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ARTICLE

The Occurrence of Vibrionaceae, Staphylococcaceae, and Enterobacteriaceae in Green Turtle *Chelonia mydas* Rearing Seawater

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Abstract

In this study, levels of Vibrionaceae, Staphylococcaceae, and Enterobacteriaceae were observed in seawater from juvenile green turtle *Chelonia mydas* rearing tanks and in the incoming coastal seawater (the water supply). Bacterial loads were compared between the incoming coastal seawater and two different rearing conditions: in cement tanks at a low stocking density and in fiberglass tanks at a high stocking density. The total bacterial counts in seawater from fiberglass tanks were statistically greater than those in cement tanks. The nonlactose and lactose fermenting enterobacteria, tellurite-reducing bacteria, and total plate counts in water from all rearing containers were greater than those in coastal seaweater by a logarithmic fold change of 2—3. Differences in bacterial population structure of the incoming coastal seawater and rearing water were also addressed. The results from biochemical identification of 344 isolates revealed that the bacteria that were commonly found in water samples were *Citrobacter* spp., *Enterobacteria* spp., *Edwardsiella* spp., *Staphylococcus* spp., *Staphylococcus aureus*, *Photobacterium* spp., *Vibrio alginolyticus*, and *Vibrio* spp. Conclusively, the microbiological monitoring of rearing water provides important and essential information on the management of aquatic animal health and husbandry.

Since 1979, the Sea Turtle Conservation Center of Thailand (STCCT), operated by the Royal Thai Navy, has regularly conducted an early intervention program for the conservation of two sea turtle species, the green turtle *Chelonia mydas* and the hawksbill turtle *Eretmochelys imbricata*. The conservation program has been carried out by collecting eggs from the nests, incubating them in safe places, and raising them until about 4–6 months of age before releasing them back to their natural habitat. In a previous study, the postmortem examination of turtle carcasses from STCCT found signs of clinical diseases due to bacterial infection (Chuen-Im et al. 2010a). The isolation and identification of bacteria from the lesions demonstrated that the infections were most commonly attributed to the following bacterial species: *Citrobacter freundii*, *Aeromonas hydrophila*, *Vibrio alginolyticus*, *V. parahaemolyticus*, *Micrococcus* spp., and β-haemolytic *Staphylococcus* spp. The isolation of bacterial species infecting both free-living and captive sea turtles has been documented in several areas around the world, including Bermuda, Australia, Hawaii, and the Canary Islands (Jacobson et al. 1986; Wiles and Rand 1987; Glazebrook and Campbell 1990a, 1990b; Raidal et al. 1998; Work et al. 2003; Orós et al. 2005).

Water quality can affect the health of aquatic animals and may be influenced by parameters such as temperature, dissolved oxygen, pH, salinity, and stocking density (George and Swingle 2006). These abiotic parameters can also have an impact on the growth of microorganisms that are present in the environment. For aquatic animals,

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infections may be attributed to both primary pathogens as well as opportunistic invaders in immunocompromised animals; they may also be aided by poor hygiene in the rearing water to which the animals are continually exposed (Cerdà-Cuéllar and Blanch 2004; Zavala-Norzagaray et al. 2015). The introduction of these bacteria into rearing water can come from several sources such as feed, water supply, and poor biosecurity practices. Hence, routine quantitative and qualitative microbiological investigation of rearing water as well as all possible routes for contamination is essential in designing preventative management practices.

According to our previous report, the most common etiologic agents of turtles at STCCT belonged to the families Vibrionaceae, Staphylococcaceae, Micrococcaceae, and Enterobacteriaceae (Chuen-Im et al. 2010a); therefore, in this study it was our interest to investigate the prevalence of these taxonomic groups in the holding tanks and in the water source at STCCT. The results obtained will provide informative sea turtle husbandry guidelines for reducing disease.

