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Indoor Aquaria and Offshore Cages Bacterial Disease of Cultivated Giant Clam

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ABSTRACT

Giant clam marine culture is commonly influenced by a wide variety of pathogenic bacteria including *Vibrio* species, resulting in high losses. The current study was organized to investigate the bacterial infections, in the Hurghada area, between the culture of *Tridacna maxima* and *Tridacna gigas* in indoor aquaria and offshore cages. Eighty samples of *T. maxima* and *T. gigas* in both indoor aquaria and offshore cages were collected for bacteriological analysis. Samples were taken from gills, gut, mantels, and gonads of *Tridacna*. The results showed reduced motion followed by loss of attractive color pattern with 25 % and 20 % mortality rate among indoor cultured *T. maxima* and *T. gigas*, respectively. About forty-six bacterial isolates were identified, using phenotypic and biochemical testing. Most of those isolates were *Vibrio logei*, *V. harveyi*, and unidentified *Vibrio*. The pathogenicity and virulence of *V. logei* seem high with a 30% mortality rate. The current study would provide useful information for better management of bacterial infections in *T. maxima* and *T. gigas*.

INTRODUCTION

Bivalve molluscs are one of the important species because of commercial benefits, distributed in the coastal and estuarine environmental zones. Therefore, their aquaculture industry production is now considered important industry that grows quickly in benefits concerning the food production process **(FAO, 2014)**. Bivalves are the rich-environmental medium for the growth and accumulation of bacterial microbiota, such as *Vibrio* species with passive transporters of human pathogenic agents **(Pruzzo *et al.*, 2005)**. Notably, giant clams are important species of coral reef ecosystems. Natural and hatchery bivalve molluscs are susceptible to infection with a wide range of microorganisms, including bacteria, algae, Perkinsozoa, Haplosporidia, Ciliophora,

Apicomplexa, Turbellaria, Trematoda, Cestoda, and Nemertea **(Vázquez & Cremonte, 2017)**.

Globally, the genus *Vibrio* is one of the major causes of mortality of commercial aquaculture. Using a wide variety of physiological mechanisms, they can adapt to environmental conditions. Many of their essential functions, including niche colonization, survival strategies, and virulence, are being regulated by cell-to-cell contact **(Mahmoud *et al.*, 2013; Girard, 2019)**.

Noticeably, *Vibrio tasmaniensis*, *V. splendidus*, and *V. neptunius* species have been considered as one of the main infectious vectors for aquaculture diseases **(Lago *et al.*, 2009)**. The *V. logei* has been reported as pathogenic microorganism to *H. harid* with 86.7% mortality rate (**Mahmoud *et al.*, 2017**). Moreover, **Fidopiastis *et al.* (1998)** revealed that light organs of *Sepiola affinis* and *Sepiola robusta* have various infectious organisms of *Vibrio* species, such as *Vibrio logei* and *V. fischeri*. The previous authors added that, the infection rates of those species ranged from 63% to 100%, respectively; with no previous detected symbiose rates for the *V. logei* species..

Specifically, *Tridacna gigas* larvae species were the most susceptible to infection with Photobacterium: *Vibrio damsel*, *V. harveyi*, *V. alginolyticus*, and *V. campbelli* causing 100% mortality **(Sutton & Garrick, 1993)**. Those infections cause significant decrease in commercial production in aquafarming. Moreover, disease outbreaks due to *Vibrio* species have a wide range of consequences on the different developmental stages from larval to post-larval stages in hatcheries and natural environment.

The current study was conducted to evaluate the bacterial diseases due to *Vibrio* species for the indoor aquarium and offshore cages of cultured *Tridacna maxima* and *Tridacna gigas*. In addition, this work considered to detect the consequential effects of changes in the environmental factors on the culture of *Tridacna sp.* and determine the pathogenicity of the recovered bacteria as well.

MATERIALS AND METHODS

1. Samples collection

Ethical approval of the present research has been conducted according to ethical criteria of the laboratory of Hurghada's Red Sea branch in NIOF during the period from December 2019 to February 2020. Forty reared specimens of each *T. maxima* and *T. gigas* (1.4- 2 kg) were harvested from cultivated cages in the reef area, approximately 130 m from the shore of the National Institute of Oceanography and Fisheries (NIOF), Hurghada (27°17'37" N, 33°47'10"E). The specimens were transferred to laboratory of Hurghada's Red Sea branch in NIOF and directly subjected to diagnostic assay. Forty reared specimens were also taken from the cultured cages, located in the sea.

