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JKAU: Mar. Sci., **Vol. 31**, No.2, 65 Pages (1442 A.H. / 2021 A.D.) **ISSN: 1012-8840**
Legal Deposit 0301/14

Journal of

KING ABDULAZIZ UNIVERSITY

Marine Sciences

Volume 31

Number 2

1442 A.H. / 2021 A.D.

Scientific Publishing Center
King Abdulaziz University
P.O. Box 80200, Jeddah 21589
Saudi Arabia
http://spc.kau.edu.sa

Contents

English Section

JKAU: Mar. Sci., **Vol. 31** No. 2 pp: 1-13 (1442 A.H. / 2021 A.D.)
DOI :10.4197/Mar.31-2.1

Antifouling Activity of Epibiotic Bacteria Associated with Soft Coral *Sarcophyton* sp. Collected from the Central Red Sea

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Abstract. The microbial communities associated with marine invertebrates are considered as a prolific source of bioactive metabolites. In this study, the bacteria associated with the soft coral *Sarcophyton* sp. collected from the Red Sea was screened for antifouling activity to identify potential natural antifouling compounds. The extract of bacterial strain *Ruegeria lacuscaerulensis* KAU-MB3 associated with the soft coral showed strong antifouling activity in laboratory assays. The crude bacterial extract reduced the growth of biofilm-forming bacteria and inhibited the biofilm formation significantly. Barnacle larval settlement assay indicated a significant reduction in the settlement of larvae treated with the crude extract. The crude extract was analysed by GC-MS to understand the chemical composition. GC-MS results revealed the presence of compounds such as hexadecane, octatriacontyl pentafluoropropionate, cis-Z-à-bisabolene epoxide and geranyl isovalerate. In conclusion, this study indicated that the bacteria associated with the soft corals could be used as one of the potential sources for the natural product antifoulants.

Keywords: Soft corals; bacterial symbionts; biofouling; bioactive compounds; microbial ecology; Red Sea.

1. Introduction

Soft corals (Alcyonacea) are the main components of the benthic communities in the reef environment with great diversity in tropical regions of the Indian and Pacific oceans, Fabricius and Alderslade 2001), including the Red sea (Reinicke 1997). Previous studies showed that many microbial species are associated with coral species mainly in the mucus, tissues and surfaces (Ritchie and Smith, 1997; Rohwer *et al*., 2001; Koren and Rosenberg, 2006). The microbes associated with the corals may have many functions based on the relationships such as mutualistic and pathogenic (Harvell *et al*., 1999; Ben-Haim *et al*., 2003). Generally, the microbial symbionts of corals provide protection to the hosts through the production of biologically active metabolites or antagonistic activity (Ritchie, 2006; Rosenberg *et al*., 2007). Besides, the microorganisms associated with the corals may have many ecological functions such as supply nitrogen and phosphorus to the host, participation in nutrient recycling *etc*. (Rosenfeld *et al*., 1999; Anthony and Fabricius, 2000).

In marine waters, almost all surfaces (both living and non-living) are colonized by microorganisms and macroorganisms. Some marine organisms have defence mechanism (antifouling defence) against unwanted colonization. The microorganisms associated with marine organisms are also reported to play important role in the antifouling defence of these organisms. These microorganisms could be a source for the isolation of novel antifouling metabolites to control the biofouling development on artificial materials

(Satheesh *et al*., 2016). Biofouling development on hard substrates is one of the serious issues for marine technology and maritime sectors due to the ecological and economical complications (Satheesh *et al*., 2016). The biofouling development process usually begins with the formation of biofilm (microbes and microalgae) on the substrates, attachment of larvae and algal spores and finally assemblage of macroorganisms (Kwon *et al*., 2002; Wesley and Satheesh, 2009; Satheesh and Wesley, 2010; Satheesh *et al*., 2016).

Many methods are used to control the biofouling development on technical objects (Qian *et al*., 2009). Tributyltin is one of the popular antifouling compounds used in coatings (Nehring, 2001). Though TBT based coatings are very effective to prevent biofouling development, the side effects to the environment and other non-target marine organisms warrant strict guidelines and complete prohibition from 2008 by International Maritime Organization (Satheesh *et al*., 2016). After the ban on TBT, many alternatives are suggested but most of those compounds are also reported to possess toxic effects on marine organisms (Nehring, 2001). The natural products from marine organisms and their microbial symbionts are reported to exhibits strong antifouling activity in laboratory assays (Qi *et al*., 2008; Qian *et al*. 2009; Viju *et al*., 2017; Salama *et al*., 2018).

