

Microbial Signature of Ocean-Going Syndrome

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Research

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Abstract

Background and aims: During ocean-going voyages, crew members are subject to complex pressures from their living and working environment, which leads to a sub-optimal health status. Although abnormal defecation was observed during voyages, the associations between the gut microbiome and the symptoms are still unclear.

Methods: Associations between the gut microbiome and the health status of 77 crew members during a 135 day-long ocean-going voyage were evaluated using shotgun metagenomics of stool samples and health questionnaires (with 24 physical and psychological indicators) taken before and after the voyage.

Results: The co-appearance of symptoms e.g. abnormal defecation frequency, insomnia, shallow sleep, nausea and over-eating best described the sub-optimal health status of the majority of crew members, and we named this as 'Ocean-Going Syndrome (OGS)'. The OGS, instead of any single symptom, has a significant effect on the gut microbiome, and it was proved to be a key factor for individual perturbation in the gut microbiome during the voyage. Co-occurrence network analysis revealed a different microbial dynamic between the OGS and non-OGS crews, and the variation of 19 bacterial species and 31 gene families were identified as microbial signature for the OGS. Moreover, using a Random Forest model, the OGS can be predicted with a high accuracy (AUC=0.91) based on 27 biomarkers from pre-voyage samples.

Conclusions: Understanding associations between the gut microbiome and health status under extreme environments could help us discover potential predictors or even therapeutic targets for dysbacteriosis related diseases.

Introduction

The challenging conditions encountered during ocean-going voyages increases the risk of poor health in crew members who have to live under these harsh conditions, e.g., cramped living quarters with high humidity, high salinity, intense UV radiation, insufficient fruit and vegetables, and a lack of physical exercise¹. This environment can cause a number of physical and psychological symptoms indicative of a sub-optimal health status²⁻⁷. It has been reported that ocean-going voyages may affect the body's immune system and cause cardiovascular diseases^{8,9}, scurvy¹⁰, oral diseases^{11,12}, digestive system diseases, musculoskeletal disorders and circulatory diseases¹³. These health threats come from both the extreme living environment and the psychological pressure. Isolation from family, limited activity space and an unhealthy diet are the main causes of mental stress^{7,14}. Some studies have found that changes in neuroticism and subjective fatigue in seafarers are significantly related to the environment at sea^{2,15}.

The gut microbiome interacts with the human immune system and plays a key role in human health and disease¹⁶. Emerging evidence has highlighted the important role of diversity amongst gut microbial species and their functional genes in various chronic diseases¹⁷. Previous studies have also shown that

long-term living in a closed or semi-closed environment (e.g., a space capsule) profoundly affects commensal bacteria, especially the gut microbiome¹⁸. Although much attention has been paid to manage the health of crew members during long sea voyages, research on the role of the microbiome remains limited. Two studies have found significant changes in oral microbial diversity after voyages^{19, 20}, while another study has reported that probiotics can maintain homeostasis of the gut microbiome of crew members during a one-month sea voyage²¹. However, how the gut microbiome responds to ocean-going voyages (i.e., voyages of longer than five months), and any association between the gut microbiome and sub-optimal health remain unexplored.

In this study, we tracked the gut microbiome and health status of 77 crew members on a 135-day-long ocean-going voyage (without landing during the voyage). Questionnaire on 24 physical/ psychological symptoms and stool samples from those crew members were collected at two time points: at day 1 (before the voyage) and day 135 (after the voyage). We found that some symptoms appeared together in a part of the crew members, and we considered this group of symptoms as the core symptoms of 'Ocean-Going Syndrome (OGS)'. Stool samples were sequenced by Whole-Genome Shotgun (WGS) sequencing. The taxonomical and functional profiles from two time points were then related to the OGS to outline a microbial signature to describe the microbial dynamic of the OGS. This study provides a fundamental basis for discovering potential predictors to address the sub-optimal health status of crews during ocean-going voyages.

Methods

Experimental design and subject recruitment. This study included 77 crew members who participated in a five-month long ocean-going voyage (135 days). Fecal samples were collected at the beginning (baseline at day 1; morning of the first day after boarding) and at the end (day 135, morning of the last day before landing) of the voyage. At the same time points, 24 physical and psychological states were defined for each participant using their responses to a questionnaire. Dietary records showed that the menu was repeated every week during the voyage; at least two kinds of non-staple food and two or three kinds of fruits were guaranteed every day to meet the crews' nutritional needs. Sample protector (CW0592M, CWBIO, China) was added to each stool sample in a ratio of 1:5 prior to storage at -20 °C until further processing.

Questionnaire design. The questionnaire consisted of three parts: (1) Physical indicators including BMI, and incidence of: backache, headache, stomach ache, pectoralgia, muscular soreness, dyspnea and palpitation; (2) Psychological indicators including: insomnia, shallow sleep, overeating, nausea, inactivity, poor appetite, feelings of loneliness, depression, self-accusation, and mistrustfulness; (3) Defecation-related indicators including stool type and incidence of: bloody stools, incomplete defecation, bowel difficulties, bowel movement, and defecation frequency (weekly). For more details about the definition of the above indicators, please refer to **Table S1**. The 24 physical and psychological indicators in the questionnaire were self-evaluated using a 0–5 points system, and then converted into grouping variables. If the indicator did not change or decrease between the two questionnaires taken at the beginning and

end of the voyage, then the crew member was recorded as 'symptomless'. If the indicator increased, then the crew member was recorded as 'symptom'. BMI was measured using the Chinese reference standard:18.5–23.9.