METHODS

Water samples.— Water samples were collected on a monthly basis for 6 months in 2017 (January through June) at STCCT, located on Sattahip Bay in the Gulf of Thailand. Sample sources included incoming coastal seawater that was used as the water supply for juvenile sea turtle rearing, and the seawater from three cement and three fiberglass rearing tanks. The water supply was pumped directly from the sea at a distance of about 10–20 m offshore. The seawater was not treated before filling the rearing tanks. The cement tanks (2 m deep × 2.5 m wide × 2.5 m long) were lined with ceramic tiles, and they housed apparently healthy juvenile turtles of 2–3 months of age at a stocking density of 1.5–3.8 g/L. The fiberglass tanks (1 m deep × 1.6 m wide × 1 m long) were made in one seamless piece, and were used to rear juvenile turtles, also 2–3 months old, at a stocking density of 3.0–8.8 g/L. All of the tanks that housed turtles used a static water system. The turtles were fed once daily with sea grapes *Caulerpa lentillifera* (cultured at STCCT in fiberglass tanks using the same water supply), and twice daily with Yellowstripe Scad *Selaroides leptolepis* from a fresh food market. The fish were rinsed with chlorinated tap water and chopped before being fed to the turtles in the morning and afternoon (totaling 10% of the turtles' body weight per day). Our preliminary study indicated that the bacterial levels in the feed were not significantly high enough to be a potential source of infection in the housing system (the aerobic plate count was $< 10^7$ CFU/g according to the protocol described by the International Commission on Microbiological Specifications for Foods (ICMSF 1986).

Feeding time lasted 30 min, after which any uneaten food was collected using a dip net. The water was drained entirely before rinsing the turtles with fresh seawater without removing them from the holding tanks. The tanks were cleaned with a brush to remove dirt and debris, and then filled with fresh coastal seawater. The water samples were collected 3 h after cleaning the tanks. For sampling, 15-mL sterile tubes were used to collect the water in duplicate at a depth of 5 cm below the surface and a distance of about 1 m apart in both types of tanks. Water samples filled the tubes and care was taken to ensure that there was no remaining air in the tubes. Samples were placed on ice during transportation and then maintained at 4°C until microbiological examination (within 10 h). The water temperature was measured at the time of sampling. The pH and salinity of the water samples were determined using a multi-parameter analyzer (Consort Medical, Hemel Hempstead, UK; Model C535) at the laboratory.

Bacterial analyses.— The investigation of heterotrophic aerobic bacteria was done using the spread plate technique (Buck and Cleverdon 1960). Each water sample was prepared using a 10-fold serial dilution technique with 0.85% solution of NaCl. Samples (0.1 mL) of 10^{-1} to 10^{-4} dilutions were spread on various growth media: nutrient agar supplemented with 1% NaCl (NA + 1% NaCl) for heterotrophic aerobic bacteria, Baird Parker agar (BPA) for tellurite-reducing bacteria, Eosin methylene blue agar (EMB) for Enterobacteriaceae, and thiosulfate citrate bile salt sucrose agar (TCBS) for Vibrionaceae. Plates were incubated aerobically at 25°C for 24–48 h prior to examination. All media used in the study were provided by HiMedia Laboratories (Mumbai, India). Distinctive colonies, categorized by their characteristics and growth patterns, were isolated to pure culture before being identified. Each isolate was considered a separate organism and subjected to the following tests: gram stain, oxidase, catalase, gelatin hydrolysis, motility, carbohydrate fermentation, decarboxylase, urease, oxidation/fermentation, IMViC, triple sugar iron, citrate utilization, hemolysis, and growth in 0, 3, 6, 8, and 10% NaCl (Buchanan and Gibbons 1974).