Marine microbes attracted the attention of researchers due to their capability to produce novel metabolites (Milinski, 1993; Bernan *et al*., 1997; Fenical, 1997). These secondary metabolites serve as model systems in the discovery of new drugs as well as ecologically relevant compounds. Bacteria associated with sponges, corals and seaweeds have been studied for their antimicrobial activity (Burgess *et al*., 1999; Boyd *et al*., 1999; Thiel and Imhoff, 2003; Radjasa *et al*., 2007; Kennedy *et al*., 2009) and many bioactive compounds have been reported (Chandramohan, 1997; Anand *et al*., 2006; Devi *et al*., 2010). However, only a few works are available on the antifouling activity of bacteria associated with soft corals. Hence, in this study, the bacterial communities associated with the surface of the soft coral species collected from the Red Sea was analysed for their antifouling activities. The results obtained in this study will improve our understanding of the chemical defence role of bacteria associated with marine invertebrates.

2. Materials and Methods

2.1 Collection of Soft Corals and Isolation of Surface-Associated Bacteria

The soft coral *Sarcophyton* sp. was collected from the Obhur Creek, Jeddah coast of central Red Sea. The collected coral samples were immediately transferred to the laboratory in a sterile container that contained filtered and sterilized seawater. In the laboratory, soft coral samples were rinsed in filtered and sterilized seawater to remove the debris and other organisms. The surface of the soft coral samples was swabbed with cotton. After that, the cotton swab was kept in 2 ml filtered and sterilized seawater and vortexed. Following this, the suspension was diluted and spread on agar plates (Marine agar). The plates were kept at 30°C for 24-48h for the development of bacterial colonies. The bacterial colonies developed on the plates were separated based on colony morphology. The isolated colonies were purified by the streak plate method on marine agar plates. After that, the purified colonies were maintained in marine agar slants at 4^{o}C for further studies.

2.2 Preparation of Crude Extract of Coral-Associated Bacteria

The coral-associated bacterial isolates were cultured in marine broth in conical flasks (250 ml). The culture broth was kept at 30°C in a shaker (100 rpm) for 3 days. Following this, the culture broth was centrifuged at 10000 x *g* at 4^{o}C for 15 min. After centrifugation, the resulting pellet was collected and washed with 10 mM phosphate-buffered saline. The cell pellet was extracted with 2 ml methanol. The

extraction process was carried out for 24 h in a shaker. After that, the supernatant was collected and concentrated under reduced pressure in a rotary evaporator. The resulting crude extract was mixed with the required amount of methanol for further assays.

2.3 Screening of Antibacterial Activity of Coral-Associated Bacterial Extracts

The traditional disc diffusion assay was used to test the antibacterial activity of the crude extracts of the coral-associated bacteria. Two biofilm-forming bacteria, *Vibrio harveyi* and *Planomicrobium* sp. isolated from the artificial materials submerged in the Obhur Creek (Balqadi *et al*., 2018) were used as target bacteria for the anti-biofilm assays. The crude extract was mixed with methanol (100 µg in 1 ml) and used for the disc assay. Paper discs (6 mm) were prepared using Whatman filter paper. About 50 µl of the extract was loaded on each treatment disc. The control discs were loaded with 50 µl of methanol. The extracts and solvent loaded discs were kept in a chamber for 1 h. After that, the paper discs were placed on marine agar plates that were seeded with biofilm-forming bacteria. Following this, the plates were kept in an incubator at 30°C for 24-48h. The plates were checked for the inhibition zones around the discs. The zone of inhibition on the plates was measured using a scale. The coral-associated bacterial extract which showed strong activity in the disc diffusion assay was selected for further studies.

2.4 Identification of Coral-Associated Bacteria

Genomic DNA from the bacteria was extracted using a commercially available DNA extraction kit by following the protocol recommended in the kit instructions. The extracted DNA samples were subjected to PCR amplification using universal 16S rRNA primers for bacteria. The PCR conditions and sequencing protocols described previously by Balqadi *et al*. (2018) was used in this study. The sequences were aligned and the bacterial strain was identified using National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI-BLAST). The sequence was submitted to NCBI Genbank.

2.5 Biofilm-forming Bacterial Growth Inhibition Assay (Spectrophotometer Method)

Bacterial growth inhibition assay was conducted using the spectrophotometric method. About 3 ml of overnight grown biofilm-forming bacterial culture was taken in test tubes. To this, two different concentrations (25 and 50 µg ml^{-1}) of coral-associated bacteria were added and incubated for 5 h. Two types of control wells were maintained, one with 25 and 50 µl ml^{-1} methanol and the other control without extract or solvents. The OD (optical density) of the test and control samples will be read at 630 nm in UV-Vis spectrophotometer at 1 h interval for 5 h duration.

$$Growth\ Rate(\%) = \frac{Final\ OD\ Value - Initial\ OD\ value}{Initial\ OD\ value} \times 100$$

2.6 Antibiofilm Activity Assay (Microtitre Plate Method)

The microtitre plate biofilm assay described by O'Toole (2011) was used to test the antibiofilm activity. In brief, overnight grown biofilm-forming bacteria culture was taken into 96 well microtitre plates. Two different concentrations of the extracts (25 and 50 µg ml^{-1}) were added to the wells and incubated for 24-72 h at 30°C in an incubator. Two types of control wells were maintained, one with 25 and 50 µl ml^{-1} methanol and the other control without extract or solvents. After incubation, the culture along with the unattached bacterial cells was removed by gently turning the plates over and washed with phosphate buffer saline. Crystal violet (0.1%) was added to the wells to stain the attached bacterial cells. The plates were incubated for 15 minutes and after that washed with distilled water to remove excess stain. The attached biofilm cells were quantified by adding 30% acetic acid to the wells followed by incubation

for 15 minutes at 30°C. The crystal violet eluted from the wells was transferred to a new microtitre plate and the optical density was measured at 550 nm in a microtitre plate reader (Biotek). The experiment was conducted in replicates (n=6) with a different batch of biofilm-bacteria culture.