2. Water quality

Water samples were taken from the indoor aquarium and the red sea in the study area. The environmental conditions of water temperature (C), salinity (%), dissolved oxygen (DO), and pH, were measured using YSI professional multiparameter. While, ammonia concentration was measured in 50 mL of water sample, using Nessler's reagent method.

3. Bacterial isolation and identification

Bacterial isolations were taken from gills, mantel, gonads, and gut of *T. maxima* and *T. gigas* under aseptic procedures. All the samples were cultured on agar plates of tryptone soya and brain heart infusion (Oxide) supplemented with 1.5% (w/v) sodium chloride (TNA) and incubated at 25°C for 48-72 hrs **(Suttona & Garrick, 1993)**. Thiosulphate citrate bile salt sucrose agar (TCBS, Difco) was used to purify the bacterial colonies followed by characterization with traditional bacterial techniques of **Nicky (2004)** and advanced characterization using API20E (bioMerieux) biochemical techniques.

4. Experimental infection

Twenty *T. gigas* individual specimens were acclimated to the environment of laboratory for two weeks in the fiberglass tanks, and subdivided into two groups (10 individuals for each). The first group was infected by bath immersion in 5L glass aquaria containing 1.5x106 CFU of V. logei/ ml for 30 minutes according to the protocol of **Martins *et al.* (2010)**. The second group acted as a control non-infected group, and both were kept under close observation for two months.

RESULTS

1. Pathological changes

The infected *T. maxima* and *T. gigas* revealed reduced movement with sluggish behavior at the tanks. The characteristic combinations of colors (blue, green, brown, purple, and yellow color) were faded out as shown in Fig. (1).

Fig. 1: *T. gigas* showing faint brown, purple color patterns.

Mortality rates were 25% and 20 % in indoor culture *T. maxima* and *T. gigas* clams, respectively. Postmortem examination revealed severe congestion in gut, mantel, and gonads of culture *Tridacna*, accompanied with distributed ulcer spots in the mantel area and scattered color changes. Additionally, green and dark lines appeared in the inner surface of the shell.

In contrast, *Tridacna* offshore culture showed no significant changes in the color profile of diagnostic or postmortem as shown in Fig. (2).

Fig. 2: *T. maxima* cultured in offshore cages showing attractive color patterns.

2. Water parameter

The findings showed a significant rise in pH and ammonia levels, parallel with a decline in dissolved oxygen in indoor aquarium water samples (Table 1).

Table 1: The measured environmental conditions in the Red Sea and indoor aquarium.

Item	Indoor aquarium sample	Red Sea sample
Water temperature	27°C	25°C
pH values	8.1	7.6
Dissolved oxygen	4.9 mg/ L	5.6 mg /L
Ammonia	0.004 mg /L	0.0007 mg /L

3. Bacteriological isolation and identification

The bacteriological identification showed that the most sensitive organ among offshore cage cultures of *T. gigas* to Vibro species infection was the gut (Ten bacterial isolates) without recorded infection in mantel, gonads, and/or gills. While, indoor aquarium of *T. gigas* organs showed more sensitivity to bacterial infection in the gut and less sensitive in mantel and gills, but the lowest rate was observed in gonads (9, 2, 2 and 1 bacterial isolates, respectively). Offshore cages cultured *T. maxima* clams revealed that, the most sensitive organ to infection with *Vibrio* species was the gut (Nine bacterial isolates). However, indoor aquarium of *T. maxima* organs showed more sensitivity to infection with high rates in the gut and less sensitive in mantel and gills, whereas the

lowest rate was recorded in gonads (7, 3, 2 and 1 bacterial isolate, respectively) as shown in Table (2).

Table 2: Bacteriological isolation from *T. gigas* and *T. maxim*a in offshore cages and indoor aquarium.

Type of culture	Samples	No. of samples	Growth on culture media	
			T. gigas	*T. maxim*a
Offshore cages	Mantel	20	0	0
	Gut	20	10	9
	Gonads	20	0	0
	Gills	20	0	0
Indoor aquarium	Mantel	20	2	2
	Gut	20	9	7
	Gonads	20	1	1
	Gills	20	2	3

The significant infection with Vibro species for the two *Tridacna* clams showed variable activity and susceptibility to pathogenicity. The results showed that the infected *T. gigas* clams with *V. logei* were 8 and 9 individuals in offshore cages and indoor aquarium, respectively. While, *V. harveyi* infection showed variable infection rates from zero to 5 infections among offshore cages cultured and indoor aquarium samples, respectively. Additionally, 2 unidentified *Vibrio* species were detected in offshore cages cultured with no recorded infection among indoor aquarium.

