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ORIGINAL ARTICLE

Meta-Analysis of Environmental Factors That Influence the Relative Decay Rate between Enterococcus and Escherichia Coli in Aquatic Environments

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Abstract

For many decades, *Escherichia coli* and *Enterococcus* are used as fecal indicator bacteria (FIB) to evaluate water quality. The decay rates of the two FIBs and hence their persistence in the environment varied significantly over space and time. There have been on-going debates as to whether the use of dual FIBs provides more accurate evaluation of health risks than the use of a single FIB, and whether one FIB is more advantageous than the other in a given type of water body (e.g. freshwater vs. marine). However, many national and international bureaux use a single set of reference standards, which are based on the concentrations of a single FIB or both FIBs, for a very wide range of geographical areas. In this study, we conducted meta-regression and meta-analysis of the data reported in a range of studies that were conducted in different geographical regions under different experiment settings to assess the effects of abiotic factors in the aquatic environment on the relative decay rates of *E. coli* and *Enterococcus*. The results of our analysis indicated that sunlight intensity and temperature were the most important abiotic factors that affect the relative decay rates between *E. coli* and *Enterococcus*. Despite the common belief that *E. coli* can persist longer than *Enterococcus* when exposed to seawater, we found that salinity has no obvious effects on the relative decay rates between the two FIBs. Our results imply that it may not be suitable to use a single FIB throughout the whole year even at a given location since the intensity and duration of sunlight irradiation vary drastically with seasons

Keywords: Escherichia coli, Enterococcus, decay, water monitoring

Introduction

Water quality monitoring is essential to protecting public from waterborne diseases. Fecal indicator bacteria (FIB) is used to indicate the microbiological quality of bathing and drinking water resources. *Escherichia coli* and *Enterococcus* has been used as FIBs as both of them can survive for a over 6 month in water [1]. For decades, many countries have been using *E. coli* as the principal FIB [2]. Nonetheless, more recently, *Enterococcus* is well-accepted worldwide as the better fecal indicator for marine water quality monitoring after the epidemiology study by Cabelli *et al*. [3]. They found that *Enterococcus* spp. (ENT) are very resistant to high salt concentration environment and thus their use as FIB can improve the reliability of marine water assessment compared to using other fecal coliforms, especially *E. coli*, which is not suitable to detect pathogenic strains in seawater due to their rapid die-off rate than ENT and other pathogens [4]. This phenomenon prompted us to look into the relationship between the decay rate of ENT and *E. coli*. If such relationship exists, what environmental parameters played a major role in the relationship? The two bacteria coexists in the aquatic environment, but their curve of growth or decay rate is not a simple log-linear relationship.

Different countries adopted different FIB for their water quality assessment. Dual FIBs - both *E. coli* and ENT - has been used in some countries. Currently, Singapore uses only E*nterococcus* for

both seawater and freshwater monitoring, and Hong Kong is still using *E. coli* for monitoring the quality of marine beach water. The World Health Organization uses *E. coli* for freshwater and *Enterococcus* for marine water monitoring respectively. In other regions of the world such as the European Union and Australia, dual indicators were used for both freshwater and marine water [5]. Considering both economics and efficiency, it is important to choose the most suitable FIB to best represent the water quality of the region concerned.

The major goal of this study is to provide an effective reference as to whether the current in-use FIB in a particular region is appropriate and sufficient, or some amendments may be considered (for example the use of another FIB, using dual FIBs instead of one etc.). Due to differences in geographical environments or even seasonal variability, environmental factors such as temperature, salinity, predation and sunlight are always changing and will affect the die-off rate for both *E. coli* and ENT. Our goal was to compare the survival rate of *E. coli* and ENT at the same period in water (fresh water, seawater, sewage etc.) and to quantify the contribution of these environment factors. A systematic search of literatures studying dual indicators in the same environment and period was conducted. Data was extracted from these studies and regression method and meta-analysis were both performed. Observations and discoveries were interpreted in the context of understanding the effects of natural factors on the decay rate of these fecal indicator microorganisms.

Materials and Methods

Literature Search and Selection Criteria

The PubMed database and Web of Science database were searched for studies related to *E. coli* and ENT. Both searches were limited to studies published in English and not restricted to a specific time range. In order to obtain studies which included at least the two bacteria, we first screened all possible studies using the name of the two bacteria in abstract and title by using the keywords combination of "escherichia-coli" OR "e. coli" and the other keywords combination "enterococcus" OR "enterococci" separately, and then the studies under overlapping results were kept for further filtering. The second abstract-title-based relevance screening was to restrict studies to the relevant indicator field, some common words (indicator-microorganisms, fecal bacteria, fecal indicator etc.) had been chosen in this process. The major focus was on studies addressing the survival of *E. coli* and ENT in different environments. All possible terms describing survival (survival, decay, decline or persistence etc) have been chosen to further screen the above overlapping studies. Remaining studies after the above selections were further filtered by artificial screening to select studies for data extraction. Data were then extracted from the final chosen articles for multivariate regression analysis and systematic meta-analysis. The whole study selection workflow is shown in Figure 1.

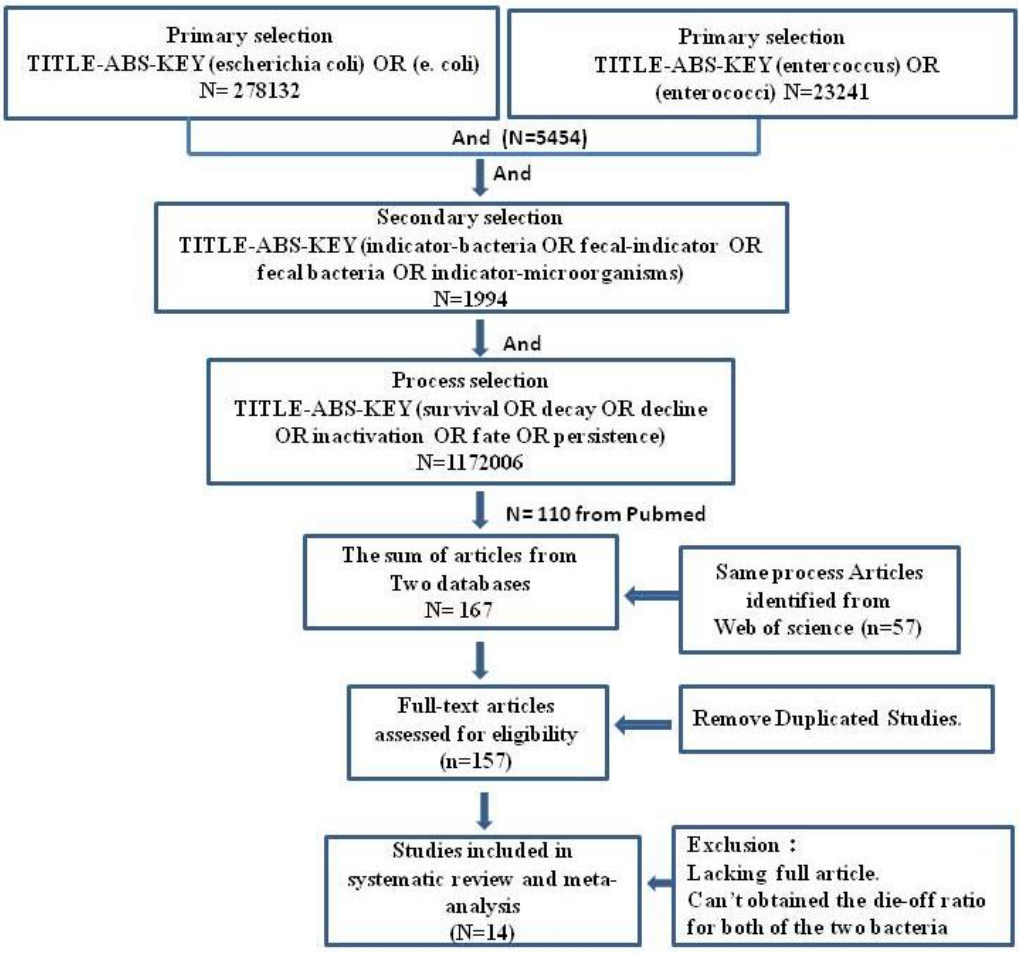

Figure 1. Data collection procedure. 157 literatures were retrieved from the first-round search. 14 qualified studies were included in the final meta-analysis.