Clustering of symptoms. The least variance method (Ward.d) from the R package 'pheatmap' (<https://cran.r-project.org/web/packages/pheatmap/index.html>) was used to cluster physical and psychological symptoms (except BMI) into groups by similarity in Ward's minimum distance method.

Metagenomic shotgun sequencing. Stool samples were thawed on ice for 1 h and three 1.5 ml bacterial suspensions from each (a total of 4.5 ml) were vortexed and used for DNA extraction. A standard QIAGEN DNA Stool Mini-Kit (QIAGEN, Hilden, Germany) was used to extract DNA following the manufacturer's instructions. Genomic DNA quality and concentration were analyzed by gel electrophoresis and Nanodrop8000 (Thermo Electron Corp., Waltham, MA, USA), respectively. The final DNA concentration was above 100 ng/μL and the 260 nm/280 nm ratio was between 1.8 and 2.0. All samples were sequenced using Illumina HiSeq 2500. After quality control and human DNA removal a mean of 22,255,766 high-quality end reads were obtained for each sample. We used mOTUs2 and HUMAnN2 on high-quality end reads for taxonomical and functional profiling with default parameters.

Association between symptoms and the gut microbiome. Firstly, Bray-Curtis distance (BCD) and root Janson Shannon distance (rJSD) were used to measure changes in the gut microbiome of an individual between the beginning (baseline) and the end of the voyage; and termed this as 'individual perturbation'. Then, for each symptom, the crew were divided into 'symptomless' and 'symptom' groups as described before. Thirdly, BCD or rJSD values for each symptom were compared between the symptomless and symptom groups using the Wilcoxon test. All statistical analysis was done using the R script in Parallel-META-3.5²².

Microbial signature and co-occurrence network analysis. For the crew members in the OGS group, the paired Wilcoxon test was used to identify microbial species and functions that changed in abundance, respectively, between the beginning and the end of the voyage. We then repeated this using the same method, for the non-OGS group. The species and functions that had changed between the beginning and end of the voyage were then compared between the OGS and non-OGS groups; species and functions that changed in the same way in both groups were considered as an effect of time point rather than the voyage and were discarded from analysis. The remaining species and functions in the OGS group were considered as the microbial signature of OGS development. Then, MetagenoNets²³ was employed for co-occurrence network analysis based on SparCC correlation inference algorithms. The co-occurrence network was trimmed by taking the relative abundance > 0.001, prevalence > 10%, and $p = 0.01$ as the threshold.

Results

Physical and psychological symptoms after a 135-day-long ocean-going voyage

To investigate major symptoms during the ocean-going voyage we used questionnaires at the beginning (day 1) and end (day 135) of the voyage. Only data from male crew members (Han nationality, China) aged 20 to 35 were analyzed statistically as they were the predominant group and this avoided the influence of age, gender and nationality. The questionnaire consisted of three parts (**Method**): (1) Physical indicators including body mass index (BMI) and the incidence of backache, headache, stomach ache, pectoralgia, muscular soreness, dyspnea and palpitation; (2) Psychological indicators including insomnia, shallow sleep, over-eating, nausea, inactivity, poor appetite, and also feelings of loneliness, depression, self-accusation and mistrustfulness; (3) Defecation related indicators including stool type, incidence of bloody stools, incomplete defecation, bowel difficulties, bowel movement (but without defecation), and defecation frequency (weekly).

In terms of physical symptoms (Fig. 1a), the highest incidence amongst crew members was for backache (46.8%) and headache (44.2%) which increased by 40.3% and 39.0%, respectively, compared to the baseline values (6.5%, 5.2%). The incidence of muscular soreness (35.1%) and stomachache (33.8%) were the next most common symptoms, with an increase of 27.3% and 29.9% respectively compared to the baseline (7.8% and 3.9%). After the voyage, 20.8% of the crew members were suffering palpitations, 19.5% had dyspnea and 19.5% had pectoralgia, the incidence rates of which had increased by 18.2%, 19.5% and 13.0%, respectively. However, the body mass index (BMI) of all crew members did not change significantly during the voyage.

As for the psychological situation (Fig. 1b), the major psychological problems reported by crew members were shallow sleep (77.9%) and insomnia (66.2%) while few had sleeping problems before the voyage. Moreover, the prevalence of over-eating (55.8%), inactivity (55.8%) and nausea (50.7%) in more than half the crew members, had increased by 46.8%, 44.2% and 48.1%, respectively compared to the baseline. In addition, crew members also suffered from poor appetite (49.4%), feeling lonely (44.2%), depression (36.4%), self-accusation (31.2%) and mistrustfulness (26.0%), each of which had increased by 33.8%, 40.3%, 31.2%, 23.4% and 24.7%, respectively. These symptoms suggest a high level of mental stress as a result of the semi-closed living and working environment during the ocean-going voyage.