T. maxima clams showed that 7 of offshore cages cultured samples and 6 of the indoor aquaria were infected with *V. logei*. Among offshore cages samples, the detected *V. harveyi* infection rate was zero whereas, seven infections were detected among indoor aquarium samples. Finally, a detection of four unidentified *Vibrio* species in offshore cages were observed (Table 3 & Fig. 3).

Table 3: Identification of bacterial isolates from an indoor aquarium and offshore cages cultured *T. gigas* and *T. maxima.*

Vibrio species	Indoor aquarium		Offshore cages cultured		Total
	T. gigas	*T. maxima*	*T. gigas*	*T. maxima*	
V.logei	9	6	8	7	30
V.harveyi	5	7	0	0	12
Unidentified *Vibrio species*	0	0	2	2	4
Total	14	13	10	9	46

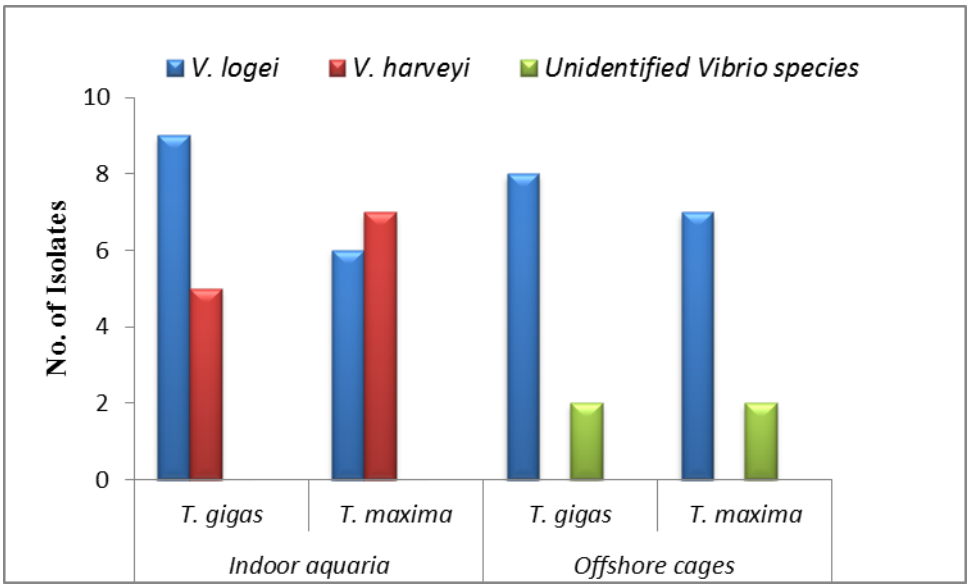

Fig. 3: Showed the number of bacterial isolates from Indoor aquaria and offshore cages *T. gigas* and *T. maxima.*

Forty-six bacterial strains were isolated from *T. gigas* and *T. maxima*. Those isolates were identified through morphology, conventional biochemical tests, and API20E tests *Vibrio logei*. The most dominant pathogen was *V. logei* (30 isolates). While, *V. harveyi* was identified in 12 isolates. Moreover, four isolates were determined as unidentified *Vibrio* species (Table 4).

4. Experimental infection

Tridacna gigas, laboratory experiments showed slow motility with a 25% mortality rate during the 60- days experimental period. The postmortem examination revealed severe congestion in the gut, gonads and ulceration in the mantel. The recovered bacteria from the gut, gonads, and mantel was identical to *V. logei*.

Table 4: Cultural and biochemical characterization of the *Vibrio logei* and *V. harveyi* isolates.

Items	*Vibrio logei*	***V.*** harveyi
Colony shape	Round	Round
Colony color	White creamy	White creamy
Motility	+	+
Gram stain	-Ve rods	-Ve slightly curved rods
Cytochrome oxidase	+ Ve	+ Ve
Catalase	+ Ve	+ Ve
0% NaCl	-	-
1.5% NaCl	+	+
3% NaCl	+	+
6% NaCl	+	+
ONPG	+	-
ADH	-	-
ODC	-	+
LDC	+	+
CIT	-	+
URE	+	+
H2S	-	-
TDA	-	+
Indole	-	+
VP	-	-
GEL	-	V
Xylose	-	+
Raffinose	-	-
Manitol	-	+
Glucose	-	+
Inositol	-	-
Sorbitol	-	+
Rhaminose	-	-
Sucrose	-	+
Malonate	-	-
Arabinose	-	-
Adonitol	-	-
Lactose	-	-
Salicin	-	V