Data Extraction and Categories

The major data extracted was the decay rate of the two bacteria in their first order decay. The collected information contains Climate class, Temperature, Sample source, Area, Study ID, Year, No. of sampling events, Season, Water Deep, Light irradiation, and the Decay rate of bacteria. Considering some detailed information about the decline rate of bacteria are lacking in some of the literatures and difficulty in requesting raw data from the authors, we assumed that at a constant rate in time [6]:

$$\mu=[\log(10)C0-\log(10)Ct]/t \qquad (1)$$

where Ct is the concentration of bacteria at a specific time, C0 is the initial bacterial concentration, t is the time [min, hour or day], μ as the coefficient on a log10 scale. In the rest of the manuscript, values of μ were collected directly or derived from reported T90 values, that is, the time to one log10 reduction and the μ is the reciprocal . Besides the decay rate, environmental factors (temperature, light, deep, season, climate, water source) had also been arranged. All of the decay rates under different environmental factors will be converted to the same unit (/min) to make direct comparison of the rates reported in these studies.

Considering the data categories is very important for assessing the impact of each environmental factor. Here, for evaluating the contribution of temperature factor to decay rate, we review all the collecting studies' temperature range from 6°C to 30°C and the former study [7] found the bacteria degraded shown significantly difference between 20°C and 14°C($p < 0.0001$). Hence we decided to use temperature around 10°C, 15°C, 20°C and above 25°C as the four reasonable major categories in temperature ,For the light category, due to the experimental recording limited, We could only assess the difference under the three major categories of light condition (light 50 W/M^2, light 200-300 W/M^2 and light 500-700 W/M^2) based on our obtained data. For the rest of the environment factors such as deep, season,

climate and water source, the regular categories are as stated in table1.

Data Processing and Statistical Analysis

In order to investigate which environmental factors and to what extent they affected the ratio of decay rate of the two bacteria, multiple regression analysis was performed by means of linear model fitting using the stargazer package [8]. The following line's corresponding coefficient of determination (R-squared) value and F-statistic value and P value etc were also generated by the stargazer package in R setting programming environment.

For regression analysis, the ratio was log-transformed for convenient calculation and visualization by ggplot2 [9]. The lm method was chosen as the smoothing method in the plots. Confidence intervals of the ratio for each factor were calculated by the Wilson score interval with continuity correction. Model predictions the trend of E. coli and ENT decay rate comparison ratio included 95% confidence intervals. Further meta-analysis for these observational studies was performed by STATA (version 13.0). Subgroup analysis was also performed for assessing the ratio affect by each corresponding environment factor. The ratio differences between the decay rate of E. coli and ENT were expressed as the standardized mean difference (SMD) at 95% confidence interval (CI). The heterogeneity among studies was estimated by I^2 index. Random effect model and fixed-effect model were adopted for the heterogeneity was statistically significant (I^2 >75%) or not statistically significant (I^2 <=75%) separately. The above calculations were run by R software invoking stargazer package and all figures were generated by the ggplot2 package. Both of the packages were performed in the R setting programming environment. Regression slopes comparison was performed by Minitab statistical software [10].

Results

Regression Models Estimate and Coefficient Comparison

The primary PubMed and Web of Science databases search returned 167 unique hits for further full-text article analysis and assessment. Articles in which the decay rate of ENT and *E. coli* were not from the same point location sample were filtered out. Articles which did not provide the die-off rate or from which die-off rate cannot be deduced based on their concentration will also be excluded from the last systematic review analysis. When an article is present in both databases, we manually removed the repeated hit. At the end, we obtained a total of 14 studies based on these criteria. These studies represented data of the two fecal indicator bacteria isolated from 14 different countries and including 18 different regions. Table 1 summarizes this information and also compute the decay rate ratio separately.

Table1: Comparison of different study groups and data collected

Climate class	Temp Class	Tm(^{O}C)	Sample source	StudyID	Year	No of sampling events	Season class	Light class	Light (W/M^2)	m1	sd1	m2	sd2	Ref
Subtropics	15±2 °C	15	seawater	Alkan	1995	8	ND	200-300	300	0.01137	0.0038	0.01206	0.0047	[11]
Subtropics	>=25 °C	25	seawater	Alkan	1995	8	ND	200-300	300	0.0154	0.004	0.01576	0.0042	[11]
Subtropics	20±2 °C	20	seawater	Alkan	1995	22	ND	500-700	500	0.01505	0.0063	0.01411	0.0059	[11]
Subtropics	15±2 °C	15	seawater	Alkan	1995	8	ND	500-700	700	0.0167	0.0049	0.0176	0.0062	[11]
Subtropics	>=25 °C	25	seawater	Alkan	1995	8	ND	500-700	700	0.01631	0.0054	0.01486	0.0034	[11]
Subtropics	15±2 °C	15	seawater	Alkan	1995	8	ND	200-300	300	0.01086	0.0038	0.0098	0.0032	[11]
Subtropics	>=25 °C	25	seawater	Alkan	1995	8	ND	200-300	300	0.01323	0.0032	0.01343	0.0044	[11]
Subtro	20±2	20	seawat	Alkan	199	22	ND	500-	500	0.025	0.019	0.024	0.0	[11]

