess the extent to which each predictor explains the
41
42
43 380 spatial patterning of dental disease. P-values, assessing whether coefficients could be distinguished from
44
45 381 0, were adjusted by controlling the false discovery rate (FDR) at 5% using the Benjamini-Hochberg
46
47 382 (BH) (Benjamini and Hochberg 1995).
48
49
50
51 383 **Analysis of oral microbial communities**
52
53
54 384 Supragingival samples from 3 low flow and 3 control patients previously described (SRA
55
56
57
58
59
60

1
2
3 385 SUB10454805) were analyzed with newly generated subgingival data from the same patients, yielding
4
5 386 a total of 427 samples. A complete description of statistical approaches to the analysis of the microbiome
6
7 387 is included in **Supplementary Methods**.
8
9
10

11 388 **Analysis of an adapted visual analog scale (VAS)**
12
13

14 389 Subjects were asked to use a visual analog scale to rate on a scale of 0 to 100 the extent to which
15
16 390 they felt dry mouth negatively impacted their ability to swallow, their overall quality of life, and their
17
18 391 ability to speak. In addition, subjects were asked to rate on a scale of 0 to 100 the overall dryness of their
19
20 392 throat, tongue, lips, and hard palate. The specific questionnaire is provided as Supplementary Data. A
21
22 393 total of 106 individuals responded to the survey, including 73 controls and 24 low flow individuals.
23
24 394 Responses from all individuals were analyzed by principal components analysis using the `prcomp`
25
26 395 function in base R. A classification tree was generated using the `rpart` function in the `rpart` package.
27
28
29
30

31 396 **Data availability**
32
33

34 397 The data supporting the results of this study are available in the NIH Short Read Archive, under
35
36 398 SRA accession number SRP126946 (<http://www.ncbi.nlm.nih.gov/sra/22016164-22016299>). The code
37
38 399 and data that were used to generate these findings can also be found at
40
41 400 <https://github.com/dmap02/spatial-pattern-dental-disease>. All other data supporting the findings of this
42
43 401 study are available within the article and its Supplementary Information files, or are available from the
44
45 402 authors upon request.
46
47
48

49 403 **Acknowledgements**
50
51

52 404 We thank colleagues at Stanford and at UCSF who provided technical support for this project,
53
54 405 including Swetha Kanukula, Lakshmi Karayil, Muneet Shoker, Divya Vadlamudi, Saba Dolatshahi,
55
56
57
58

1
2
3 406 Anchita Venkatesh, Nicole Davis, and Felix Chen. This work was supported by the National Institutes
4 of Health (R01DE023113 to D.A.R.), the Chan Zuckerberg Biohub Microbiome Initiative (D.A.R.), and
5
6 407 by the Thomas C. and Joan M. Merigan Endowment at Stanford University (D.A.R.). Survey data were
7
8 408 collected and managed using REDCap electronic data capture tools hosted at Stanford University.
9
10 409
11
12
13 410 **Author contributions**

14
15
16
17 411 DMP, PML, GCA, MIR, DAR, SPH: Conceived of study/developed IRB protocols. DMP:
18
19 412 Wrote the first draft of the manuscript. MEM: performed literature review. DMP, CS, ARB, SW, TJ,
20
21 413 JW, SS, MEM: performed data analysis. YR, MIR, DAR, SPH: assisted with the interpretation of data.
22
23 414 CS and SPH guided statistical analysis. All authors reviewed and approved of the manuscript.
24
25
26 415
27
28 416
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 417 **References**
4
5 418
6
7
8 419 Algarni AA, Ungar PS, Lippert F, Martinez-Mier EA, Eckert GJ, Gonzalez-Cabezas C, Hara AT. 2018.
9
10 420 Trend-analysis of dental hard-tissue conditions as function of tooth age. *J Dent.* 74:107-112.
11
12 421 Almstah IA, Wikstrom M, Stenberg I, Jakobsson A, Fagerberg-Mohlin B. 2003. Oral microbiota
13
14 422 associated with hyposalivation of different origins. *Oral Microbiol Immunol.* 18(1):1-8.
15
16
17 423 Almstahl A, Wikstrom M, Kroneld U. 2001. Microflora in oral ecosystems in primary Sjögren's
18
19 424 syndrome. *J Rheumatol.* 28(5):1007-1013.
20
21
22 425 Baudet-Pommel M, Albuisson E, Kemeny JL, Falvard F, Ristori JM, Fraysse MP, Sauvezie B. 1994.
23
24 426 Early dental loss in sjogren's syndrome. Histologic correlates. European community study group
25
26 427 on diagnostic criteria for sjogren's syndrome. *Oral Surg Oral Med Oral Pathol.* 78(2):181-186.
27
28 428 Baum BJ. 1981. Clinical science: Evaluation of stimulated parotid saliva flow rate in different age
29
30 429 groups. *J Dent Res.* 60(7):1292-1296.
31
32
33 430 Becks H, Wainwright WW. 1943. Human saliva: XIII. Rate of flow of resting saliva of healthy
34
35 431 individuals. *Journal of dental research.* 22(5):391-396.
36
37
38 432 Ben-Aryeh H, Miron D, Berdicevsky I, Szargel R, Gutman D. 1985. Xerostomia in the elderly:
39
40 433 prevalence, diagnosis, complications and treatment. *Gerodontology.* 4(2):77-82.
41
42 434 Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: A practical and powerful approach
43
44 435 to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological).*
45
46 436 57(1):289-300.
47
48
49 437 Bowen WH, Madison KM, Pearson SK. 1988. Influence of desalivation in rats on incidence of caries in
50
51 438 intact cagemates. *J Dent Res.* 67(10):1316-1318.
52
53
54 439 Dawes C. 2004. How much saliva is enough for avoidance of xerostomia? *Caries Res.* 38(3):236-240.
55
56
57
58
59
60

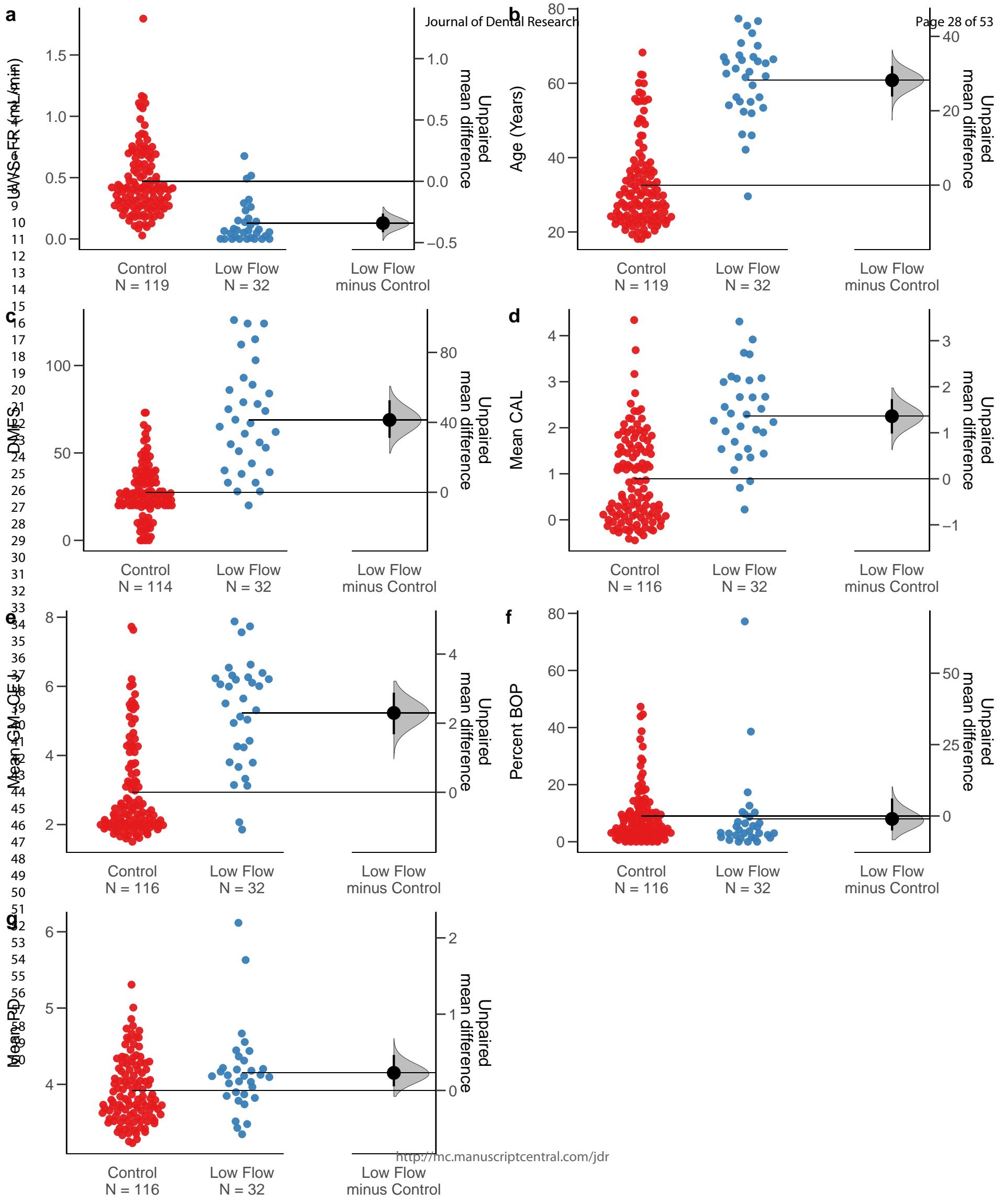
- 1
2
3 440 Dreizen S, Brown LR, Daly TE, Drane JB. 1977. Prevention of xerostomia-related dental caries in
4
5 441 irradiated cancer patients. *J Dent Res.* 56(2):99-104.
6
7
8 442 Eliasson L, Carlen A, Almstahl A, Wikstrom M, Lingstrom P. 2006. Dental plaque pH and micro-
9
10 443 organisms during hyposalivation. *J Dent Res.* 85(4):334-338.
11
12 444 Featherstone JD. 2000. The science and practice of caries prevention. *J Am Dent Assoc.* 131(7):887-
13
14 445 899.
15
16
17 446 Fernandez CE, Aspiras MB, Dodds MW, Gonzalez-Cabezas C, Rickard AH. 2017. The effect of
18
19 447 inoculum source and fluid shear force on the development of in vitro oral multispecies biofilms.
20
21
22 448 *J Appl Microbiol.* 122(3):796-808.
23
24 449 Fure S, Zickert I. 1990. Salivary conditions and cariogenic microorganisms in 55, 65, and 75-year-old
25
26 450 swedish individuals. *Scand J Dent Res.* 98(3):197-210.
27
28
29 451 Ho J, Tumkaya T, Aryal S, Choi H, Claridge-Chang A. 2019. Moving beyond p values: Data analysis
30
31 452 with estimation graphics. *Nat Methods.* 16(7):565-566.
32
33 453 Kassebaum NJ, Smith AGC, Bernabe E, Fleming TD, Reynolds AE, Vos T, Murray CJL, Marques W,
34
35 454 Collaborators GBDOH. 2017. Global, regional, and national prevalence, incidence, and
36
37
38 455 disability-adjusted life years for oral conditions for 195 countries, 1990-2015: A systematic
39
40 456 analysis for the global burden of diseases, injuries, and risk factors. *J Dent Res.* 96(4):380-387.
41
42 457 Kitamura M, Kiyak HA, Mulligan K. 1986. Predictors of root caries in the elderly. *Community Dent*
43
44
45 458 *Oral Epidemiol.* 14(1):34-38.
46
47 459 Marsh PD. 2006. Dental diseases -- are these examples of ecological catastrophes? *Int J Dent Hyg.* 4
48
49 460 Suppl 1:3-10; discussion 50-12.
50
51
52 461 Mathews SA, Kurien BT, Scofield RH. 2008. Oral manifestations of sjogren's syndrome. *J Dent Res.*
53
54 462 87(4):308-318.
55
56
57
58
59
60