Statistical analyses.— Statistical analyses were carried out using SPSS (IBM, Armonk, New York; version 21). Initially, the data were subjected to the Kolmogorov–Smirnov test for normality and homogeneity of variance. In the case of a normal distribution, significant differences of bacterial counts between water samples were tested using a one-way ANOVA. The Tukey's test was employed for multiple comparisons of means. For the data with nonnormal distribution, a nonparametric Kruskal–Wallis test was used for pairwise comparisons. An independent

sample *t*-test was used to analyze significant differences of total plate counts on NA + 1% NaCl between the rearing water from cement and fiberglass tanks. A significance level of $P < 0.05$ was used for all tests.

RESULTS

Abiotic Parameters

The abiotic parameters of the water samples, including salinity, pH, and temperature, are summarized in Table 1. The temperature of the coastal seawater and the rearing water from January through June of 2017 ranged from 28.0°C to 31.0°C. The temperature measurements of water from all rearing tanks were slightly lower than those of natural seawater since the rearing tanks were placed under a roof. The range of salinity of all water samples was 28.0–34.5‰. The pH ranged from 6.6 to 7.8. Although no significant difference was observed in these parameters among the rearing tanks, the pH of seawater in the fiberglass tanks was always lower than that of the cement tanks, whereas the other parameters fluctuated among all water samples.

Identification of Heterotrophic Aerobic Bacteria in Seawater From Captive Juvenile Green Turtle Holding Tanks

Table 1 reports enumeration of heterotrophic aerobic bacteria, nonlactose and lactose fermenting enterobacteria, tellurite-reducing bacteria, and total plate counts on TCBS in water samples from incoming coastal seawater and seawater from the cement and fiberglass tanks from January through June of 2017. On NA + 1% NaCl, the bacterial loads (mean ± SD) in cement and fiberglass tanks ranged from 4.3 ± 0.25 to 5.5 ± 0.47 log CFU/mL, and 5.9 ± 1.21 to 6.9 ± 0.32 log CFU/mL, respectively. Tellurite-reducing bacterial counts (mean ± SD) on BPA from incoming coastal seawater and seawater from the cement and fiberglass tanks ranged from <0.01 to 1.70 ± 2.41 log CFU/mL, 1.40 ± 1.31 to 3.90 ± 0.18 log CFU/mL, and 4.10 ± 1.26 to 5.80 ± 0.67 log CFU/mL, respectively. Nonlactose fermenting enterobacteria from incoming coastal seawater and seawater from the cement and fiberglass tanks ranged from <0.01 to 1.70 ± 0.12 log CFU/mL, 0.60 ± 0.98 to 2.50 ± 0.49 log CFU/mL, and 1.00 ± 0.95 to 2.80 ± 0.92 log CFU/mL, respectively. Lactose-fermenting enterobacteria from incoming coastal seawater and seawater from the cement and fiberglass tanks ranged from <0.01 to 2.40 ± 0.04 log CFU/mL, 1.30 ± 0.45 to 3.70 ± 0.07 log CFU/mL, and 2.80 ± 0.26 to 5.10 ± 0.06 log CFU/mL, respectively. Finally, the bacterial counts of water samples from incoming coastal seawater and seawater from the cement and fiberglass tanks on TCBS ranged from 1.30 ± 1.85 to 2.80 ± 0.37 log CFU/mL, 2.90 ± 0.21 to 4.60 ± 0.73 log CFU/mL, and 4.30 ± 0.08 to 5.90 ± 0.42 log CFU/mL, respectively.

A comparison of bacterial counts between incoming coastal seawater and seawater in tanks housing captive turtles demonstrated that the bacteria isolated from TCBS, including *Vibrio* spp. and other unidentified colonies, were predominant in the incoming coastal seawater in January, March, April, May, and June of 2017. In contrast, Enterobacteriaceae, containing both nonlactose and lactose fermenting bacteria, were predominant in seawater from tanks housing turtles in January, February, March, April, and June of 2017 for the cement tanks, and in January, February, April, and June of 2017 for the fiberglass tanks (Table 1). It should be noted that all investigated bacterial loads in the fiberglass tanks were always higher than those of cement tanks (Table 1). However, the levels of heterotrophic aerobic bacteria on NA + 1% NaCl, tellurite-reducing bacteria on BPA, and total plate counts on TCBS were significantly different between the cement tanks and the fiberglass tanks whereas counts of nonlactose and lactose fermenting enterobacteria were not.