pics	°C		er		5			700		3	1	3	191	
Subtropics	15±2 °C	15	seawater	Alkan	1995	8	ND	500-700	700	0.01578	0.0039	0.0151	0.0037	[11]
Subtropics	>=25 °C	25	seawater	Alkan	1995	8	ND	500-700	700	0.01526	0.0041	0.01435	0.0039	[11]
Subtropics	>=25 °C	30	freshwater	HKUST	2013	3	summer	500-700	500	0.0188	0.0045	0.0213	0.0031	[12]
Subtropics	>=25 °C	30	freshwater	HKUST	2013	3	summer	500-700	500	0.0226	0.0055	-0.005	0.0023	[12]
Temperate	10±2 °C	9.07	freshwater	Ariamalar	2003	2	ND	ND	ND	0.0004	0.0002	0.0003	0.002	[13]
Temperate	20±2 °C	19.87	freshwater	Ariamalar	2003	2	ND	ND	ND	0.0014	0.0005	0.0016	0.0006	[13]
Temperate	>=25 °C	29.32	freshwater	Ariamalar	2003	2	ND	ND	ND	0.0023	0.0012	0.0025	0.007	[13]
Temperate	20±2 °C	20	freshwater	Ariamalar	2003	2	ND	0-50	0	0.0006	0.0003	0.0007	0.002	[13]
Temperate	>=25 °C	26.17	freshwater	Ariamalar	2003	2	ND	200-300	208.6	0.0023	0.0027	0.0033	0.011	[13]
Temperate	>=25 °C	26.17	freshwater	Ariamalar	2003	2	ND	500-700	552.3	0.0027	0.0038	0.0031	0.025	[13]
Subtropics	15±2 °C	15.1	sewage	Maraccini	2016	15	winter	0-50	35.69	0.189	0.015	0.166	0.01	[14]
Subtropics	20±2 °C	23.4	sewage	Maraccini	2016	15	summer	0-50	29.76	0.054	0.003	0.068	0.005	[14]
Temperate	10±2 °C	6	freshwater	Minji	2015	2	ND	ND	ND	0.0208	0.0087	-0.0079	-0.004	[15]
Temperate	20±2 °C	20	freshwater	Minji	2015	2	ND	ND	ND	0.051	0.006	0.125	0.021	[15]
Subtropics	>=25 °C	25	seawater	Gregory	2011	1	winter	ND	ND	0.0196	0.0000	0.0111	0.0000	[16]
Temperate	>=25 °C	25-30	freshwater	Jin	2003	1	summer	0-50	0	0.092	0.0000	0.065	0.0000	[17]
Temperate	ND	15-30	freshwater	Cristianr	2009	1	winter	ND	ND	0.09	0.01	0.1	0.02	[18]
Tropics	20±2 °C	22-24	seawater	Zhang	2015	1	ND	ND	ND	0.0196	0.0000	0.0316	0.0000	[19]
Subtropics	20±2 °C	20	seawater	Noble	2004	12	winter	ND	ND	0.029	0.0006	0.02	0.0014	[20]
Subtropics	20±2 °C	20	seawater	Noble	2004	6	winter	ND	ND	0.03	0.0028	0.019	0.0017	[20]
Subtropics	ND	ND	seawater	Noble	2004	8	summer	ND	>300	0.1373	0.0099	0.2572	0.0074	[20]
Subtropics	ND	ND	seawater	Noble	2004	8	summer	200-300	<=300	0.048	0.0035	0.2434	0.0221	[20]
Subtropics	ND	ND	freshwater	Noble	2004	8	summer	ND	>300	0.1346	0.0092	0.2724	0.0068	[20]
Subtropics	ND	ND	freshwater	Noble	2004	8	summer	200-300	<=300	0.0542	0.0039	0.2434	0.0133	[20]
Subtropics	15±2 °C	14	seawater	Noble	2004	12	winter	ND	ND	0.021	0.001	0.013	0.0017	[20]
Subtropics	15±2 °C	14	seawater	Noble	2004	6	winter	ND	ND	0.021	0.003	0.016	0.021	[20]
Tropics	>=25	27.6	freshwater	Bordal	2002	1	winter	ND	ND	0.0109	0.0000	0.00077	0.0000	[21]

Subtropics	20±2 °C	23	sewage	John	2005	2	winter	ND	ND	0.016	0.0049	0.0093	0.0056	[22]
Subtropics	10±2 °C	9	sewage	John	2005	2	summer	ND	ND	0.0037	0.0014	0.0056	0.0012	[22]
Temperate	20±2 °C	18.4	seawater	Mattioli	2016	5	summer	ND	ND	0.00333	0.0007	0.0046	0.0038	[23]
Temperate	15±2 °C	15.6	seawater	Mattioli	2016	5	winter	ND	ND	0.0027	0.0009	0.0027	0.009	[23]
Temperate	20±2 °C	18.4	sewage	Mattioli	2016	5	summer	ND	ND	0.00118	0.0001	0.00166	0.004	[23]
Temperate	15±2 °C	15.6	sewage	Mattioli	2016	5	winter	ND	ND	0.00153	0.0003	0.00097	0.003	[23]
Temperate	15±2 °C	15.6	sewage	Mattioli	2016	5	winter	ND	ND	0.00208	0.0011	0.0023	0.006	[23]
Subtropics	15±2 °C	15.8	sewage	Ahmed	2014	3	winter	0-50	17.9	0.00031	0.0000	0.00037	0.000	[24]
Subtropics	15±2 °C	15.8	sewage	Ahmed	2014	3	winter	0-50	17.9	0.0004	0.0000	0.00037	0.000	[24]

ND: No corresponding information in the paper. m1 to represent the decay rate mean value of E. coli, sd1 to represent the standard error of the mean in the study. m2 to represent the decay rate mean value of ENT, sd2 to represent the standard error of the mean in the study. ND to represent can't find the specific information in the study.

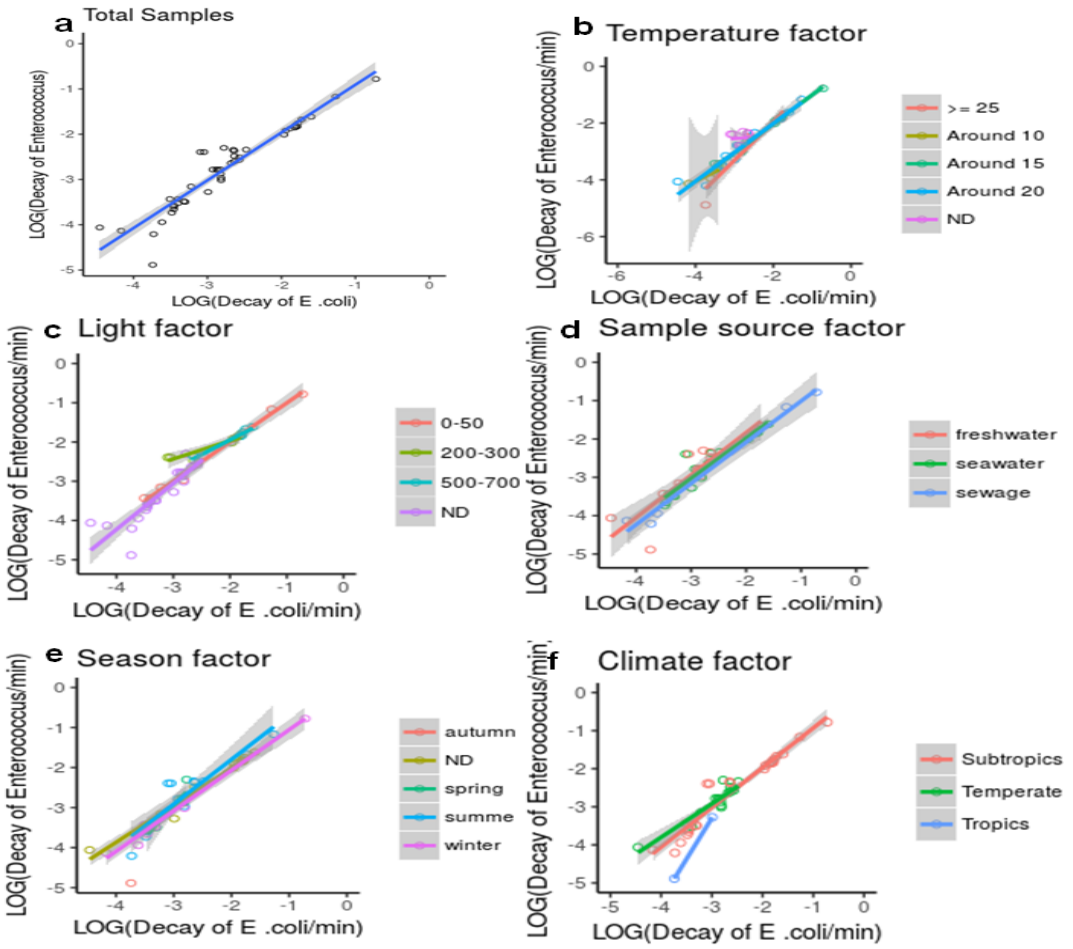

Figure 2. Linear relations of the decay rate ratio of ENT/E.coli with different environment factors. (a) The generally relationship of decay ratio of ENT/E.coli in different environment; (b) The relationship between the temperature and the decay ratio of ENT/E.coli; (c) The relationship between the sunlight and the decay ratio of ENT/E.coli; (d) The relationship between the sample source and the decay ratio of ENT/E.coli; (e) The relationship between the season and the decay ratio of ENT/E.coli; (f) The relationship between the Climate and the decay ratio of ENT/E.coli;. The above all creating model objects for two Ordinary Least Squares (OLS) models (using the lm() command in R) and the shaded areas around lines represent 95% confidence intervals.