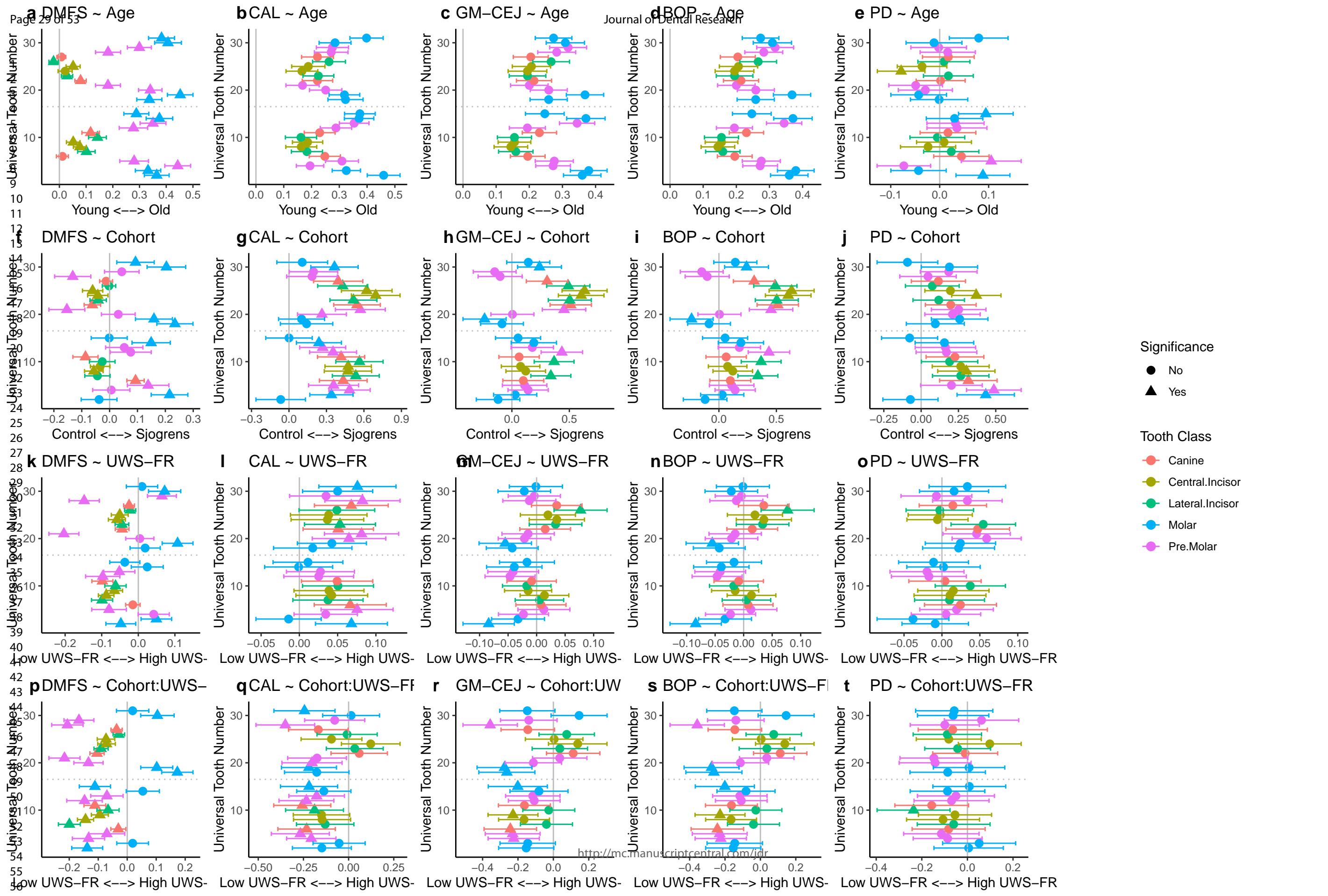
- 1
2
3 463 Mignogna MD, Fedele S, Lo Russo L, Lo Muzio L, Wolff A. 2005. Sjogren's syndrome: The diagnostic
4 potential of early oral manifestations preceding hyposalivation/xerostomia. *J Oral Pathol Med.*
5
6 464 34(1):1-6.
7
8 465
9
10 466 Niklander S, Veas L, Barrera C, Fuentes F, Chiappini G, Marshall M. 2017. Risk factors, hyposalivation
11
12 467 and impact of xerostomia on oral health-related quality of life. *Braz Oral Res.* 31:e14.
13
14
15 468 Pai S, Ghezzi EM, Ship JA. 2001. Development of a visual analogue scale questionnaire for subjective
16 assessment of salivary dysfunction. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.*
17 469
18
19 470 91(3):311-316.
20
21
22 471 Pedersen W, Schubert M, Izutsu K, Mersai T, Truelove E. 1985. Age-dependent decreases in human
23
24 472 submandibular gland flow rates as measured under resting and post-stimulation conditions. *J
25 Dent Res.* 64(5):822-825.
26 473
27
28 474 Proctor DM, Fukuyama JA, Loomer PM, Armitage GC, Lee SA, Davis NM, Ryder MI, Holmes SP,
29
30 Relman DA. 2018. A spatial gradient of bacterial diversity in the human oral cavity shaped by
31 475
32
33 476 salivary flow. *Nat Commun.* 9(1):681.
34
35 477 Ravid N, List T. 1998. Caries and periodontal conditions in patients with primary sjogren's syndrome.
36
37
38 478 *Swed Dent J.* 22(3):97-103.
39
40 479 Segal B, Bowman SJ, Fox PC, Vivino FB, Murukutla N, Brodscholl J, Ogale S, McLean L. 2009.
41
42 480 Primary Sjögren's syndrome: Health experiences and predictors of health quality among patients
43
44
45 481 in the united states. *Health Qual Life Outcomes.* 7:46.
46
47 482 Shiboski SC, Shiboski CH, Criswell L, Baer A, Challacombe S, Lanfranchi H, Schioldt M, Umehara H,
48
49 483 Vivino F, Zhao Y et al. 2012. American college of rheumatology classification criteria for
50
51 484 Sjögren's syndrome: A data-driven, expert consensus approach in the Sjögren's international
52
53
54 485 collaborative clinical alliance cohort. *Arthritis Care Res (Hoboken).* 64(4):475-487.
55
56
57
58
59
60