Beyond physical and psychological symptoms, we found that defecation was abnormal for most crew members (Fig. 1c). More than half (53.3%) of the crew members had an abnormal frequency of defecation (weekly) compared to only 1.3% at baseline. In addition, 42.9% of crew had incomplete defecation (a 29.9% increase from baseline) and/or bowel movements that did not result in defecation (a 16.9% increase from baseline). Furthermore, 42.9% had bowel difficulties, which was an increase of 10.4% compared with baseline (32.5%). Notably, 13.0% of crew members had bloody stool. Together these results indicate a sub-optimal health status amongst crew members as a result of the ocean-going voyage and including multiple physical and psychological symptoms as well as defecation disorders.

Definition of Ocean-Going Syndrome

Although the extreme living and working environment endured by the crew was the same, it has resulted in different symptoms in different individuals. To explore the correlation among the symptoms, we conducted cluster analysis on the change of physical, psychological and defecation indicators of crew members during the ocean-going voyage (Fig. 1d, **Method**). We found that the change of those symptoms were clustered into four distinct groups: Group **A**: the crews suffering from abnormal defecation frequency (weekly), insomnia, shallow sleep, nausea and over-eating; Group **B**: in addition to the symptoms of Group A, some of the crews also reported to have stomach ache, headache, incomplete defecation, backache and self-accusation; Group **C**: in addition to the symptoms of Group A, a part of the crews also have poor appetite, abnormal stool type (e.g. watery stool), bloody stool, bowel difficulties, bowel movements (without defecation), muscular soreness, feeling of tiredness leading to inactivity, feeling depressed, palpitations, pectoralgia and mistrustfulness; Group **D**: the crew members that feels lonely and dyspnea instead of symptoms in other groups. Amongst the four groups, the symptoms of Group A had the highest incidence and were most strongly correlated with each other, followed by the symptoms of Group B and Group C; the incidence of symptoms in A, B and C showed a gradually decreasing (Fig. 1e). Thus, we considered that the co-appearance of symptoms in Group A (e.g. abnormal defecation frequency, insomnia, shallow sleep, nausea and overeating) as core characteristic of this newly-discovered sub-optimal health status for long voyage crews, which we name here as 'Ocean-Going Syndrome (OGS)'. In addition to the core symptoms that almost every OGS crew suffers from, OGS crews also have a chance of suffering from symptoms in Group B and Group C.

Association between the gut microbiome and the Ocean-Going Syndrome

To explore any relationship between the OGS and the gut microbiome, we collected stool samples and sequenced the microbiome within them at two time points i.e. at day 1 (before voyage) and day 135 (after voyage) using WMS (Illumina HiSeq 2500). After quality control, each sample contained a mean of 22,255,766 clean reads which were profiled using mOTUs2 and HUMAnN2 to identify the taxonomical and functional structure of the gut microbiome (**Method**).

Then, we used Permanova test to determine the effect size of the OGS on the gut microbiome of crew members. Although Host ID had the greatest influence on the gut microbiome (Bray-Curtis distance, BCD: $F = 2.690$, $p = 0.001$; root Jensen Shannon distance, rJSD: $F = 2.520$, $p = 0.001$), the influence of the OGS as a factor on the gut microbiome was significant (Table 1, BCD: $F = 1.946$, $p = 0.020$; rJSD: $F = 1.810$, $p = 0.024$) and its significance was greater than the influence of time point (BCD: $F = 1.762$, $p = 0.043$; rJSD: $F = 1.481$, $p = 0.063$). Notably, based on Permanova, no effect sizes for any symptoms were positive (Table 1), suggesting only a weak influence of single symptom on the gut microbiome during the voyage. The above results indicated that the crews' gut microbiome associated with a group of symptoms i.e. the OGS instead of any single symptom.

Table 1
 Permanova test on the gut microbiome using different physical and psychological health indicators (symptoms) as factors.

Factors	Bray-Curtis		rJSD	
	Adonis.F	Adonis.P	Adonis.F	Adonis.P
Host ID	2.689	0.001	2.523	0.001
Ocean-going syndrome	1.946	0.020	1.810	0.024
Time points	1.762	0.043	1.481	0.063
Self-accusation	1.588	0.072	1.375	0.100
Feeling depressed	1.585	0.054	1.424	0.083
Dyspnea	1.519	0.086	1.418	0.085
Bloody stool	1.464	0.081	1.354	0.096
Bowel difficulties	1.405	0.136	1.394	0.104
Backache	1.324	0.147	1.186	0.189
Defecation frequency	1.290	0.159	1.137	0.245
Insomnia	1.181	0.222	1.082	0.314
Palpitations	1.169	0.257	1.142	0.229
Inactivity	1.167	0.259	1.199	0.191
Shallow sleep	1.157	0.237	1.042	0.347
BMI	1.130	0.282	1.072	0.322
Nausea	1.115	0.298	0.967	0.487
Overeating	0.978	0.431	1.006	0.379
Stool type	0.934	0.497	0.863	0.649
Mistrustfulness	0.913	0.527	0.950	0.488
Pectoralgia	0.913	0.533	0.904	0.572
Headache	0.903	0.552	1.008	0.383
Poor appetite	0.858	0.603	0.974	0.442
Incomplete defecation	0.855	0.594	0.867	0.616
Bowel movement	0.845	0.631	0.961	0.456
Feeling lonely	0.841	0.637	0.841	0.679

Factors	Bray-Curtis		rJSD	
	Adonis.F	Adonis.P	Adonis.F	Adonis.P
Stomach ache	0.811	0.662	0.917	0.540
Muscular soreness	0.565	0.955	0.701	0.912

Ocean-Going Syndrome as a key factor for individual perturbations during the voyage

Given individual (Host ID) has the biggest effect size on the gut microbiome, we sought to investigate the individual perturbations during the voyage. Although there was no significant difference in alpha diversity (Shannon index, $p = 0.73$) nor obvious clustering in PCoA (Fig. 2a) in the gut microbiome between day 1 and day 135, individual perturbation between the two time points, indicated by BCD and rJSD, showed a significant change in the gut microbiome (Fig. 2b); this indicates a profound change in the gut microbiome within individual during the voyage.