Figure 2 summarizes the linear relationship between the decay rate of ENT and E. coli with different environmental factors. The lines show a general relationship of decay rate ratio of ENT/E. coli under various environmental parameters. See the statistical results in Table 2 and Table3 summary the regression equation for each line.

Table 2: The estimate results of regression models using stargazer

Variable name	Observations	R^2	Adjusted R^2	Residual Std. Error	F Statistic	P value
Total sample	**47**	**0.664**	**0.485**	**0.656**	**3.703**	**<0.01**
10±2°C	3	0.003	-0.994	0.438	0.003	>0.1
15±2°C	12	0.775	0.381	0.139	1.966	>0.1
20±2°C	13	0.729	-0.086	0.57	0.894	>0.1
>=25°C	**11**	**0.990**	**0.951**	**0.08**	**25.268**	**<0.05**
Light0-50	6	0.737	0.562	0.144	4.209	>0.1
Light200-300	**7**	**0.988**	**0.975**	**0.285**	**80.311**	**<0.01**
Light500-700	**10**	**0.958**	**0.924**	**0.112**	**28.548**	**<0.01**
seawater	24	0.774	0.481	0.642	2.638	<0.1
freshwater	17	0.713	0.081	0.99	1.28	>0.1
sewage	6	0.583	-0.042	0.364	0.934	>0.1
summer	**12**	**0.976**	**0.935**	**0.384**	**23.714**	**<0.01**
winter	10	0.534	-0.399	0.288	0.572	>0.1
temperate	17	0.343	-0.501	0.741	0.406	>0.1
subtropics	**28**	**0.797**	**0.711**	**0.573**	**9.3**	**<0.01**

Table 3: Regression equations for each factor

condition name	Regression equation
Subtropics	Log(m2) = 0.113 + 1.0419*Log(m1)
Temperate zone	Log(m2) = -0.273 + 0.882*Log(m1)
Tropics	Log(m2) = 3.16 + 2.151*Log(m1)
10±2°C	Log(m2)=-1.25+0.701*Log(m1)
15±2°C	Log(m2) = 0.016 + 1.0231 *Log(m1)
20±2°C	Log(m2) = 0.026 + 1.0198*Log(m1)
>=25	Log(m2) = 0.736 + 1.3491*Log(m1)
fresh water	Log(m2) = 0.374 + 1.106*Log(m1)
seawater	Log(m2) = 0.099 + 1.0432*Log(m1)
sewage	Log(m2) = 0.062 + 1.0736*Log(m1)
summer	Log(m2) = 0.431 + 1.115*Log(m1)
winter	Log(m2) = -0.042 + 1.0132*Log(m1)
0-50 w/m^2	Log(m2) = -0.008 + 0.9966*Log(m1)
200-300w/m^2	Log(m2) = -1.032 + 0.467*Log(m1)
500-700w/m^2	Log(m2) = -0.458 + 0.744*Log(m1)

Note: m1 to represent the mean decay rate of *E. coli*, m2 to represent the mean decay rate of ENT.

Generally speaking, the linear regression model can be used to explore the proportionate amount of variation in the response y explained by the other independent variables X and the larger the coefficient of determination (R2) is, the more variability can be explained by the regression model. From Figure 2a, the linear regression shows the first order die-off rate ratio to all the experimental data used here and the regression line for predicting the average decay rate ratio is almost the 45-degree line (R2=66.4%, p<0.01) (Table 2). This result implies that ENT and E. coli own a fix decay rate ratio in general situation. Random choose one of them as the FIB could be

accepted. Considering these data were collected by different researchers under different settings and from different habitats. In order to investigate the mean decay rate ratio for these data, we clustered the data by different environmental factors (light, sample source, temperature, season, climate).

Figure 2b shows the slope situation for four different temperature range (10±2°C, 15±2°C, 20±2°C and >=25°C), we can find that the slopes difference between the around 20°C group and >=25°C group own a statistically significant difference ($p<0.01$). This implies that the ENT will die-off quickly than *E. coli* when the temperature increased from 20°C to 25°C. For the temperature from 15°C to 20°C, we can't find the slopes difference between them have a statistically significant meaning ($p>0.5$). This means the two FIBs' decay rate ratio were stable during this temperature range. However, the line of 10±2°C comparing with the line of 20±2°C were also shown statistically significant difference ($p<0.05$), It displays the decay rate of ENT decreases slower than the decay rate of E. coli when the temperature declined from 20°C to 10°C below.

The same analysis measure have been used in figure 2c, the line slope of light 200-300W/M^2 (R^2=98.8%, $p<0.01$) (table2) is less low than the line slope of light 500-700W/M^2 (R^2=95.8%, $p<0.01$) (table2). And the line slope of 0-50 W/M^2 lacking statistical power (R^2=73.7%, $p>0.1$) (table 2). When assess the corresponding slope difference among them, statistically significant difference can be found between the slope of light 200-300 W/M^2 and light 0-50 W/M^2($p<0.05$).For the slope difference between light 500-700 W/M^2 and light 0-50 W/M^2 can't obtain statistical support ($p > 0.05$). This implies that the two FIBs die-off rate ratio are almost the same under very low light level or high light level.

For the figure 2d, we will detect whether or not the different sample sources can affect the mean decay rate ratio. Same statistical measure have been used here and no statistical significant difference can be found among the three slopes of sample sources line($p>0.05$).

Turns on the season factor study, Figure 2e presents the observed *E. coli* and *Enterococcus* decay log value during the summer and winter season, the slope's difference between the summer group and the winter group also exist a statistical power ($p<0.05$) , All of the corresponding predicted values were generated by the regression equations which present in table 3.

The figure 2e and figure 2f which estimate the season factor and climate factor separately, most of the slopes lacking statistical significance ($p>0.1$) (table 2) exclude the slope of subtropics line (R^2=79.7%, $p<0.01$). There is no meaning to compare the slope difference by statistical method. That's why the following meta-analysis method by STATA will be used here to detect the relationship between factors and FIB's decay rate.

Meta-Analysis Results

The meta-analysis is a statistical technique for summarizing and reviewing previous research, here we use it to obtain a better understanding of how well the factor can affect the decay rate ratio between the two FIBs. The standard error and the number of samples can both be considered to estimate the average ratio relationship with factors in the process. Due to the heterogeneity between the studies found in the meta-analysis were higher than 75% (http://handbook.cochrane.org/chapter_9/9_5_2_identifying_and_measuring_heterogeneity.htm) , the random effect model had been adopted in here. The forest plots are graphical representations of the meta-analysis, Here we use meta-analysis to study the relationship between factors and decay ratio, a series of forest plot figures had been generated (figure 3,4,5,6,7).