The Compositions of Bacterial Flora in Water Samples

To investigate the bacterial composition of Staphylococcaceae, Vibrionaceae, and Enterobacteriaceae from water samples, isolates from incoming coastal seawater and seawater from the cement and fiberglass tanks at STCCT were subjected to biochemical tests. Results from identification and frequencies of detection are shown in Table 2. From a total of 126 tellurite-reducing isolates, *Staphylococcus* spp. were found in all water samples. These bacteria were found in every monthly sample from both cement and fiberglass tanks, whereas in the incoming coastal seawater, bacteria were present in 5 out of 6 months. *Staphylococcus aureus* could be detected in rearing seawater from both cement and fiberglass tanks but not in incoming coastal seawater, while *Micrococcus* spp. were detected only twice in cement tanks.

The results of the biochemical tests of 99 TCBS isolates revealed that 10 species of *Vibrio* (including *V. alginolyticus*) and other genera (including *Aeromonas* spp., *Photobacterium* spp., and *Aliivibrio* spp.) were found in all sample sources (Table 2). *Vibrio alginolyticus* (19 isolates) was also the most frequently identified species in the water samples. In addition to *Vibrio* spp., *Photobacterium* spp. was another predominant bacterial species that was observed in the seawater from both cement and fiberglass tanks.

For the enterobacteria, *Citrobacter* spp., *Enterobacter* spp., and *Edwardsiella* spp. were detected in all sample sources as the first, second, and third most frequent bacteria, respectively. *Citrobacter* spp. was detected in cement tanks in each monthly sample, and in fiberglass tanks for 5 out of 6 months. *Escherichia* sp., *Obesumbacterium* sp.,

TABLE 1. Summary of bacterial counts (log CFU/mL; mean ± SD) in water samples collected from coastal incoming water and turtle rearing tanks. Different lowercase letters in each column indicate significant differences between bacterial loads in water samples; "N/A" denotes that the value was not determined.