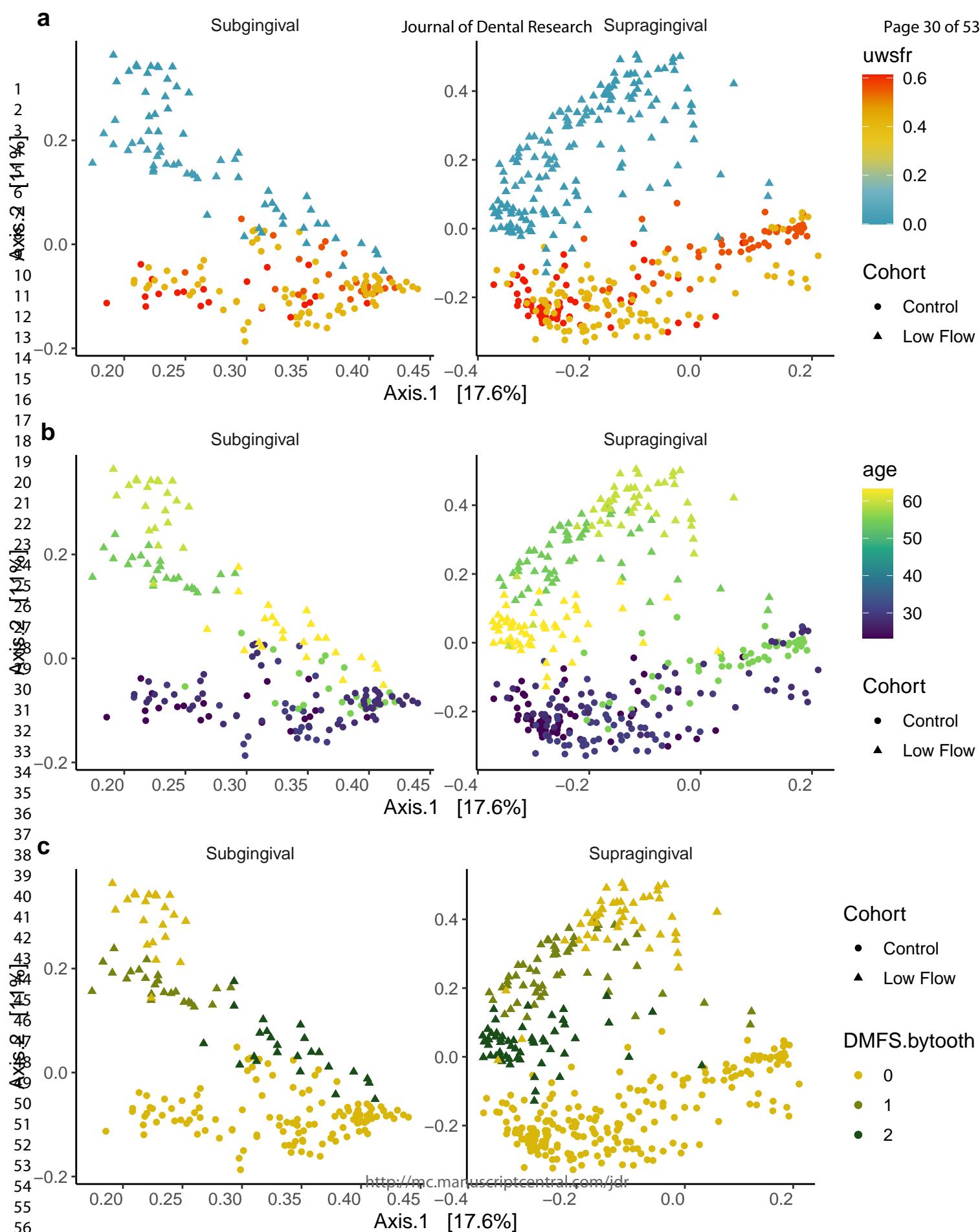
- 1
2
3 486 Sreebny LM, Valdini A. 1988. Xerostomia. Part i: Relationship to other oral symptoms and salivary
4
5 487 gland hypofunction. *Oral Surg Oral Med Oral Pathol.* 66(4):451-458.
6
7
8 488 Sumney DL, Jordan HV, Englander HR. 1973. The prevalence of root surface caries in selected
9
10 489 populations. *J Periodontol.* 44(8):500-504.
11
12 490 von Bultzingslowen I, Sollecito TP, Fox PC, Daniels T, Jonsson R, Lockhart PB, Wray D, Brennan MT,
13
14
15 491 Carrozzo M, Gandera B et al. 2007. Salivary dysfunction associated with systemic diseases:
16
17 492 Systematic review and clinical management recommendations. *Oral Surg Oral Med Oral Pathol*
18
19 493 *Oral Radiol Endod.* 103 Suppl:S57 e51-15.
20
21
22 494
23
24 495
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

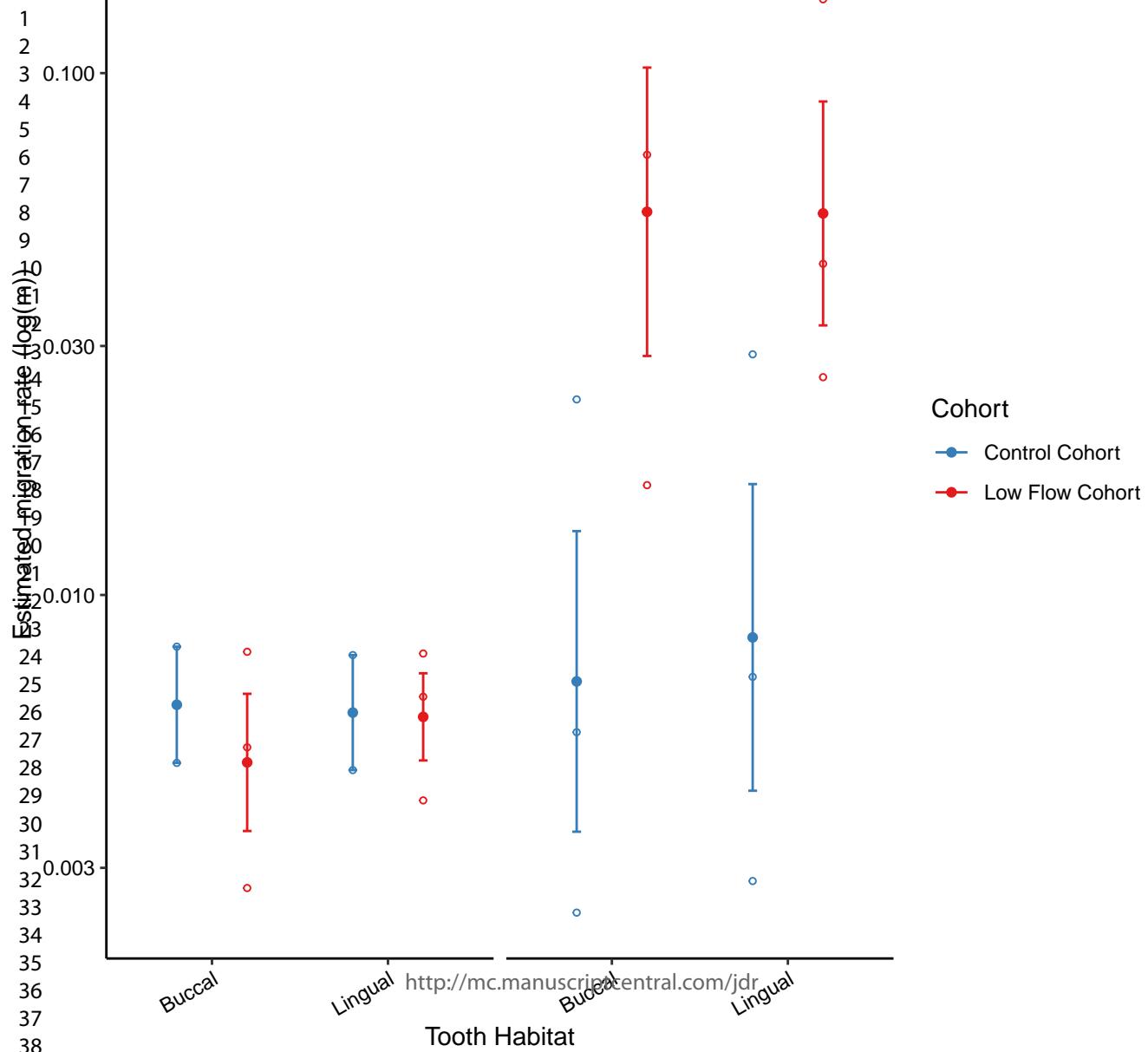
1
2
3 496 **Figure Legends**
4
5 497
6
7
8 498 **Figure 1.** Gardner-Altman estimation plots reveal significant demographic and clinical differences
9
10 499 between low flow and control cohorts. Data for control and low flow cohort patient Swarm plots of a)
11
12 500 age, b) UWS-FR, c) DMFS, d) CAL, e) GM-CEJ, f) BOP, and g) PD are plotted on the left-aligned
13
14 501 axis. The right-aligned axis displays the point estimate of the unpaired mean difference between
15
16 502 groups, surrounded by their 95% confidence intervals (95% CI).
17
18
19 503
20
21
22 504 **Figure 2.** Spatial modeling reveals independent effects of age and patient cohort on the spatial pattern
23
24 505 of dental disease. Coefficients with 95% confidence intervals are plotted as a function of universal
25
26 506 tooth number for a series of per-tooth linear models where DMFS (a, f, k, p), CAL (b, g, l, q), GM-CEJ
27
28 507 (c, h, m, r), BOP (d, i, n, s), and PD (e, j, o, t) were regressed against age, patient cohort, UWS-FR and
29
30 508 the interaction between UWS-FR and cohort. Intercepts were calculated, but not visualized for each
31
32 509 model. Colors map to different tooth classes. Triangles denote estimates with adjusted p-values < 0.05,
33
34 510 while squares denote estimates with adjusted p-values > 0.05. P-values were adjusted by controlling
35
36 511 the false discovery rate (FDR) at 5% using the Benjamini-Hochberg (BH).
37
38 512
39
40 513 **Figure 3.** Subgingival and supragingival communities from 3 control and 3 low flow subjects
41
42 514 segregate by UWS-FR while age imperfectly separates supragingival communities. Principal
43
44 515 coordinates analysis on Bray Curtis dissimilarity was performed on the combined subgingival and
45
46 516 supragingival dataset. The PCoA is displayed as a facet wrap with subgingival and supragingival
47
48 517 samples in left and right panels, respectively. a) Communities segregated by UWS-FR along the
49
50 518 second coordinate which explained 12.8% of the variance. b) Samples clustered by age for
51
52
53
54 519
55
56
57
58
59
60