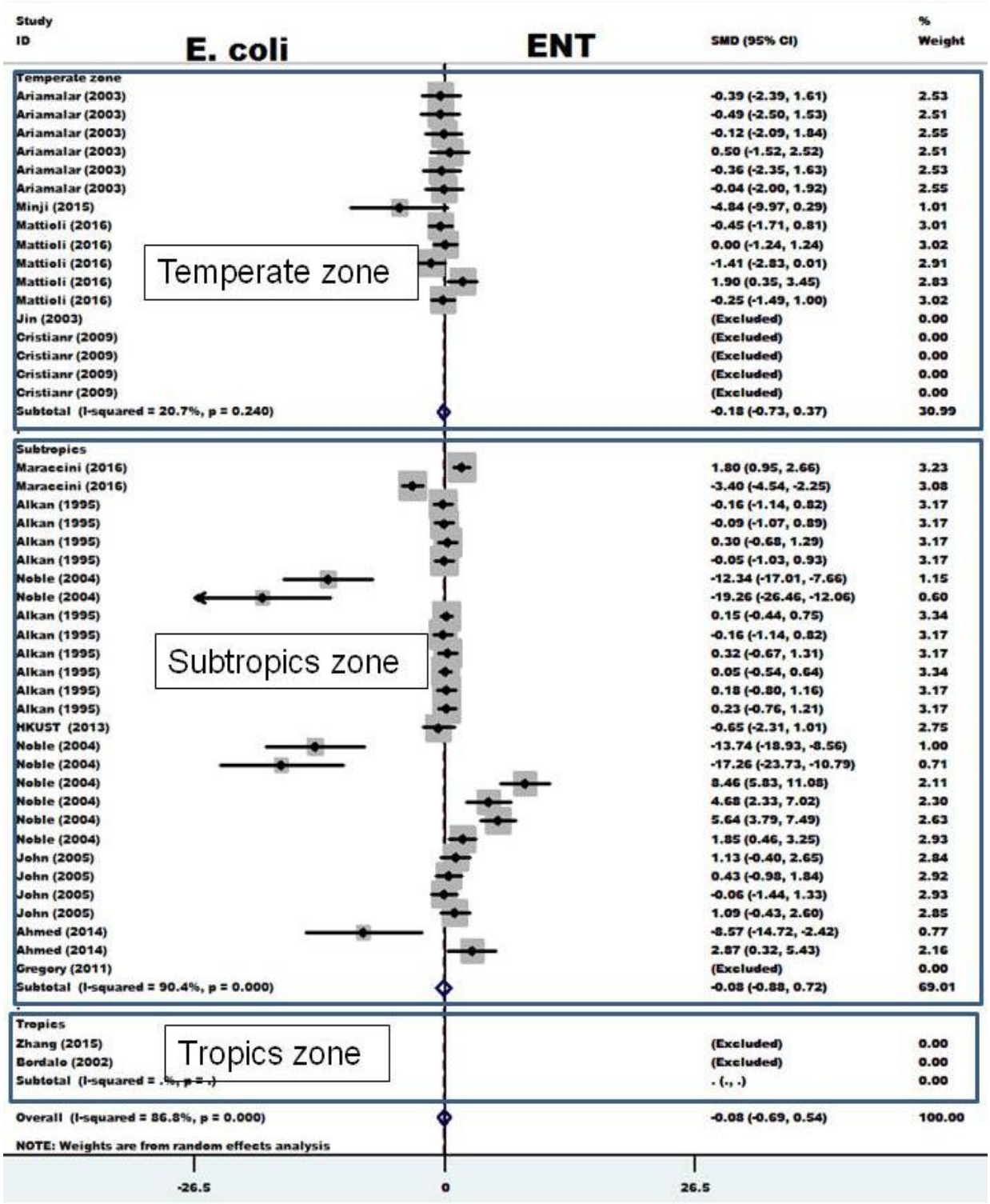

Figure 3. Forest plots of standardized mean differences (SMD) for the association between different climate class and a variety of studies under different settings. The grey shade area to represent the effect estimate and weight for each study. Line to represent the confidence interval of an effect estimate. The diamond at the bottom calculates overall effect estimate in each subgroup. Diamonds are crossed with the vertical line in the middle, this imply that there is no any significant difference for the decay rate changing between E. coli and ENT in each subgroup.

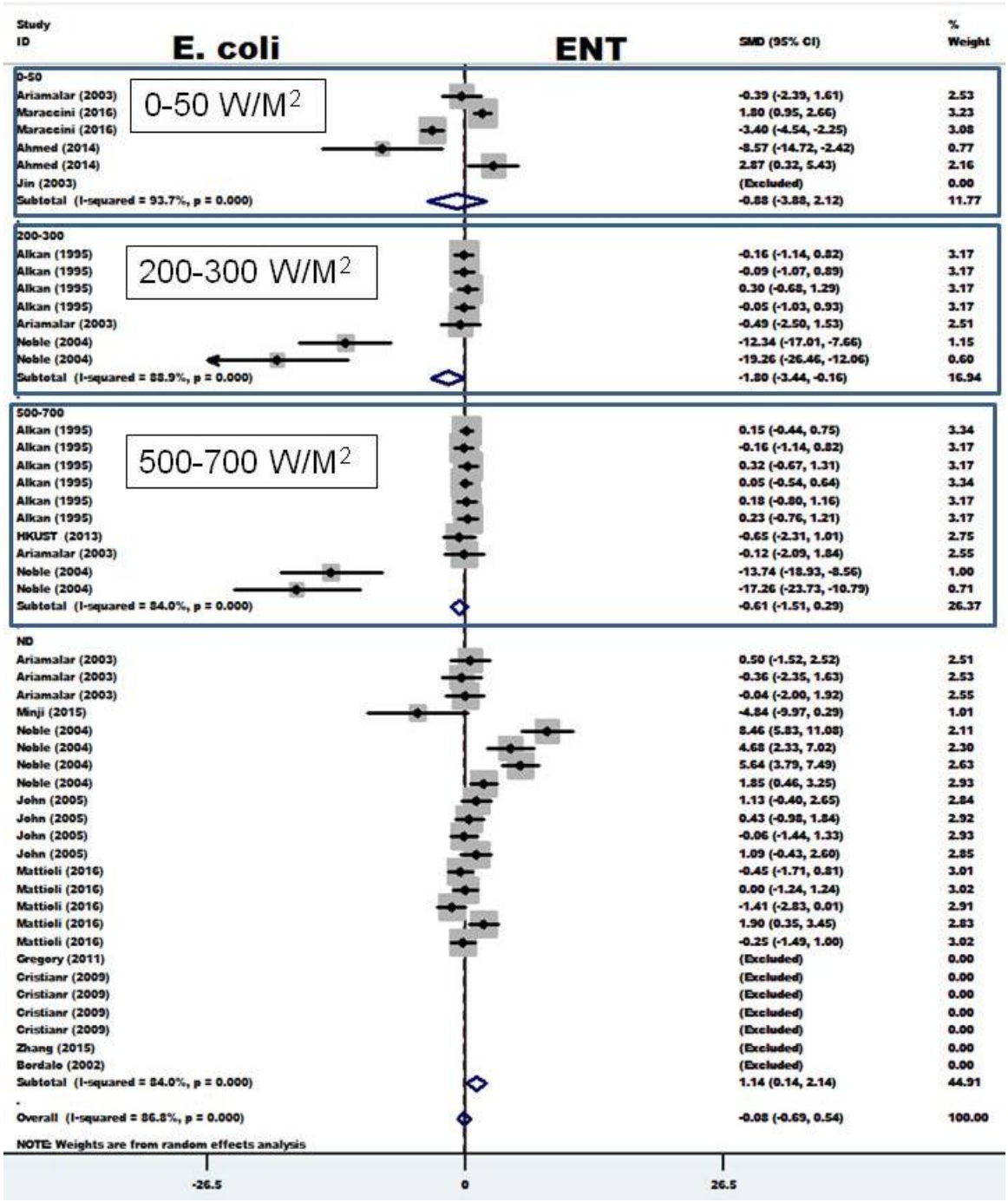

Figure 4. Forest plots of standardized mean differences (SMD) for the association between a different light conditions and a variety of studies under different settings. The grey shade area to represent the effect estimate and weight for each study. Line to represent the confidence interval of an effect estimate. The diamond at the bottom calculates overall effect estimate in each subgroup. ND to represent not determine the information. *E. coli* can survive well than ENT under the light density 200-300w/m^2 based on the diamond location in the subgroup.