Then we compared individual perturbations in the gut microbiome between crew members suffering the OGS (individuals that clustered in Group A, Fig. 1d) with non-OGS (crew members that not clustered in Group A, Fig. 1d) and found that the former were significantly different to the latter (BCD: $p = 0.024$; rJSD: $p = 0.021$, Fig. 2c). These results demonstrate a strong association between the OGS and individual change of the gut microbiome during the voyage. However, such correlation was not found between the gut microbiome and any single symptom. Specifically, crew members were grouped by symptom (e.g. shallow sleep vs. symptomless) and the individual perturbations were grouped accordingly and compared (Fig. S1, Method). There was no strong correlation between any physical or psychological symptom and individual perturbation in the gut microbiome during the voyage, suggesting the crews with OGS had greater individual perturbations in the gut microbiome during the voyage, and no single symptom can introduce such significant perturbations.

Microbial signature for Ocean-Going Syndrome during the voyage

To identify the microbial biomarkers contributing to the development of the OGS, we evaluated how microbial species and their functions differed in abundance and expression, respectively, between the OGS group (the crew members in Group A) and the non-OGS group from beginning to the end of the voyage (Fig. 3a, Method). Overall, 15 species were significantly enriched ($p < 0.05$) in the OGS group compare to the non-OGS group during voyage: *Bifidobacterium pseudocatenulatum* ($p = 0.010$), *Clostridium* sp. 3680 ($p = 0.029$), *Collinsella aerofaciens* ($p = 0.008$), *Coprococcus comes* ($p = 0.010$), *Dorea longicatena* ($p = 0.001$), *Enterobacteriaceae* sp. 96 ($p = 0.043$), *Eubacterium ramulus* ($p = 0.030$), *Eubacterium* sp CAG 180 ($p = 0.020$), *Faecalibacterium* sp. 12325 ($p = 0.016$), *Firmicutes bacterium* CAG 227 ($p = 0.043$), *Firmicutes* sp. 3641 ($p = 0.044$), *Ruminococcaceae* sp. 12259 ($p = 0.004$), *Ruminococcus* sp. CAG 254 ($p = 0.031$), *Ruminococcus torques* ($p = 0.004$) and *Sutterella wadsworthensis* ($p = 0.013$). In

contrast, the abundance of four species decreased in the OGS group compare to the non-OGS group during the voyage e.g. *Faecalibacterium* sp. 12303 ($p = 0.044$), *Clostridium* sp. CAG 138 ($p = 0.021$), *Clostridium* sp. CAG 217 ($p = 0.028$) and *Bacteroides coprocola* ($p = 0.019$).

As for differential microbial functions, the abundance of 31 gene families from 14 pathways was found decreasing in the OGS group compare to the non-OGS group during the voyage. These pathways involved (Fig. 3a): Protein families: genetic information processing (09182); Protein families: metabolism (09181); Protein families: signaling and cellular processes (09183); Membrane transport (09131); Signal transduction (09132); Translation (09122); Infectious disease: bacterial (09171); Amino acid metabolism (09105); Carbohydrate metabolism (09101); Global and overview maps (09105); Lipid metabolism (09103); Poorly characterized (09194); Unclassified: signaling and cellular processes (09193), and Immune system (09151). These suggested that a microbial signature can be captured in the OGS crews during the voyage, which can be quantified as change of 15 taxonomical and 31 functional biomarkers.

To better understand the microbial dynamic difference between the OGS and non-OGS groups, co-occurrence network analysis was employed for the stool samples at both before and after the voyage (Fig. S2, Table S2, Method). Taking relative abundance > 0.001 , prevalence $> 10\%$, and $p = 0.01$ as the threshold, we compared the nodes, edges and density of the four co-occurrence networks (Fig. 3b). We found that: (1) before the voyage at day 1, the OGS and non-OGS shared a large number (170/190 and 170/199 respectively) of nodes while the edges based on those nodes are barely overlapped (5/237 and 5/216 respectively); (2) after the voyage at day 135, similarly, the OGS and non-OGS shared a large number (179/198 and 179/200 respectively) of nodes and the edges still barely overlapped (5/227 and 5/253 respectively); (3) from day 1 to day 135, within the OGS and non-OGS group, we observed a large of shared nodes but limited shared edges as well. On the other hand, we found a decreasing of network density (from 0.013 to 0.012) in the OGS group and an increasing of network density (from 0.011 to 0.013) in the non-OGS group from day 1 to day 135. Collectively, the above results suggest that the OGS and non-OGS have unique microbial dynamic, and the correlation of microbial species in the two groups are different even at the beginning of the voyage.