VP= Voges-Proskauer, GEL= gelatin hydrolysis, TDA= tryptophane deaminase, ONPG, o-nitrophenyl-b-d-galactopyranoside, v= variable, LDC= lysine, V= variable decarboxylase, ADH= arginine dihydrolase, ODC= ornithine decarboxylase, CIT = citrate, URE= urea hydrolysis

DISCUSSION

The microbiome of giant clams is particularly interesting because clams are exposed to an extreme abundance and diversity of microbes through filter-feeding, and they live in symbiosis with dinoflagellate algae **(Guibert *et al.*, 2020)**. Limited information is available in literature concerning the disease affecting both cultured and wild *Tridacna* species. Hence, a total number of 80 *T. maxima* and *T. gigas* was collected to be examined from the indoor aquarium of NIOF and offshore cage cultures in the Red Sea at Hurghada. The diagnostic and postmortem pictures revealed a decrease in motality and color changes with severe congestion in the gut, mantel, and gonad.While, no postmortem changes was observed in offshore cages cultured *Tridacna*.

The present findings agree with those of **Tubiash and Otto (1986)**, who reported that the typical signs of vibriosis in clams included decreased motility in circles, velum disturbance and visceral atrophy. In addition, lesions in the other organs were correlated with decreased dissolved oxygen and increased ammonia level in water. Variations in the calcification phase on the inner surface of the valves and the presence of a typical brown deposit between the edge of the shell and the pallial line define clam vibriosis **(Borrego *et al.*, 1996)**. It is worthy to mention that, the sensitivity of bivalves promotes the pathogenesis of the disease, but external stress associated with low water quality elevated organic matter, and other stimuli promote the dissemination of possible bacterial infections. Moreover, mortality can occur through the overgrowth of opportunist bacterial in several incidents **(Tubiash & Otto, 1986)**.

In the indoor cultured species, 46 bacterial strains were isolated from the gut, mantel, gills, and gonads regarding bacteriological analyses. But the species cultivated in the offshore cages were collected from the gut only. Those findings suggest that the ideal bivalve aquaculture environment induces bacterial production **(Brown & Tettelbach, 1988)**. Thirty samples were classified as *V. logei*, while 12 isolates were classified as *V. harveyi* by the population, morphological and biochemical characters, including the API20E studies. **Al-Sunaiher *et al.* (2010)** and **Mahmoud *et al.* (2017)** have identified a strong correlation. Larval clams are mostly pathogenic to bacteria belonging to the genera *Vibrio* and *Aeromonas* and those in other genera are less commonly pathogenic.

In the current research, the dominant bacterial isolates of both indoor aquarium and offshore cage cultivated species were *V. logei* from the gut. The mantles, gonads, and gills of indoor tank culture organisms were also triggered by *V. harveyi*. These results are consistent with those of **Fidopiastis *et al.* (1998)** and **Edward and Kyu-Ho (1998)**, who suggested that several pathogenic and competitive interactions with marine species are established by *V. fischeri*, *V. logei*, *Photobacterium phosphoreum*, and *P. leiognathi*. Those bacteria are widely recognized as disease causative agents for marine invertebrates, as they are typical representatives of microbial inhabitants of intestinal tract. The current results agree with **Mahmoud *et al.* (2017)** who reported that, *V. logei* was the dominant bacterial pathogen for *H. harid*. Because it was already isolated from

most samples of infected oysters, *V. harveyi* is known to be a causative agent of summer mortality in oysters and adult clams, and it induces mortality during experimental infection.

Not only can mortality be related to infection with bacterial pathogenes, but there was also a complex relationship between the genetic and/or biological functions of bivalve species, the state of the ecosystem and the existence of more than one opportunistic pathogenic species of *Vibrio* **(Pruzzo *et al.*, 2005)**. In the present experiment, it was noticed that *V. logei* was pathogenic to *T. gigas*. Diagnostic illustration and P.M lesions, identical to those of the naturally infected ones with 25 % mortality, were revealed by the experimentally pathogenic bacteria. Remarkably, similar results have been documented in experimental infections with various *Vibrio* species **(Beaz-Hidalgo, 2010)** in juvenile and adult clams.

CONCLUSION

In conclusion, the offshore cages are much more appropriate for aquaculture of *Tridacna* species compared to indoor aquariums. In the aquaculture of *Tridacna* species, opportunistic pathogens such as *V. logei* and *V. harveyi* must be considered as the cause of severe losses, particularly when combined with the degradation of environmental factors.

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