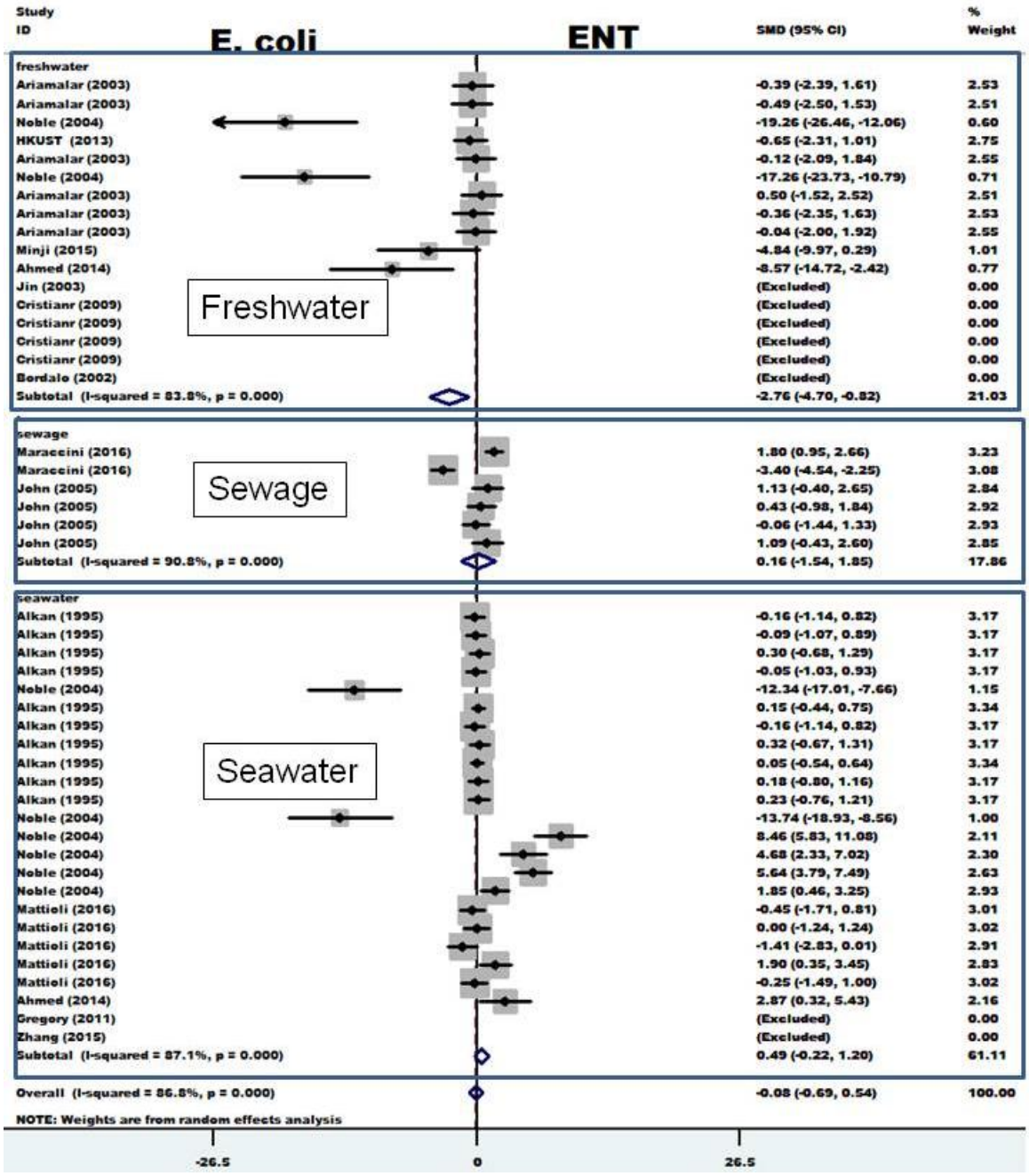

Figure 5. Forest plots of standardized mean differences (SMD) for the association between different type of samples and a variety of studies under different settings.The grey shade area to represent the effect estimate and weight for each study. Line to represent the confidence interval of an effect estimate. The diamond at the bottom calculates overall effect estimate in each subgroup. E. coli and ENT can survive well in the freashwater and seawater respectively and no significant different decay rate in sewage.