Prediction of Ocean-Going Syndrome before the voyage

To explore whether the OGS can be predicted, we investigated the microbial compositions of the OGS before the voyage. Although Permanova test showed that there was no influence of the OGS on overall taxonomic structure (BCD: $F = 1.23$, $p = 0.215$; rJSD: $F = 1.169$, $p = 0.241$) or functional structure (BCD: $F = 0.676$, $p = 0.648$; rJSD: $F = 0.687$, $p = 0.691$) before the voyage, six species (*Bacteroides massiliensis* ($p = 0.028$), *Parabacteroides distasonis* ($p = 0.019$), *Eubacterium rectale* ($p = 0.035$), *Bacteroides rodentium* ($p = 0.023$), *Clostridiales* sp. 12229 ($p = 0.035$), *Bacteroides stercoris* ($p = 0.029$)) and two gene families (Protein families: genetic information processing (09182, $p = 0.035$); Membrane transport (09130, $p = 0.040$)) were identified as biomarkers between the OGS and non-OGS at baseline. The differential abundance of the above taxon and functions can potentially be used to predict the onset of the OGS at baseline.

Moreover, to explore whether the OGS could be predicted, Random Forest was used to build a prediction model that distinguished between the OGS group and the non-OGS group based on microbial taxonomic and functional profiling of samples taken before the voyage began. Performance improvement was minimal once the top 27 most discriminatory species and functions were included (**Table S3**, Fig. 4a). Ultimately samples from the OGS could be distinguished from non-OGS samples with 87.0% accuracy (ten-fold cross validation AUC = 0.91, Fig. 4b). On the other hand, RF models using baseline data from microbial species, microbial functions, or questionnaire separately could only predict the onset of the OGS with 80.5% (AUC = 0.85), 83.1% (AUC = 0.85) and 68.8% (AUC = 0.67) accuracy (Fig. 4b). Thus, biomarkers from baseline gut microbiome data alone can be used to predict the likelihood of the OGS developing, without the need for determining physical or psychological status.

Discussion

During ocean-going voyages, crew members are subject to complex pressures from their living and working environment. These pressures lead to a series of physical and psychological disorders or, as we call it, a sub-optimal health status, which only appear during the voyage and associated with the length of voyage^{24, 25}. In previous studies, abnormal defecations were observed while the association of the sub-optimal healthy status and the gut microbiome is rarely studied²¹. This study is designed to test whether the sub-optimal health status during long voyages are associated with changes in their gut microbiomes.

Questionnaires clearly showed that crew members had a sub-optimal health status after the ocean-going voyage as indicated by the multiple physical and psychological symptoms they suffered, including defecation disorders. However, none of the symptoms taken individually can positively relate to the gut microbiome since individuals can respond differently to the same stimulus/treatment²⁶. This led us to consider that crew members' responses to an identical extreme environment may also vary for the same reason, or even, similar changes in the gut microbiome could trigger different physical and psychological responses. To test this hypothesis and rule out the noise introduced by individual differences, we performed cluster analysis on the symptoms in the first place, which is rarely performed in previous studies due to sample size limitation and questionnaire design. Then, we for the first time found that the co-appearance of symptoms of abnormal defecation frequency, insomnia, shallow sleep, nausea and over-eating (symptoms in Group A) best described the sub-optimal health status of the majority of crew members, i.e. 'Ocean-Going Syndrome (OGS)', and a strong association between the OGS and the gut microbiome was proved. Then a microbial signature of OGS was identified, which supports the hypothesis that similar changes in the gut microbiome can trigger both similar (symptoms in Group A) and different physical and psychological responses e.g. stomachache, headache, incomplete defecation, backache, self-accusation (symptoms in Group B) and poor appetite, stool types, bloody stools, bowel difficulties, bowel movement, muscular soreness, inactivity, feel depressed, palpitation, pectoralgia, mistrustfulness (symptoms in Group C).

On the other hand, to bridge the concept of the OGS to application, we built a model that could predict the likelihood of ocean-going syndrome developing with 87.0% accuracy (AUC = 0.91) before the voyage, which would be a useful health risk assessment for crew members and potential criteria for sailors selection. Studying the association between the gut microbiome and sub-optimal health status in extreme environments will give us a better understanding of the interactions between the gut microbiome and dysbacteriosis related systemic or even chronic diseases which is an important health problem in today's society. This study provides a fundamental basis for discovering potential predictors for the sub-optimal health status caused by extreme environments and demonstrates how pre-treatment of symptoms could facilitate the study of dysbacteriosis related diseases with multi-symptoms under the influence of individual differences.