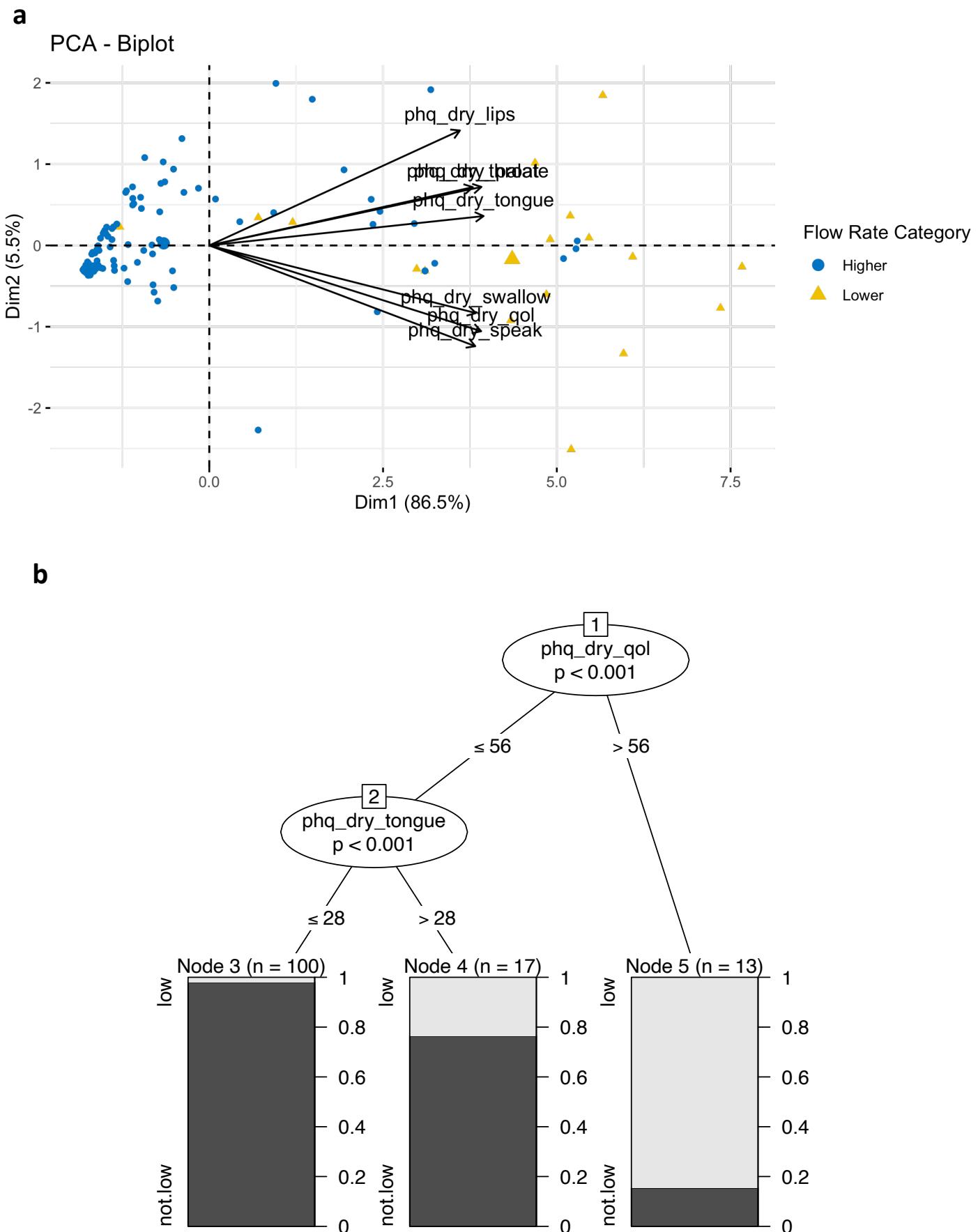
1
2
3 519 subgingival, but not supragingival sites. c) Supragingival communities from sites within low flow
4
5 520 subjects with 1 or 2 DMFS segregated from sites with 0 DMFS.
6
7
8 521
9
10 522 **Figure 4.** Estimated migration rates by habitat within each individual. Rates were inferred using the
11
12 523 Sloan Community Neutral Model. Each hollow point represents the estimated migration rate for each
13
14 524 of the defined metacommunities for each individual, while the solid point represents their average with
15
16 525 standard error bars. Colors correspond to patient cohort.
17
18
19 526
20
21
22 527 **Figure 5.** Symptoms of dry mouth predict low salivary flow. a) Principal component analysis of
23
24 528 subject responses to visual analog scale revealed two groups of questions – questions that segregate
25
26 529 subjects who complain of impacts to quality of life (dry_speak, dry_qol, dry_swallow) and questions
27
28 530 that indicate feelings of dry mouth (dry_tongue, dry_throat, dry_palate, dry_lips). b) Conditional
29
30 531 inference tree was used to identify the variables most predictive of separating patients into groups
31
32 532 based on low (UWS-FR < 0.1ml/min) and not low (UWS-FR > 0.1 ml/min) subgroups.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60











Supplementary Information

Spatial patterns of dental disease in patients with low salivary flow

Diana M. Proctor^{1,2}, Christof Seiler^{3,6}, Adam R. Burns¹, Samuel Walker⁴, Tina Jung⁴, Jonathan Weng⁴, Shanne Sastiel⁴, Yoga Rajendran⁴, Yvonne Kapila⁴, Meredith E. Millman⁴, Gary C. Armitage⁴, Peter M. Loomer⁵, Susan P. Holmes⁶, Mark I. Ryder⁴, David A. Relman^{1,7,8*}

Affiliations

¹Division of Infectious Disease & Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305 USA

²National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892 USA

³Department of Data Science and Knowledge Engineering, Mathematics Centre Maastricht, Maastricht University, The Netherlands

⁴Division of Periodontology, University of California, San Francisco School of Dentistry, San Francisco, CA 94143 USA

⁵ School of Dentistry, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229 USA

⁶Department of Statistics, Stanford University, Stanford, CA 94305 USA

⁷Infectious Diseases Section, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304 USA

⁸Department of Microbiology & Immunology, Stanford University School of Medicine, Stanford, CA 94305 USA

*Corresponding author: relman@stanford.edu; Address: Encina E209, 616 Jane Stanford Way, Stanford, California 94305-6165; Phone: 650-736-6822; Fax: 650-852-3291

Table of Contents

Supplementary Data.....	4
Supplementary data file 1	4
Supplementary data file 2.....	4
Supplementary data file 3.....	4
Supplementary Methods	5
Inclusion and exclusion criteria	5
Clinical data measurements.....	6
Sample collection sites and protocol	7
DNA extraction and barcoded sequencing of the 16S rRNA gene.....	8
Demultiplex and quality filtering	8
Decontaminating the ASV table.....	9
Impact of clinical variables on community composition.....	9
Estimation of microbial migration	9
Supplementary Tables.....	12
Supplementary Table 1.....	12
Supplementary Table 2.....	13
Supplementary Figures	14
Supplementary Figure 1	14

1		
2		
3	Supplementary Figure 2	15
4		
5		
6	Supplementary Figure 3	17
7		
8		
9	Supplementary Figure 4	18
10		
11		
12	Supplementary Figure 5	19
13		
14		
15	Supplementary Figure 6	20
16		
17		
18	Supplementary Figure 7	21
19		
20		
21	Supplementary References	22
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		
33		
34		
35		
36		
37		
38		
39		
40		
41		
42		
43		
44		
45		
46		
47		
48		
49		
50		
51		
52		
53		
54		
55		
56		
57		
58		
59		
60		

Supplementary Data

Supplementary data file 1

Supplementary data file 1 is a .xlsx file that can be used to build a duplicate version of the Visual Analog Scale deployed in this study in REDCap.