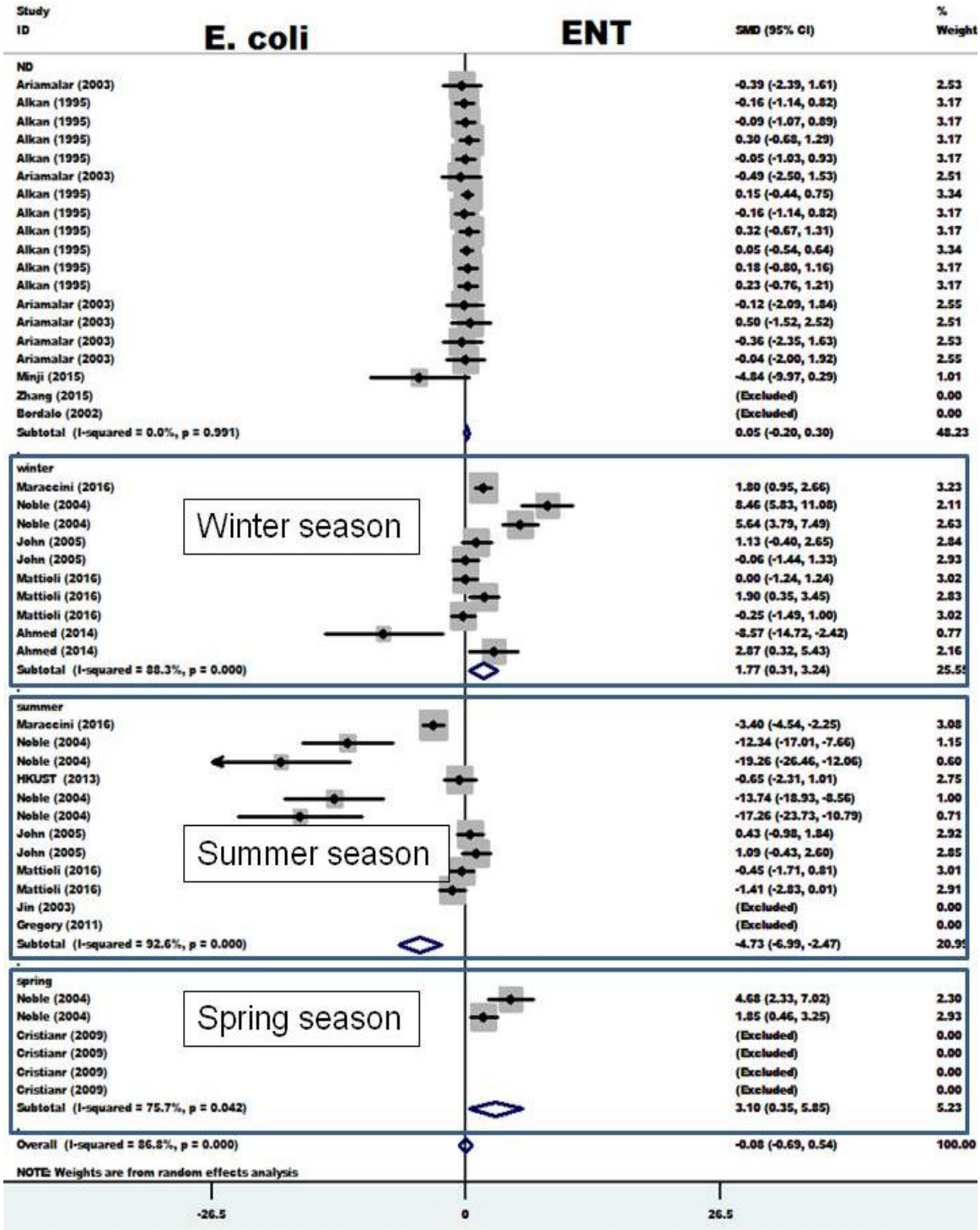

Figure 6. Forest plots of standardized mean differences (SMD) for the association between different season and a variety of studies under different settings. The grey shade area to represent the effect estimate and weight for each study. Line to represent the confidence interval of an effect estimate. The diamond at the bottom calculates overall effect estimate in each subgroup. ND to represent not determine the information. E. coli's decay rate is smaller than ENT in summer and vice versa in winter.

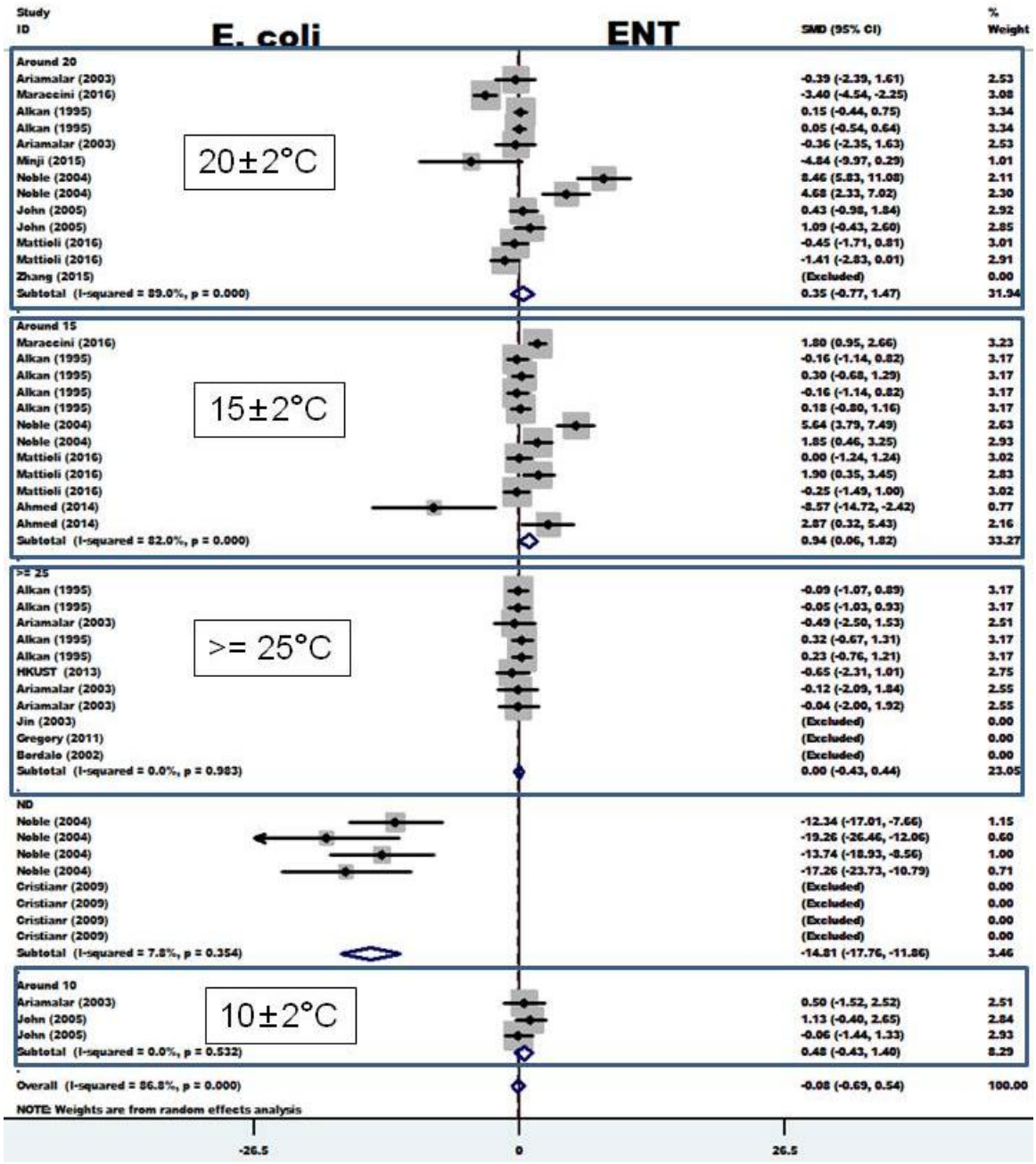

Figure 7. Forest plots of standardized mean differences (SMD) for the association between different temperature and a variety of studies under different settings. The grey shade area to represent the effect estimate and weight for each study. Line to represent the confidence interval of an effect estimate. The diamond at the bottom calculates overall effect estimate in each subgroup. ND to represent not determine the information.

For each study, If the line falls on the left-hand side of the graph (labeled *E. coli*) represent the standard mean difference (SMD) is a negative value, which tells us that the decay rate of *E. coli* in this study is smaller than the decay rate of ENT. On the contrary, if the horizontal line falls on the right-hand side of the graph (labeled ENT) represent the standard mean difference is a positive value, which tells us that the decay rate of *E. coli* in this study is larger than the decay rate of ENT. The grey shade area represent the effect estimate and weight for each study. On the whole, the diamond at the subgroup bottom calculates overall effect estimate with a statistical model. whether the diamond on each subgroup analysis sits on the left side or right side of the graph, this will imply the corresponding decay rate size between the two water monitoring strains. Take the figure3 as example, all of the diamond are