Conclusion

Studying the association between the gut microbiome and sub-optimal health status in extreme environments will give us a better understanding of the interactions between the gut microbiome and dysbacteriosis related systemic or even chronic diseases which is an important health problem in today's society. In our study, 77 crew members were recruited, and 24 physical and physiological conditions that they suffered from were recorded based on their responses to questionnaires made at the beginning and end of an ocean-going voyage. Results clearly showed that crew members had a sub-optimal health status during the ocean-going voyage as indicated by the multiple physical and psychological symptoms they suffered, including defecation disorders. The co-appearance of symptoms e.g. abnormal defecation frequency, insomnia, shallow sleep, nausea and over-eating best described the sub-optimal health status of the majority of crew members, and we named this as 'Ocean-Going Syndrome (OGS)'. To explore the association between the gut microbiome and the sub-optimal health status or the OGS of crew members, we used WMS to record the microbiome in the stool samples of crew members collected at the beginning and end of the ocean-going voyage. Then we found that the crews' gut microbiome was influenced by the OGS instead of any single symptom in the questionnaire. Moreover, the OGS is the key factor for individual perturbations (in the gut microbiome) during the voyage instead of any single symptom. Furthermore, we built a model that could predict the likelihood of ocean-going syndrome developing with 87.0% accuracy (AUC = 0.91) at the beginning of the voyage, which would be a useful health risk assessment for crew members and potential criteria for crew selection. This study provides a fundamental basis for discovering potential predictors for the sub-optimal health status caused by extreme environments and demonstrates how pre-treatment (clustering) of symptoms could facilitate the study of dysbacteriosis related diseases with multi-symptoms under the influence of individual differences.

Declarations

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Author contributors

HZ, ZHS and ZS designed the study. JZ, YJ, HC and ML collected the samples and conducted the experiments. ZS, MZ and ML analyzed the data and prepared the manuscript. All authors approved the final version of the manuscript.

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Availability of data and materials

Data are available in a public, open access repository. All sequence data from this study has been submitted to Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra>) and can be accessed through the BioProject IDs: PRJNA629464.

Ethics approval and consent to participate

Written informed consent was obtained from all subjects. The study protocol was approved by the Ethical Committee of the Inner Mongolia Agricultural University (Hohhot, China).

Consent for publication

All authors have given consent to publish this manuscript in Microbiome.