Supplementary data file 2

Supplementary data file 2 is an Excel workbook that contains 2 integrated datasets.

- ASV table, taxonomy file, and sample data mapping file for supragingival samples described in Proctor et al (2018) and the subgingival samples, generated for this manuscript.

Supplementary data file 3

Supplementary data file 3 contains the code and figures used to analyze the dataset.

Supplementary Methods

Inclusion and exclusion criteria

Sjögren's Syndrome (SS) subjects were included in the study if they were adults over 18 years old, complained of dry mouth, and had been diagnosed at least three months ago with Sjögren's Syndrome. The diagnosis date of SS was also collected verbally and verified with subjects' documentation. Otherwise, healthy control subjects were included in the study if they were healthy, non-smoking adults over the age of 18 years.

Exclusion criteria for the SS group were 1) having fewer than 15 non-implant teeth; 2) smoke or use chewing tobacco or snuff or quit using tobacco products within the 6 months preceding enrollment; 3) being treated by a physician for an uncontrolled chronic medical condition; 4) have symptoms of or treatment of asthma or acid reflux in the last 3 months; 5) history of radiation therapy to the head or neck; 6) history of oral, systemic antibiotics or antifungals use within the 6 month period preceding enrollment; 7) required to take antibiotics before dental treatment; 8) history of stimulant or heroin abuse or of eating disorders; 9) lactating, pregnant, or intending to become pregnant; 10) any dental treatment during the one month period preceding enrollment and cannot or will not abstain from dental treatments during their enrollment; 11) have fixed dental appliance (retainers, fixed dentures, braces, orthodontic wires); 12) periodontitis, candidiasis, halitosis, tooth pain, or any other disease in the mouth (to patient's knowledge); 13) diagnosed with Sjogren's syndrome by the American European Consensus Criteria or the American College of Rheumatology Classification Criteria fewer than 3 months prior to the date of enrollment.

Exclusion criteria for the control group were 1) having fewer than 15 natural, non-implant teeth; 2) missing both central incisors, both canines, or both first molars in either the maxilla or the mandible; 3) crown or implant replacing both central incisors, both canines, or both first molars in

either the maxilla or the mandible; 4) currently being treated by a physician for any chronic medical condition, including asthma and acid reflux; 5) history of radiation therapy to the head or neck; 6) take any medication on a daily basis other than birth control; 7) history of oral, systemic antibiotics or antifungals use within the 6 month period preceding enrolment; 8) required to take antibiotics before dental treatment; 9) history of stimulant or heroin abuse or of eating disorders; 10) lactating, pregnant, or intending to become pregnant; 11) any dental treatment during the 1 month period preceding enrollment and cannot or will not abstain from dental treatments during their enrollment; 12) fixed dental appliance (retainers, fixed dentures, braces, orthodontic wires); 13) experienced dry mouth for a full week at any time in the past 6 months; 14) periodontitis, candidiasis, halitosis, tooth pain, or any other disease in the mouth (to patient's knowledge).

Clinical Data Measurements

A calibrated dentist performed a comprehensive dental exam to evaluate the oral and dental health status of each subject. Dental health was evaluated by measuring decayed, missing, and filled surfaces (DMFS), probing depth (PD), gum recession (GM-CEJ), and bleeding on probing (BOP). These measurements were taken at the mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual sites on each tooth (except third molars) using a North Carolina probe. Clinical Attachment Loss (CAL) was calculated using the following formula: PD + GM-CEJ. Mucosal surfaces in the oral cavity were examined for any sign of disease (e.g., oral candidiasis). No subject had active dental disease at the time of sample collection. No subject had used antibiotics in the 6 months preceding enrollment.

Unstimulated whole salivary flow rate (UWS-FR; mL/min) was measured as part of an oral health exam at the UCSF School of Dentistry. Participants were asked to refrain from eating, drinking anything but water, or performing oral hygiene 2 hours prior to oral screening. UWS-FR was collected

1
2
3 for all subjects over a period of 5 minutes by a data recorder following a standardized procedure.
4
5
6
7

8 In addition, subjects completed 4 surveys to gather information on personal history, medication
9 usage, medical history, and demographical data, and for the visual analog scale (VAS). Study data were
10 collected and managed using REDCap electronic data capture tools hosted at Stanford University (Harris
11 et al. 2019). REDCap (Research Electronic Data Capture) is a secure, web-based software platform
12 designed to support data capture for research studies, providing 1) an intuitive interface for validated
13 data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export
14 procedures for seamless data downloads to common statistical packages; and 4) procedures for data
15 integration and interoperability with external sources. The REDCap template for the VAS is included
16 (Supplementary Data File 1).

31 Sample collection sites and protocol 32

33 Subjects were asked to refrain from eating, drinking, or performing oral hygiene within 2 hours
34 of sample collection. For each of 3 control and 3 low flow subjects, samples of the buccal and lingual
35 surfaces of index teeth (universal tooth numbers 3, 6 , 8, 9, 11, 14, 19, 22, 24, 25, 27, 30) were collected
36 by a dentist at the UCSF School of Dentistry. Supragingival plaque samples were collected with
37 Epicentre Foam Swabs (Madison, WI, Item #QEC091H). Swabs were applied to either the buccal or
38 lingual tooth aspect in a circular motion with moderate pressure for 5-20 seconds. For subgingival
39 samples, each tooth was sampled independently with two paper points per tooth, one inserted into the
40 mesio-buccal (or mesio-lingual) sulcus and one inserted into the distal-buccal (or distal-lingual) sulcus.
41 Each ISO-45 Paper Point was inserted to the base of the subgingival sulcus or pocket at the mesio-buccal
42 site, until resistance was felt. The paper point was left in place for 20 seconds when it was removed to a
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58

1
2
3 sterile collection tube with cotton pliers.
4
5
6
7

8 DNA extraction and barcoded sequencing of the 16S rRNA gene. 9

10 Genomic DNA was extracted from all samples using the MoBio PowerSoil DNA Isolation kit
11 (product #12888–100, Carlsbad, CA) according to the manufacturer's instructions. The V4 region of the
12 16S rRNA gene was PCR-amplified with barcoded primers, as previously described (DiGiulio et al.
13 2015; Proctor et al. 2018), pooled in batches of roughly 800 samples per run, and sequenced on the
14 Illumina HiSeq 2500 platform (University of Illinois Roy J. Carver Biotechnology Center, Urbana, IL).
15
16
17
18
19
20
21
22
23

24 Demultiplex and quality filtering 25

26 Forward and reverse reads were independently de-multiplexed using the split_libraries_fastq.py
27 command in Qiime 1 with parameters tuned to prevent quality filtering. Sequences were parsed into
28 sample-specific files using the split_sequence_file_on_sample_ids.py command in Qiime before import
29 into R-3.6.1 for quality filtering with the R package dada2 (Callahan et al. 2016); the first 10 nucleotides
30 (5') of each read were trimmed followed by the truncation of forward and reverse reads at lengths of 240
31 and 160 nucleotides, respectively. Reads were eliminated if the maximum expected error exceeded 2;
32 reads were also truncated at the first instance in the sequence where the quality score was less than 2.
33 Sequences were de-replicated before inference of sequence-specific errors and elimination of
34 problematic reads. Dereplicated and filtered forward sequences were subsequently merged with their
35 paired-end reads before construction of an amplicon sequence variant (ASV) table (**Supplementary**
36 **Data File 2**). The removeBimeraDenovo function of dada2 set to the 'consensus' method was used to
37 filter chimeras from each ASV table. Taxonomic assignment was then performed down to the species
38 level where possible using the dada2 implementation of the RDP Naive Bayesian Classifier 3 trained on
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58

1
2
3 the RDP database (version 14.0).
4
5
6
7

8 Decontaminating the ASV table 9

10 To identify taxa in the subgingival dataset, we calculated an enrichment score for taxa enriched
11 in controls compared to true samples, as previously described (Proctor et al 2018). A total of 23 technical
12 controls were analyzed in concert with 223 true samples (**Supplementary Data File 3**). Filtering using
13 this method reduced the data table for the subgingival dataset from 741 to 625 taxa.
14
15
16
17
18

19 Impact of clinical variables on community composition. 20 21

22 Constrained correspondence analysis was used to evaluate the extent to which UWS-FR, SWS-
23 FR, and the VAS explained variation in community structure in the microbiome.
24
25
26
27
28
29
30
31

32 Estimation of microbial migration 33

34 We used neutral community assembly models to estimate microbial migration rates (Munoz,
35 Couteron, and Ramesh 2008) (Sloan et al. 2006). These models assume that the structure of communities
36 is determined by a combination of neutral, or competitively equivalent growth dynamics and random
37 immigration from a shared source “pool” of potential microbial migrants. Neutral community models
38 make few to no assumptions about the physical nature of the source pool (indeed, the microorganisms
39 that make up the source pool may derive from multiple different environments), but rather assume that
40 all habitats are equivalent and that the composition and relative abundances of taxa within the source
41 pool can be approximated by averaging the composition of multiple communities that share the same
42 source pool. Here, we refer to a group of individual, local communities that share a source pool as a
43 “metacommunity”.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For our purposes, we defined metacommunities in our dataset as all of the community samples belonging to the same habitat type within an individual (for example, all supragingival, buccal tooth surface samples within a single individual would constitute a metacommunity in our analysis). This was based on our assumption that communities within these groups would both be the most likely to share a source pool, since they inhabit the same mouth, and also be more likely to have similar environmental characteristics, thus increasing the likelihood that differences among them are the result of neutral, rather than selective, dynamics. Thus, we defined four metacommunities for each individual: supragingival buccal, supragingival lingual, subgingival buccal, and subgingival lingual samples, resulting in a total of thirty-two metacommunities across eight subjects.