crossed with the vertical line in the middle, this imply that there is no any significant difference for the decay rate changing between E. coli and ENT in each subgroup. We can't get any prediction for which one is a better water monitoring strain under different climate conditions, climate is not the key factor for changing their decay rate. For the figure4, the diamond locations demonstrate the effect estimate for the subgroup of 200-300 W/M^2 (SMD=-1.80, 95% CI -3.44,-0.16; I squared=88.9%, p= 0.032) and the subgroup of 500-700 W/M^2 (SMD=-0.61, 95% CI -1.51,0.29; I squared=84.2%, p= 0.182) respectively. This implies that E. coli can survive a little better than ENT under the light condition 200 W/M^2 , and follow the light density reach to above 500W/M^2. The decay rate of E. coli also increase faster. For the figure5, we could observe that the type of water also can affect the decay rate of the two bacteria (*E. coli* and ENT).The effect size can be quantified by the standardized mean difference (SMD) between the two groups. The seawater subgroup own a high SMD value (SMD=0.49, 95% CI -0.22,1.20; I squared=87.1%, p= 0.025) and the fresh water subgroup own a low SMD value (SMD=-2.76, 95% CI -4.70,-0.82; I squared=90.8%, p= 0.021) when comparing the decay rate between *E. coli* and ENT. The higher SMD value means the lower decay rate of ENT in this subgroup and vice versa for E. coli. In other words, this suggests that in order to get a reliable water monitoring results, E. coli and ENT should be recognized as the better water monitoring strain in freshwater and seawater separately. For figure 6, the season as a factor for estimating the decay rate between this two strains is obvious, the meta-analysis results display the diamonds which represent the overall effect estimate are more inclined to sit on the right-hand side of the graph (labeled ENT) in the winter subgroup and to locate on the left-hand side of the graph (labeled *E. coli*) separately. More specifically, in the summer group, subtotal diamond sits display a lower SMD value (SMD=-4.73, 95% CI -6.99,2.47; I squared=92.6%, p= 0.000) which imply the decay rate of E. coli is far more less than ENT and in the winter group, it displays a higher SMD value (SMD=1.77, 95% CI 0.31,3.24; I squared=88.3%, p= 0.018) which imply that ENT's decay rate is lower than *E. coli*. From the above, we could see that the season factor also play a major role for the contribution the bacterial decay change. In specific, the decay rate ratio (ENT/*E. coli*) was increased drastically from summer to winter with statistically significant changing. This was proved by a recent study which confirms that *E. coli* and *Enterococcus* decay are dependent on season and sunlight together [23].

In a short conclusion, we should take ENT as the suitable water monitoring strain in winter and take E. coli in summer respectively. For the last figure 7, only in the subgroup around 15°C and subgroup around 10°C the diamond locations demonstrate some tendency with two little higher SMD values(SMD=0.94, 95% CI 0.06,1.82; I squared=82%, p= 0.031 and SMD=0.48, 95% CI -0.43, 1.40; I squared=0.0%, p= 0.419). This implies that the decay rate of ENT is lower than the decay rate of *E. coli* when the temperature is close to 15°C or smaller than 10°C.

Discussion and Conclusions

We used systematic reviews and meta-analysis on a set of published articles which has studied the factors influencing the decay rates between ENT and *E. coli* at the same period in water. We mainly focused on the following abiotic factors: climate, season, light, temperature and sample source. The biotic factors including predators and microbial competitors can also contribute a lot impact on the decay rate of FIB [25]. However, due to insufficient data available to measure their significance, these factors (such as nutrient, DO, predator etc.) were not included in this analysis but future studies is warranted when data is sufficient. Generally speaking, the decay ratio (ENT/*E. coli*) almost equaled to one based on the summary of the total sample's linear regression model result. This means that the die-off rate change own a same speed between E. coli and ENT in most situation, choose any of them as the FIB strain can obtain a consistent water report conclusion,. when the decay ratio (ENT/*E. coli*) was higher than 1, this indicates that ENT die-off faster than *E. coli* in this geographical environment and vice versa. If we still choose the strain whose decay rate is very higher than the other one as the water monitoring strain, then the water quality conclusion will become not reliable.

From the above simple linear regression model results, we can see that the data were classified based on their own features and the corresponding

slope represents a linear relation of the decay ratio of ENT/*E. coli* under different environment factors. Each study point of decay ratio will contribute to the form of the slope. It's easy to get to the conclusion that climate, season, light density and temperature had an impact on the decay ratio based on the linear regression line model, and due to various variation in the data, lead to wide credible intervals appearing in the figures. Analysis of covariance (ANCOVA), which is available in minitab17 has been used here to access the slope difference among these regression lines. ANCOVA analysis suggests that, in terms of climate factor, there's no statistically significant difference between subtropics and temperate zone but comparison of both of them with the tropics was significant. In particular, E. coli and ENT have similar decay rates in the subtropics and temperate zone areas. However, in the tropics, this balance can be disrupted due to the fact that ENT can't tolerate as high temperature as E. coli(http://www.octomarine.net/speedybreedy/speedybreedy_culture_media.php).

We know the intensive and duration of sunlight irradiation vary drastically with seasons. According to season factor, significant slope value change can be found between the winter and summer lines. For light factor, the water's temperature also can be influenced by light absorption[26], We can find that the two line's slope(light 0-50 W/M^2 and light 500-700W/M^2) are not change significantly. Only the line slope of light 200-300 W/M^2 shows significant difference compared with the others. This phenomenon can be interpreted that both of the two FIB's decay rate are very lower under dark environment, accompanies the light density reach to 200-300 W/M^2,the *E. coli*'s die-off rate increase faster than ENT's which result in the decay ratio (ENT/*E. coli*) become smaller. However, when the light density increase to 500-700 W/M^2,the ENT's decay rate also arise fast, which result in the decay ratio (ENT/*E. coli*) increase again. Specific for temperature factor, the slopes of temperature range from 13 to 22 were not a significant difference but that temperature lower than 10 or raised close to 30 degrees can obtain statistical significant change support. Temperature as a major impact on the decay rate of FIB has also been previously reported [7, 27].

From the former studies, people usual find that the ENT could be a better fecal indicator strain in the seawater and E. coli could be a better fecal indicator strain in the fresh water [28]. However, in the figure 2d result by clustering there data based on the type of water (freshwater, seawater and sewage). Their slopes difference among them show nothing statistical power ($p>0.1$) due to the data limited, and the decay ratio was only computed by the mean die-off rate of each bacteria, without considering sample number and standard error. Therefore we further applied meta-analysis method as a good complement to prove and correct above conclusion and find possible significant changing which can't be found by linear regression model analysis alone. We found that the type of water is also another important factor affecting the survival of these two bacteria. ENT can usually survival better than *E. coli* in seawater.

In general, most studies detected high ENT decay rate in summer and lower ENT decay rate in winter[23]. All of these suggest that in order to get a reliable water quality report, we must consider specific situations including the type of water (freshwater vs marine), time and place when choosing the right bacteria as FIB.

At present, Singapore, which is located in the tropics (whole year average temperature is around 26 °C) and has no significant seasonal change, all these stable environment predict the decay ratio balance between *E. coli* and ENT are seldom to broke significant. This means choose one of them as a single indicator for water assessment in this kind of stable environment for the whole year is acceptable. However, considering the type of water factor, we still suggest those local related agencies use ENT and *E. coli* as the better fecal indicator bacteria in winter and summer separately and not using ENT for both seawater and freshwater. For Hong Kong, which is located in subtropics and experiences significant seasonal changes, the local government uses only E. coli as the water monitoring FIB. Considering the effect of dual factors (type water and season), for beach water, using ENT as the fecal indicator especially in the winter season maybe should be given consideration. Some developed countries, such as the European Union and Australia adopted dual indicators for both freshwater and marine water. This would be the safest and reliable method for

determining water bacteriological quality. However, considering the economy and effective, single indicator approach is still valuable for some countries based on their regionally specific feature and local studies.

In summary, this study do not attempt to stop the discussion of FIB chosen, but rather to summarize the important factors that could disrupt the stable relationship of concentration of FIB and then further affect the accuracy of water quality assessment. Especially for those regions where using single indicator for their water monitoring. We compared the decay ratio (ENT/*E. coli*) changing between different studies based on some common important environmental variables (temperature, light, salinity, season etc) and we used the statistical methods to identify the two usual indicator ratio changing association with these variables. Here we found that the decay ratio was closed related with temperature, season and Light density. And the combination of double effect on the ratio of season and water type should be considered seriously. For winter season and ocean water, we suggest using ENT as the proper FIB and for summer season and fresh water, the *E. coli* will be better. And in the end, this study can give a good reference for choosing the better water indicator at the right time to detect the pathogens strain concentration and then to assess the public health risks in water environment of a certain region.

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