Month	Samples	Temperature (°C)	Salinity (ppt)	pH	Total plate count (NA ± 1% NaCl)	Tellurite-reducing bacteria (BPA)	Enterobacteriaceae		Total plate counts on TCBS
							Nonlactose fermenters	Lactose fermenters	
January	Seawater	28.0	33.4	7.3	N/A	1.0 ± 1.47 z	<0.01 z	0.7 ± 0.92 z	2.8 ± 0.06 z
	Cement	27.0 ± 0.0	33.8 ± 0.6	7.4 ± 0.1	5.5 ± 0.47 z	3.9 ± 0.18 y	2.5 ± 0.49 y	2.8 ± 0.13 y	4.0 ± 0.39 y
	Fiberglass	27.0 ± 0.0	34.1 ± 0.3	7.2 ± 0.1	6.4 ± 0.37 y	5.8 ± 0.67 x	2.8 ± 0.92 y	3.5 ± 0.18 y	5.2 ± 0.08 x
February	Seawater	29.0	34.5	7.8	N/A	1.3 ± 0.00 z	<0.01 z	2.4 ± 0.04 z	1.3 ± 1.85 z
	Cement	27.0 ± 0.0	33.5 ± 0.3	7.7 ± 0.0	4.4 ± 0.13 z	1.6 ± 1.65 y	0.8 ± 0.76 y	2.4 ± 1.26 y	2.9 ± 0.21 y
	Fiberglass	27.5 ± 0.5	34.3 ± 0.6	7.0 ± 0.1	6.0 ± 0.20 y	5.2 ± 0.76 x	1.9 ± 0.53 y	3.5 ± 1.08 y	4.4 ± 0.61 x
March	Seawater	30.0	33.4	7.2	N/A	0.7 ± 0.92 z	<0.01 z	<0.01 z	1.6 ± 0.16 z
	Cement	28.0 ± 0.0	33.6 ± 0.6	7.2 ± 0.0	4.3 ± 0.25 z	3.0 ± 0.79 y	0.6 ± 0.98 y	3.7 ± 0.07 y	3.0 ± 1.47 y
	Fiberglass	28.0 ± 0.0	33.9 ± 0.2	7.0 ± 0.0	6.3 ± 0.62 y	4.1 ± 1.26 x	1.7 ± 0.50 y	3.7 ± 0.55 y	5.7 ± 0.57 x
April	Seawater	29.0	30.0	7.3	N/A	<0.01 z	<0.01 z	1.4 ± 0.55 z	2.7 ± 0.14 z
	Cement	27.0 ± 0.0	30.1 ± 0.6	7.3 ± 0.3	4.5 ± 0.72 z	1.5 ± 0.04 y	2.1 ± 0.75 y	3.3 ± 0.28 y	4.6 ± 0.73 y
	Fiberglass	26.0 ± 0.0	30.4 ± 0.5	6.6 ± 0.1	6.0 ± 0.03 y	4.3 ± 1.26 x	2.2 ± 0.67 y	5.1 ± 0.06 y	5.1 ± 0.20 x
May	Seawater	31.0	29.9	7.3	N/A	1.7 ± 2.41 z	<0.01 z	<0.01 z	2.5 ± 0.25 z
	Cement	28.0 ± 0.0	29.3 ± 0.5	7.3 ± 0.1	4.8 ± 0.66 z	3.1 ± 1.22 y	0.8 ± 0.68 y	1.3 ± 0.45 y	3.6 ± 0.40 y
	Fiberglass	28.0 ± 0.0	29.8 ± 0.3	7.0 ± 0.1	5.9 ± 1.21 y	4.2 ± 0.58 x	1.0 ± 0.95 y	2.8 ± 0.26 y	4.3 ± 0.08 x
June	Seawater	29.0	28.0	6.9	N/A	1.5 ± 0.34 z	1.7 ± 0.12 z	1.9 ± 0.16 z	2.8 ± 0.37 z
	Cement	27.5 ± 0.0	28.4 ± 0.7	7.2 ± 0.1	4.5 ± 0.19 z	1.4 ± 1.31 y	0.7 ± 1.22 y	3.2 ± 0.28 y	3.8 ± 0.55 y
	Fiberglass	27.0 ± 0.0	28.8 ± 0.2	6.8 ± 0.1	6.9 ± 0.32 y	5.0 ± 0.88 x	1.8 ± 1.58 y	5.0 ± 0.45 y	5.9 ± 0.42 x

TABLE 2. Bacterial species and frequency of detection in water samples collected over the period of January through June of 2017. The number in parentheses indicates the number of isolates detected. Cells that do not contain numbers indicate that no isolates were detected.