To estimate migration rates for samples within each of these metacommunities, we used two different methods and compared the results. The first estimates migration rates from measures of community differentiation in a manner analogous to measuring rates of gene flow in populations from measures of population differentiation, such as F_{ST} or G_{ST} (Muñoz, Couteron, and Ramesh 2008). The other method estimates migration rates by fitting the data to a neutral community assembly model developed by Sloan et al. (referred to here as the Sloan Neutral Community Model, or SNCM) (Sloan et al. 2006). This model predicts the frequency of occurrence for each taxon, or the proportion of samples in the metacommunity in which each taxon is found, as a function of its abundance in the source pool (approximated by its average abundance across samples in the metacommunity) and a migration rate parameter, which is parameterized by fitting the model to the observed relationship between occurrence frequency and average abundance.

Samples were rarefied to depths ranging between 1000 reads per sample to 50,000 reads per

sample at 1,000 read increments in order to determine a depth threshold yielding stable estimates of microbial migration for the Sloan model.

Supplementary Tables

Supplementary Table 1. Demographic composition of patient population. Race (top), ethnicity (middle) and sex (bottom) of patients broken down by the control and low flow cohorts.

Race	Control Cohort	Low Flow Cohort
American Indian or Alaskan Native	4	2
Asian	60	9
Black or African American	7	0
Pacific Islander	1	0
White	44	20
Multiracial	9	2
Prefer not to reply	3	3

Ethnicity	Control Cohort	Low Flow Cohort
Hispanic or Latino	14	5
Not Hispanic or Latino	102	26
Prefer not to reply	3	2

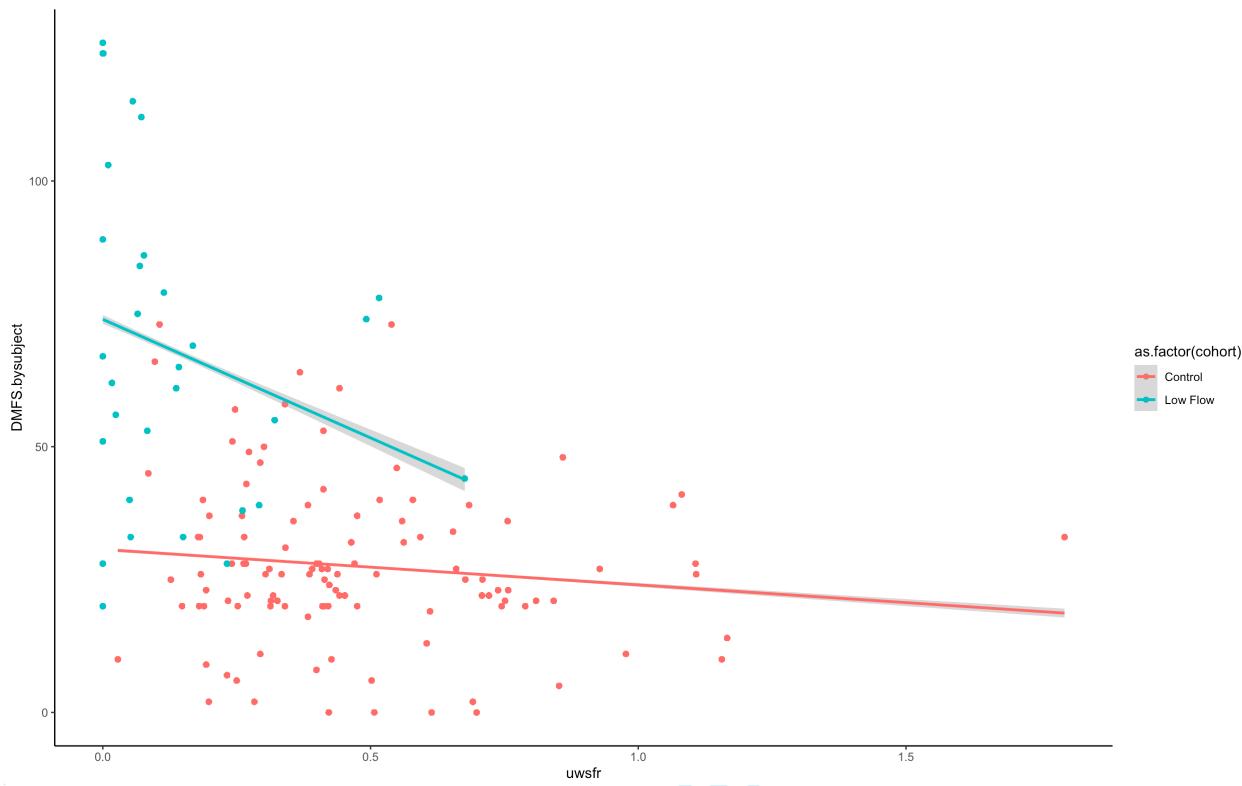
Sex	Control Cohort	Low Flow Cohort
Female	73	31
Female to Male Transgender	0	0
Male	45	1
Male to Female Transgender	0	1
Prefer not to answer	1	0

1
2
3 **Supplementary Table 2. Analysis of variance on estimated migration rates.** Estimated migration
4 rates were significantly higher in supragingival compared to subgingival habitats (Habitat_Class);
5 however there was no significant difference between buccal and lingual tooth surface habitats
6 (Tooth_Aspect). The effect of cohort (Aim) was also significant. There was a significant interaction
7 between cohort (Aim) and Habitat_Class, indicating the difference between healthy individuals and
8 those with Sjögren's Syndrome was substantial in supragingival habitats but far less pronounced in
9 subgingival habitats.
10
11
12
13
14
15
16
17
18

	Df	Sum Sq	Mean Sq	F value	p (>F)
Habitat_Class	1	0.0073	0.0073	6.303	0.025
Tooth_Aspect	1	0.00E+00	0.00E+00	0.0278	0.8699
Cohort	1	0.0063	0.0063	5.4572	0.0349
Habitat_Class:Tooth_Aspect	1	0.00E+00	0.00E+00	0.0129	0.9111
Habitat_Class:Cohort	1	0.0052	0.0052	4.4883	0.0525
Tooth_Aspect:Cohort	1	0.00E+00	0.00E+00	0.008	0.9299
Habitat_Class:Tooth_Aspect:Cohort	1	0.00E+00	0.00E+00	0.0032	0.9556
Residuals	14	0.0162	0.0012		