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

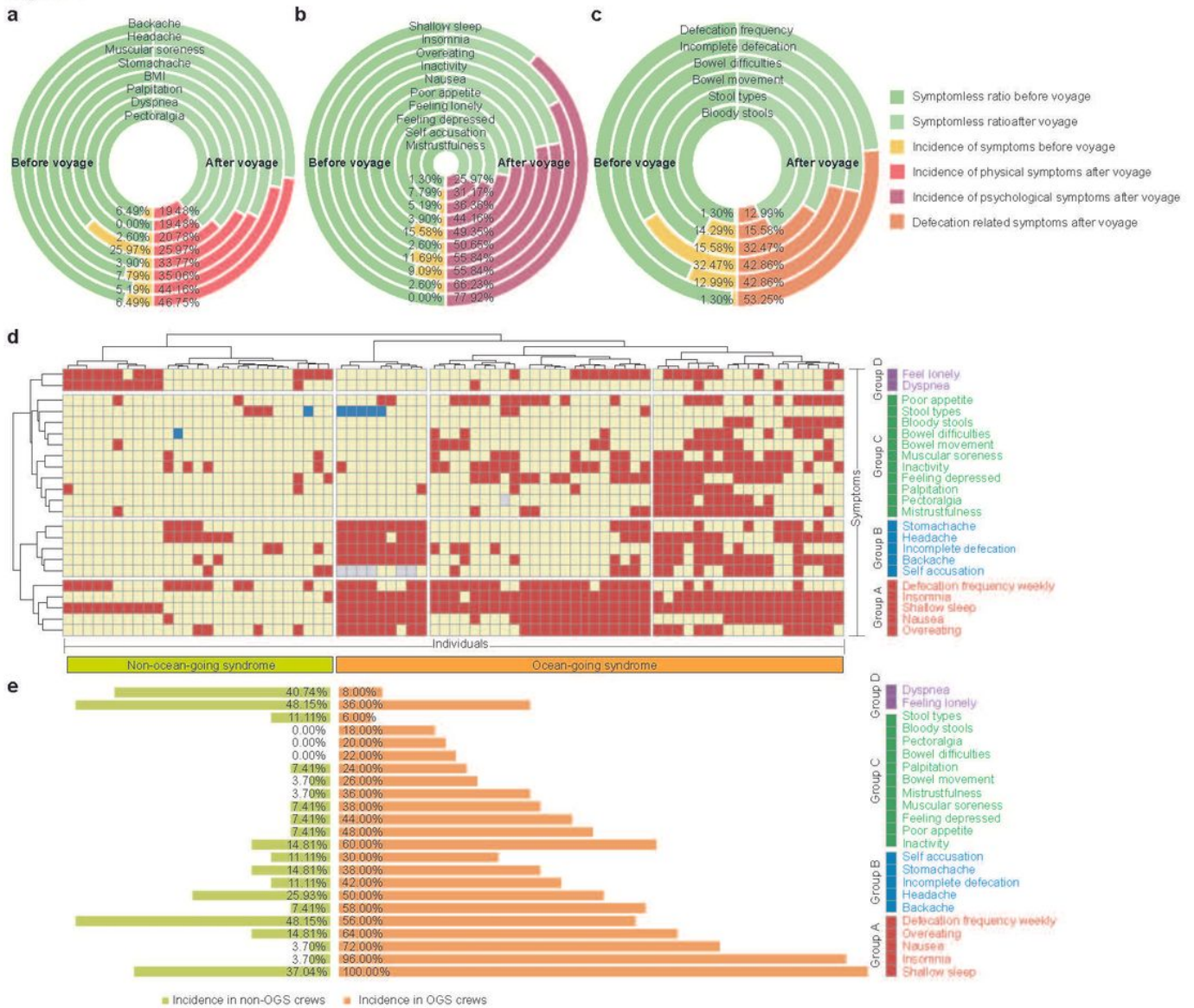


Figure 1

Sub-optimal health status during the voyage and definition of Ocean-Going Syndrome. Changes in self-assessed indicators of physical (a), psychological (b) and defecation-related (c) health status are presented separately. The left part of each circle refers to the health status before sailing while the right part of each circle refers to the incidence of sub-optimal health indicators (symptoms) after the voyage. (d) Heatmap showing the change of different health indicators (symptoms) during the voyage adjusted by clustering results (red for increased severity of the specific symptom, yellow for non-change and blue for decreased symptoms). Four groups of symptoms were classified, and Group A was considered to be the core symptoms of the OGS. The crew members (individuals) clustered in orange area are the ones suffering with the OGS, while the light green indicates the 'symptomless' crew members i.e. non-ocean-going syndrome. (e) Comparison of the incidence of symptoms between the OGS and non OGS groups rearranged by clustering results.

Figure 2

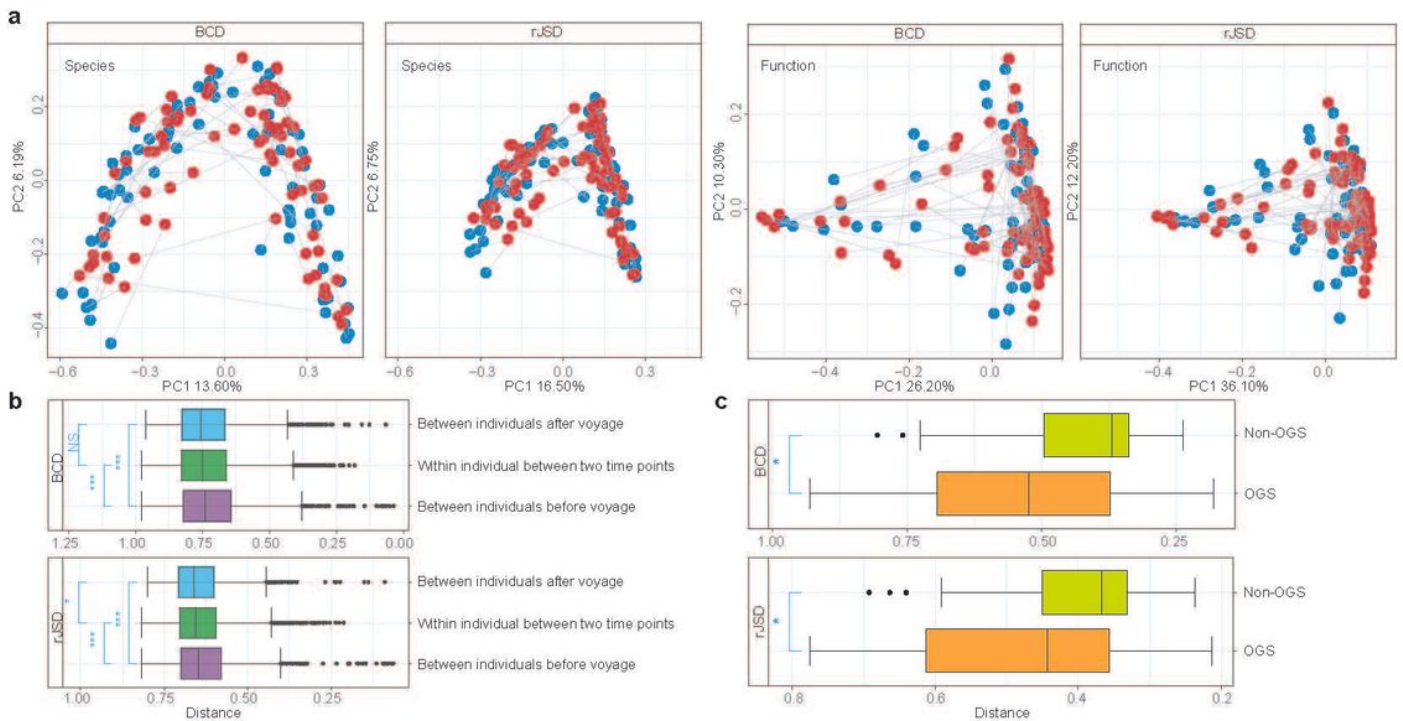


Figure 2

Impact of voyage on the diversity of the gut microbiome. (a) Principal Coordinate Analysis based on both the taxonomical and functional profiles between two time points. The developing trajectory for each individual during the voyage was connected by grey lines. (b) The BCD and rJSD between individuals at the beginning and end of the voyage, and the distance within individual between two time points were compared. (c) Boxplots of the individual perturbations in the gut microbiome between the OGS and non-OGS groups based on BCD, rJSD (BCD: $p = 0.024$, rJSD: $p = 0.021$).