	Incoming coastal seawater	Cement tanks	Fiberglass tanks	Number of isolates detected/total isolates examined
Isolates on BPA				
Staphylococcus spp.	4 (7)	6 (29)	6 (43)	79/126 (62.70%)
Staphylococcus aureus	–	3 (6)	3 (13)	19/126 (15.08%)
Micrococcus spp.	–	2 (6)	–	6/126 (4.76%)
Unknown	1 (2)	6 (11)	5 (9)	22/126 (17.46%)
Total number of species/genera detected	2	4	3	–
Isolates on TCBS				
Vibrio spp.	2 (2)	5 (7)	4 (11)	20/99 (20.20%)
V. alginolyticus	3 (4)	3 (11)	3 (4)	19/99 (19.19%)
V. parahaemolyticus	3 (3)	2 (3)	–	6/99 (6.06%)
V. cincinnatiensis	–	2 (4)	–	4/99 (4.04%)
V. tapetis	–	1 (2)	1 (2)	4/99 (4.04%)
V. scophthalmi	–	–	2 (2)	2/99 (2.02%)
V. vulnificus	1 (1)	1 (1)	–	2/99 (2.02%)
V. sinaloensis	–	2 (2)	–	2/99 (2.02%)
V. ordalii	1 (1)	–	–	1/99 (1.01%)
V. metschnikovii	–	1 (1)	–	1/99 (1.01%)
V. fluvialis	–	–	1 (1)	1/99 (1.01%)
Photobacterium spp.	–	4 (18)	2 (5)	23/99 (23.23%)
Aliivibrio spp.	2 (2)	–	1 (4)	6/99 (6.06%)
Aeromonas spp.	1 (1)	1 (3)	2 (2)	6/99 (6.06%)
Unknown	–	–	1 (2)	2/99 (2.02%)
Total number of species/genera detected	7	10	9	–
Isolates on EMB				
Citrobacter spp.	3 (8)	6 (32)	5 (16)	56/119 (47.06%)
Enterobacter spp.	3 (4)	4 (11)	3 (5)	20/119 (16.81%)
Edwardsiella spp.	2 (2)	2 (4)	2 (5)	11/119 (9.24%)
Proteus spp.	–	2 (4)	3 (4)	8/119 (6.72%)
Salmonella spp.	–	2 (7)	–	7/119 (5.88%)
Cedecea spp.	–	1 (2)	1 (3)	5/119 (4.20%)
Obesumbacterium sp.	–	1 (3)	–	3/119 (2.52%)
Serratia sp.	–	1 (1)	1 (1)	2/119 (1.68%)
Klebsiella sp.	1 (1)	–	1 (1)	2/119 (1.68%)
Yersinia spp.	–	–	1 (2)	2/119 (1.68%)
Escherichia sp.	–	1 (1)	–	1/119 (0.84%)
Unknown	–	1 (2)	–	2/119 (1.68%)
Number of species/genera detected	4	10	8	–
Number of genera detected/total of genera identified and unknown	13/31 (41.94%)	24/31 (77.42%)	20/31 (64.52%)	–

and *Salmonella* spp. could be detected only in cement tanks whereas *Yersinia* spp. and *Klebsiella* sp. were found in only fiberglass tanks (Table 2). Although the bacterial counts in the seawater from fiberglass tanks were always higher than that of the cement tanks (Table 1), the seawater from cement tanks showed a higher diversity of bacterial flora (77.42%) than that of the fiberglass tanks (64.52%; Table 2).

Further study of bacterial populations showed that of the enterobacteria, *Citrobacter* spp. (47.06%) was the most prevalent genus followed by *Enterobacter* spp. (16.81%) and *Edwardsiella* spp. (9.24%; Table 2). Other genera of Enterobacteriaceae detected were *Proteus* sp. (6.72%), *Salmonella* spp. (5.88%), and *Cedecea* spp. (4.20%). Regarding the tellurite-reducing bacteria, 62.70% of isolates belonged to the Staphylococcaceae family, from which *Staphylococcus aureus* was the most prevalent at 15.08%. In addition, *Micrococcus* spp. was identified (4.76%). Finally, the majority of the bacteria isolated on TCBS were *Photobacterium* spp. (23.23%) and *Vibrio* spp. (20.20%) followed by *V. alginolyticus* (19.19%), *V. parahaemolyticus* (6.06%), *Aliivibrio* sp. (6.06%), *Aeromonas* spp. (6.06%), *V. cincinnatiensis* (4.04%), and *V. tapetis* (4.04%).