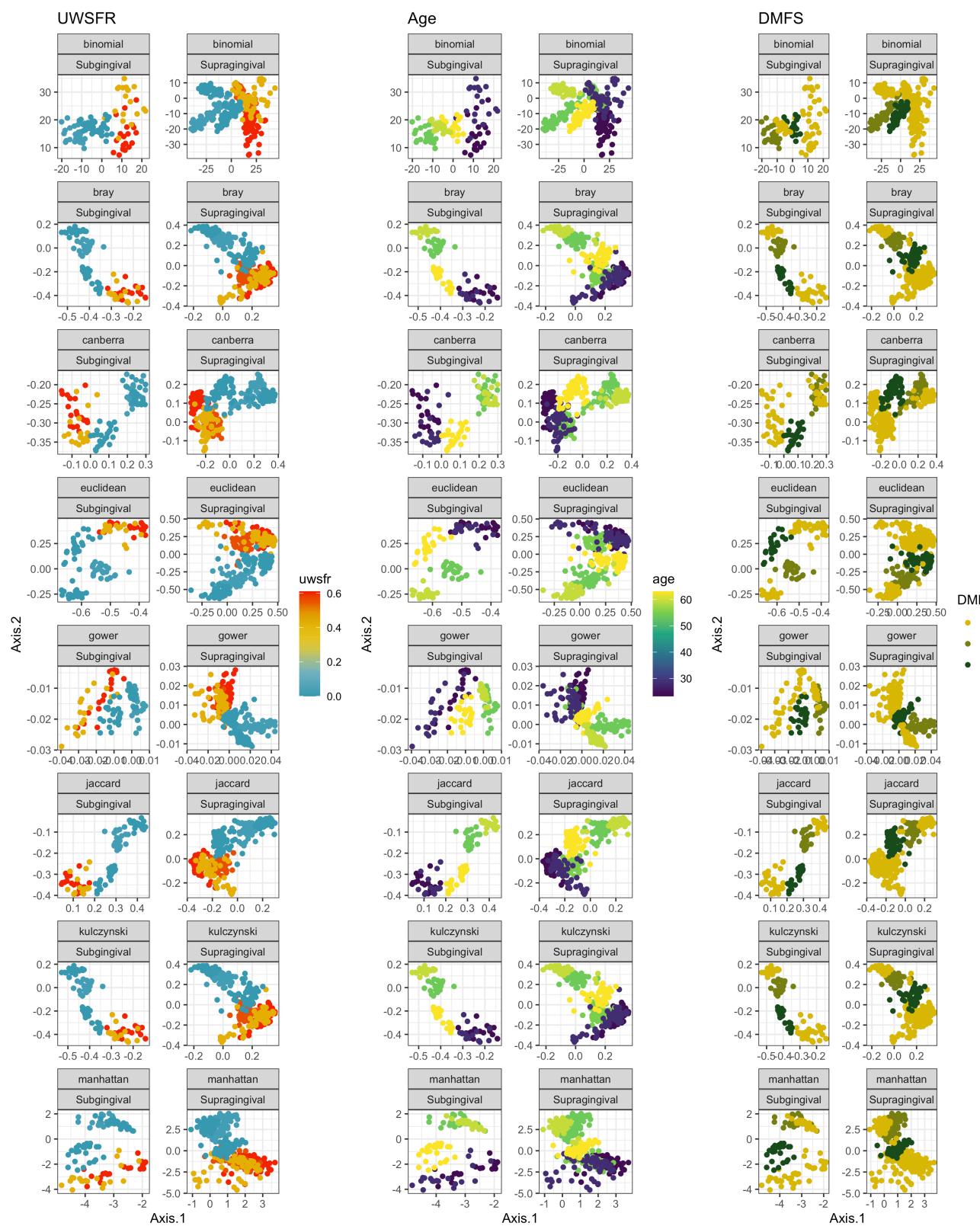
Supplementary Figures

Supplementary Figure 1



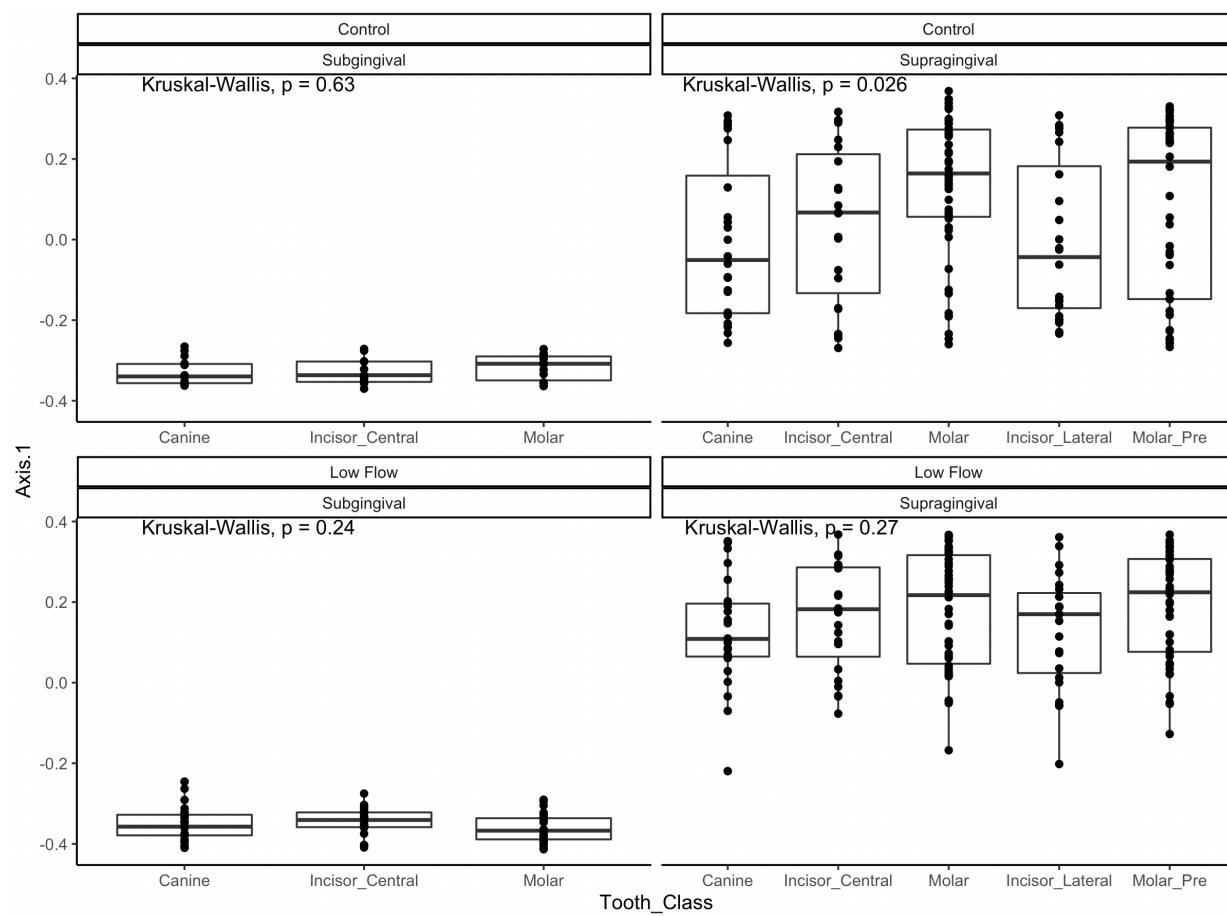
Supplementary Figure 1. Scatterplot of DMFS as a function of UWS-FR. Linear models were fit for each cohort. As flow rate decreased, the number of DMFS increased at a greater rate in the low flow cohort (blue) compared to controls (orange).

Supplementary Figure 2



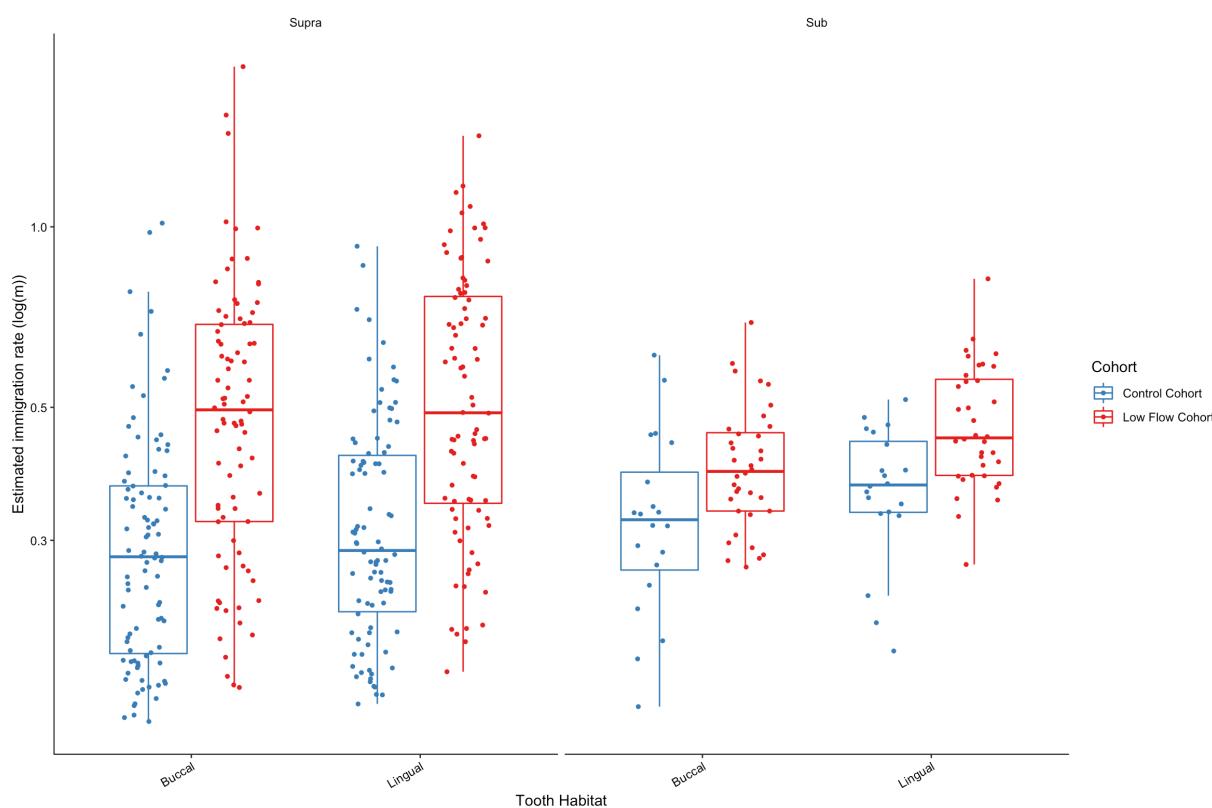
1
2
3 **Supplementary Figure 2. Patterns observed in Figure 3 can be seen using a variety of different**
4 **ecological distance metrics.** Principal coordinates analysis on various dissimilarity metrics (bray,
5
6 Canberra, Euclidean, Manhattan, kulzynski, gower) using the combined subgingival and
7
8 supragingival dataset. Each independent PCoA on each distance metric is displayed as a facet wrap
9
10 with subgingival and supragingival samples in left and right panels, respectively. Left panels
11
12 represent samples shaded by UWS-FR, middle panels are shaded by participant age, and right panels
13
14 are shaded by the average DMFS per tooth.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Supplementary Figure 3