Figure 3

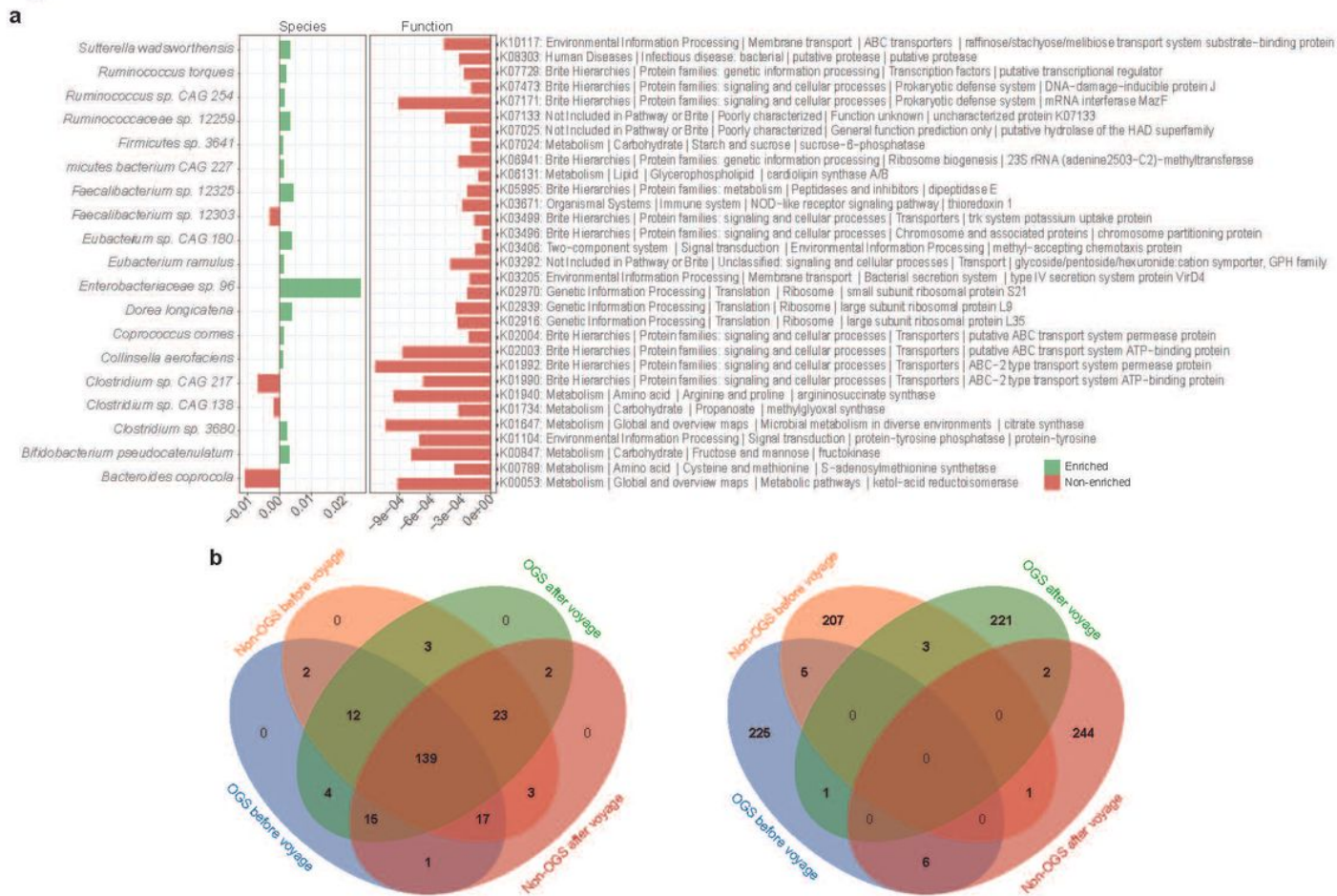


Figure 3

Microbial signature of ocean-going syndrome. (a) Bar plots of microbial species and functions that changed in abundance during the voyage (green represents species/ functions that increased while red represents species/ functions that decreased after the voyage). (b) Venn diagram for nodes (left panel) and edges (right panel) of four co-occurrence networks: the OGS and non-OGS at beginning (day 1) and end (day 135) of the voyage.

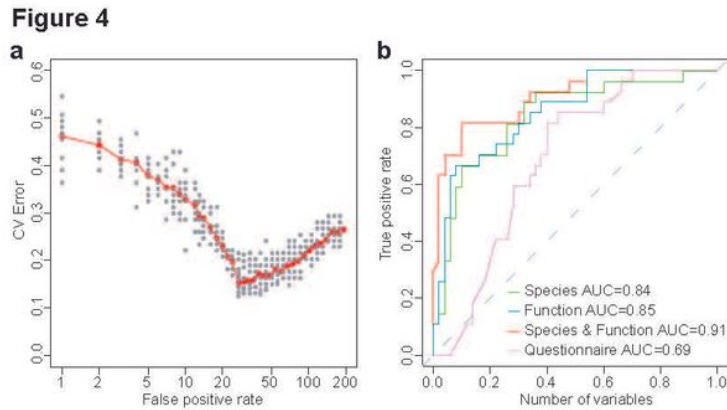


Figure 4

Random Forest model for predicting the likelihood that crew members would develop ocean-going syndrome. (a) Selection of biomarkers based on the gut microbiome for RF model to predict the likelihood of ocean-going syndrome developing. The relationship between the number of variables in the RF model and model performance were analyzed; 27 biomarkers with the most discriminating power were selected. (b) Prediction performance of RF models using different biomarkers at baseline (e.g. only microbial species, only microbial functions, only questionnaire before the voyage, and microbial species plus gene functions), as assessed via the Area Under the Receiver Operating Characteristic Curve (AUC).

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