DISCUSSION

This study investigated the microbiological quality of seawater from tanks housing captive turtles at STCCT, focusing on three bacterial families: Staphylococcaceae, Vibrionaceae, and Enterobacteriaceae. According to the National Marine Fisheries Service Sea Turtle Facility (NMFS STF) in Galveston, Texas, the temperature, pH, and salinity of seawater in sea turtle housing tanks in a static system should be maintained at between 26°C and 30°C, 7.5 and 8.1, and 14‰ and 32‰, respectively (Higgins 2003). The abiotic parameters of seawater in the rearing tanks from January through June of 2017 at STCCT including temperature, pH, and salinity ranged between 26.0 ± 0.0°C and 28.0 ± 0.0°C, 6.6 ± 0.1 and 7.7 ± 0.0, and 28.4 ± 0.7‰ and 34.3 ± 0.6‰, respectively. Considering the NMFS STF guidelines, the temperature and salinity levels in seawater in the STCCT rearing tanks were within the recommended range, but the pH was lower than the recommended guidelines in every sample except for the cement tank in February. It should be mentioned that the pH of the incoming coastal seawater was below 7.5 in all months except February. It is possible that the low pH of the rearing water was partly a result of the low pH of the incoming water, and was also influenced by acid fermentation products from microbial metabolic pathways. The acidity of the coastal seawater might have resulted from pollution by terrestrial runoff or municipal sewage, which is 5 km away from STCCT (Spanton and Saputra 2017). To combat the issue of low tank pH, we recommended extending the inflow pipe to deeper water to obtain unpolluted water or percolating the water over carbonate to increase the alkalinity, as well as cleaning the rearing tanks more frequently (e.g., 3–4 times per day). Furthermore, the tanks should be covered with transparent UV materials to prevent access from birds or other animals, which may be a source of contamination with other organisms.

Cement tanks are known to result in more alkalinity due to the leaching of carbonate into the water. Newly casted tanks should be rinsed prior to installation to remove the carbonate residues, which can affect water pH. When exposed to freshly casted cement specimens, the pH level of water will significantly increase, and then diminish with time (Setunge et al. 2009). Despite the STCCT cement tanks being used for over 7 years, it is possible that there was carbonate leaching from the tanks that buffered the water, which resulted in the higher pH levels. Moreover, the results from this study demonstrated the influence of the tank surface on the bacterial loads in the rearing water. There are several publications reporting the ability of various bacteria, including *Escherichia coli* and *Staphylococcus aureus*, to adhere and colonize at different degrees on a variety of surfaces such as glass, stainless steel, and polystyrene (Visvalingam and Holley 2013; Di Ciccio et al. 2015); however, more experiments are required to draw firm conclusions on the appropriate tank material.

Animal rearing greatly affects the bacterial composition of the water. Compared with natural seawater (Table 1), the levels of bacteria that were isolated on TCBS as well as tellurite-reducing bacteria increased by a logarithmic fold change of 1–2, and for Enterobacteriaceae, 3–4. The predominant bacterial group changed from species growing on TCBS to Enterobacteriaceae. Enterobacteriaceae are the most commonly reported gram-negative bacteria that are isolated from the nasal cavity and cloaca of green turtles (Santoro et al. 2007).

At STCCT, rearing of juvenile turtles is carried out using two different approaches, leading to differences in the stocking density and the rearing tank material. Rearing animals at an appropriate stocking density is an essential condition for animal health management. Based on the results from this study and the NMFS STF guidelines, the ideal appropriate stocking density for housing green turtles that are between 2 and 3 months old should be between 3.8 and 5.5 g/L (Higgins 2003). Inappropriate stocking density can considerably affect turtle health, resulting in biting and other undesirable physical interactions between animals in the rearing tank. Water samples from the fiberglass tanks contained higher bacterial loads than those of the cement tanks whereas the pH values of the fiberglass tank water were always lower than those of the cement tanks, but this was not statistically significant. One explanation is that the higher stocking density in the fiberglass tanks might strongly influence the bacterial loads in the seawater. This finding is in accordance with our previous observation and with observations of other groups (Chuen-Im et al. 2010b; Konghae et al. 2016). Konghae et al. (2016) found that at a high stock density the water quality was significantly decreased.