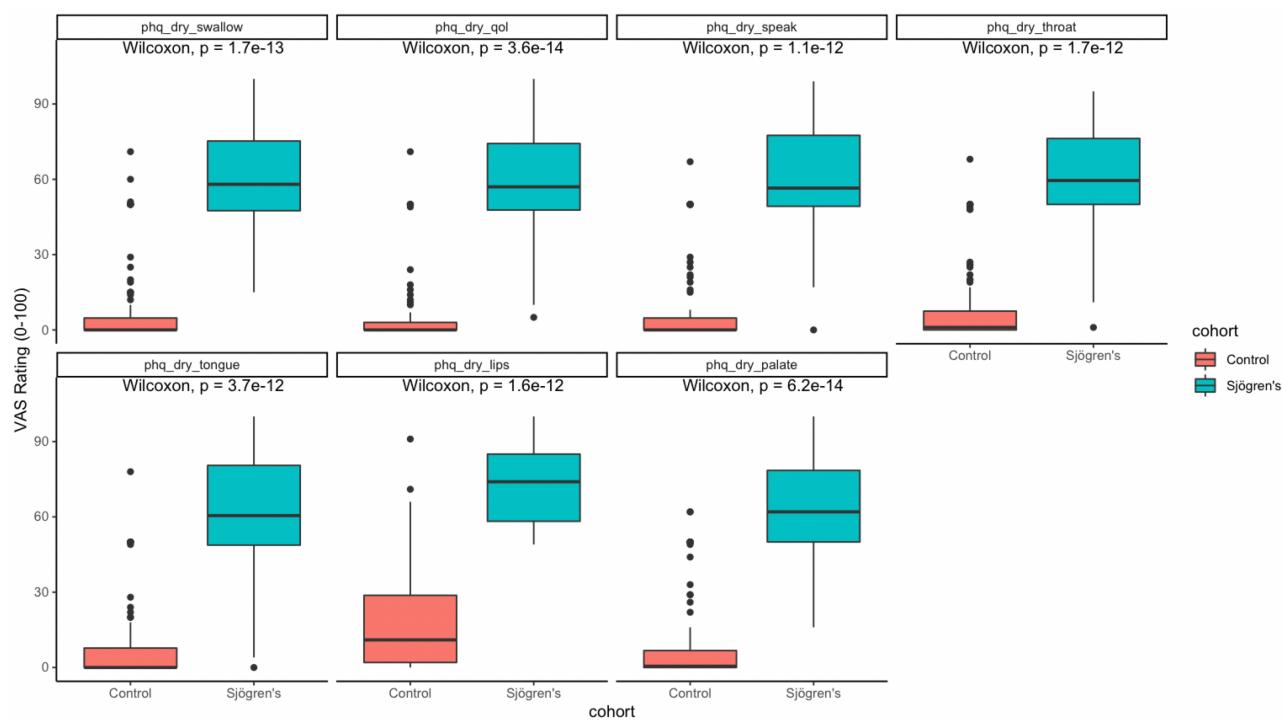
Supplementary Figure 3. Axis 1 scores plotted as a function of tooth class. Supragingival communities differed significantly in the control cohort, compared to the low flow subjects. Site-to-site variability was not observed for subgingival sites in either cohort.

Supplementary Figure 5



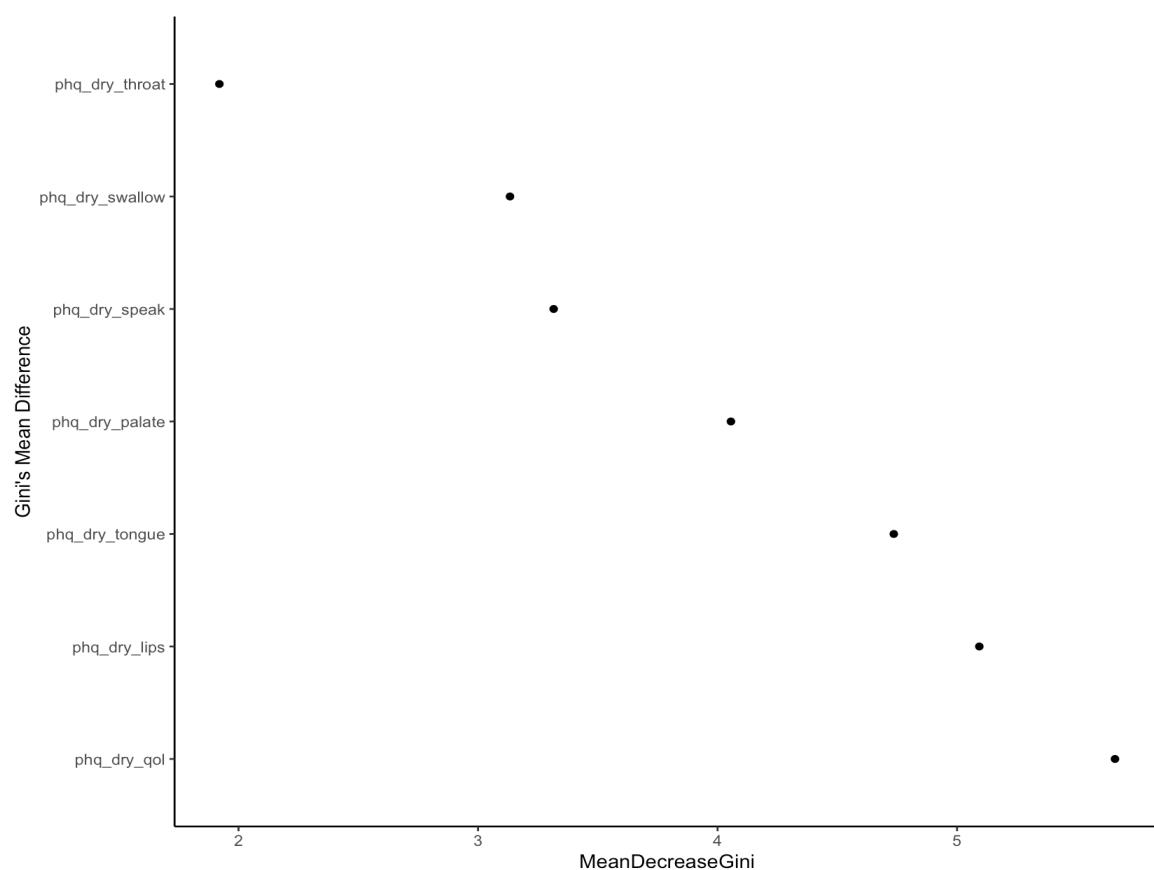
Supplementary Figure 5. Estimated migration rates (Gst) for each tooth habitat and tooth aspect. Colors correspond to cohort (control cohort; low flow cohort). In these boxplots, outer edges of boxes encompass the first and third quartiles. The horizontal line bisecting the center of each box demarcates the median. Each point represents an individual sample/community within each of the defined metacommunities.

1
2
3 **Supplementary Figure 6**
4
5



29
30 **Supplementary Figure 6. Boxplots of VAS reveal significant differences in responses between**
31 **groups.** Each question on the Visual Analog Scale querying the symptomatic complaints of dry mouth
32 is plotted as an individual panel. The midline of each boxplot represents the median while the outer
33 edges encompass the first and third quartiles. Points on the graph indicate outliers. Colors correspond
34 to patient cohort (control, low flow). Across all questions, respondents in the low flow cohort rated
35 their impairment and subjective complaints of dry mouth as more severe than the patients in the control
36 cohort (Wilcoxon rank sum tests, $p < 0.05$).
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Supplementary Figure 7



Supplementary Figure 7. Random forest analysis identifies dryness of lips and impacts on quality of life as the most discriminant features. Random forest analysis was used to identify the most discriminant features included in the Visual Analog Scale. The out-of-box error rate for the random forest model was 8.46%. The most discriminant features were the impact of dry mouth on the overall quality of life (phq_dry_qol), and the dryness of the lips (phq_dry_lips) consistent with the classification tree.

Supplementary References

- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. 2016. Dada2: High-resolution sample inference from illumina amplicon data. *Nat Methods.* 13(7):581-583.
- DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, Sun CL, Gotsman DS, Wong RJ, Shaw G et al. 2015. Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci U S A.* 112(35):11060-11065.
- Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, McLeod L, Delacqua G, Delacqua F, Kirby J et al. 2019. The redcap consortium: Building an international community of software platform partners. *J Biomed Inform.* 95:103208.
- Proctor DM, Fukuyama JA, Loomer PM, Armitage GC, Lee SA, Davis NM, Ryder MI, Holmes SP, Relman DA. 2018. A spatial gradient of bacterial diversity in the human oral cavity shaped by salivary flow. *Nat Commun.* 9(1):681.
- Sloan WT, Lunn M, Woodcock S, Head IM, Nee S, Curtis TP. 2006. Identifying the roles of immigration and chance in shaping prokaryote community structure. *Environ Microbiol.* 8(4):732-740.