Identification of isolates from STCCT water samples revealed potential primary pathogens, opportunistic bacteria, and bacterial species that have never been reported to cause disease in sea turtles or marine animals. Several of the detected bacterial species included *Micrococcus* spp., *V. alginolyticus*, *V. parahaemolyticus, A. hydrophila*, *Citrobacter* spp., *Staphylococcus* spp., *Enterobacteria* spp., and *Edwardsiella* spp. found to infect both juvenile and adult sea turtles (Glazebrook and Campbell 1990a, 1990b; Work et al. 2003; Orós et al. 2005; Chuen-Im et al. 2010a). The dominant genus of Enterobacteriaceae that was isolated from turtle rearing seawater was *Citrobacter* spp. This is not surprising as these environmental bacteria were repeatedly reported for isolation from both free-living and captive sea turtles (Aguirre et al. 1994; Raidal et al. 1998; Chuen-Im et al. 2010a). The second most frequently isolated genus in this family was *Enterobacter* spp. Similar to *Citrobacter* spp., this bacterial genus has been commonly cultured from diseased sea turtles (George 1997). In addition, *Edwardsiella* spp., *Proteus* sp., *Salmonella* spp., *Yersinia* spp., *Serratia* sp., and *Klebsiella* sp. were also detected in water samples at STCCT. These bacteria are naturally occurring microflora of sea turtles but on occasion may opportunistically infect and cause disease when conditions are appropriate and the animals are in immunocompromised (McArthur 2004; Chuen-Im et al. 2010a; Zavala-Norzagaray et al. 2015). Kim and Lee (2017) reported a strong correlation between the level of bacteria in the aquaculture water and in the animal tissues. However, in this study there was no record of morbidity or mortality on animal rearing in each rearing tank. Whether high numbers of bacterial agents in the rearing water significantly promoted infection in animals in the tanks was not investigated.

In addition to Enterobacteriaceae, several species of Vibrionaceae were isolated from the water samples (Table 2). Likewise, both pathogenic and nonpathogenic *Vibrio* were observed; *V. alginolyticus* and *V. paraheamolyticus* were the species most frequently isolated in the water from juvenile turtle rearing tanks. These bacteria are well-known pathogens of aquatic animals including sea turtles (Aguirre et al. 1994; Raidal et al. 1998; Chuen-Im et al. 2010a). Other *Vibrio* spp. were found at a lower frequency. *Vibrio scophthalmi* has been reported from Turbot *Scophthalmus maximus* larvae, brine shrimp *Artemia* spp., and also water from the fish rearing tanks (Cerdà-Cuéllar and Blanch 2004; Blanch et al. 2009). *Vibiro metschnikovii* can be found in common seafood such as squid, shrimp, mussels, crab, and cockles (Elhadi et al. 2004). *Vibrio cincinnatiensis* has been detected in nature at low frequencies including in river and estuarine water (Venkateswaran et al. 1989).

A limitation of this study was that several water quality parameters, including ammonia, nitrite, nitrate, and dissolved oxygen, were not measured. All of these factors can influence the health of animals that are housed in a static system. To minimize the risk of introduction and the spreading of infectious disease to animals in the facility, husbandry practices should be consistent with standard aquaculture biosecurity. At STCCT, we recommend a regular analysis of microbiological water quality at least monthly, as well as daily measurements of parameters such as temperature, salinity, pH, ammonia, nitrite, nitrate, and dissolved oxygen (Higgins 2003; Bluvia and Eckert 2010; USFWS 2013). Finally, because the housing facilities have been publicly accessible by visitors, the health implications associated with exposure to animals should always be a concern in terms of potential turtle-associated human pathogenic agents (Warwick et al. 2013).

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