2.7 Barnacle Larval Settlement Assay

The barnacle *Amphibalanus amphitrite* adults were collected from the Obhur Creek and maintained in a glass tank in the laboratory. The barnacles were kept in the tank with moderate aeration and provided a mixed diet consisted of *Artemia* nauplii microalga (*Chaetoceros*). The nauplii released by the adults were collected and transferred to small tanks. The nauplii were reared up to cypris stage (settling stage) using a mixed algal diet according to the conditions outlined in Salama *et al*. (2018). The cypris larvae were used for settlement assays. The settlement assay was conducted in 6-well plates using 25 larvae in each well. The extracts in two different concentrations (25 and 50 µg ml^{-1}) were added to the wells. Two types of control wells were maintained, one with 25 and 50 µl ml^{-1} methanol and the other control without extract or solvents. The plates were kept in dark at room temperature and the number of cyprids settled was counted under a microscope after 24, 48 and 72 h. The percentage of settlement for each concentration was calculated after deducting the values observed in the methanol control. The experiment was conducted in replicates (n=3) using a different batch of barnacle larvae.

2.8 GC-MS Analysis of Coral-Associated Bacteria Extracts

The column purified extract of the soft-coral associated bacteria was carried out using Shimadzu GC-MS QP 2010 according to the protocol described previously (Viju *et al*., 2020). In brief, the sample was injected using the carrier gas helium at the flow rate 1ml/ minute. The temperature of the injection port was kept at 250ºC, while the temperature of the ion source was managed at 280ºC. The column oven temperature was held at 110ºC for 2 minutes then programmed at 10ºC / minute and the ending temperature was increased to an isothermal point at 280ºC/9 minute. Subsequently, the mass spectra of the fraction were taken at 2-minute scan interval and the compounds identification was made by comparing their mass spectra with the mass spectral library of NIST (National Institute of Standards and Technology).

2.9 Statistical Analysis

The data obtained from bacterial growth inhibition and antibiofilm activity were subjected to one-way ANOVA (analysis of variance) to find the variation between control and treatment samples. The barnacle larval settlement assay data was analysed by two-way ANOVA using extract concentration and exposure time as factors. The statistical analysis was conducted using MS-Excel ($P<0.05$ was considered significant).

3. Results

3.1 Antimicrobial Activity and Identification of Coral-Associated Bacteria

In this study, five coral-associated bacterial isolates were recovered from the surface of soft coral *Sarcophyton* sp. The crude extracts of all 5 isolates from soft coral were tested for their antibacterial activity against the two biofilm-forming bacteria. All the isolates showed antibacterial activity against the biofilm-forming bacteria (Fig. 1). However, the strain KAU-MB3 exhibited strong activity (inhibition zone size: 18 mm) than the other surface-associated bacterial strains. Hence, this bacterial strain was selected for further antifouling studies.

3.2 Identification of Bacterial Strain KAU-MB3

The 16S rRNA gene sequences obtained from the strain KAU-MB3 showed 99.91% similarity with the bacterium *Ruegeria lacuscaerulensis* in the NCBI database (GenBank). The phylogenetic analysis in

presented in Fig. 2. The sequence of the strain KAU-MB3 was submitted to NCBI GenBank (accession number: MW881524).

3.3 Growth Inhibitory Activity of the Coral-Associated Bacterial Extract Against Biofilm-Forming Bacteria

The results of the present study indicated that the extract of *R. lacuscaerulensis* inhibited the growth of both *V. harveyi* and *Planomicrobium* sp. (Fig. 3). The growth rate of *V. harveyi* was observed as 77.66% for a period of 5 h under laboratory conditions. The *V. harveyi* culture treated with 25 and 50 µg ml^{-1} of the coral-associated bacterial extract showed a growth of 57.33 and 37.33 % respectively. One-way ANOVA revealed a significant variation in the growth of *V. harveyi* treated with different concentrations of coral-associated bacterial extract ($F=46.33$, $df=2,6$; $P<0.001$). Further, post-hoc Tukey test results indicated significant variation in the growth of *V. harveyi* between the control and treatments (Table 1).

The *Planomicrobium* sp. control culture showed a growth rate of 81.33% during the 5h period under the laboratory conditions (Fig. 3). However, the *Planomicrobium* sp. cultures treated with 25 and 50 µg ml^{-1} of coral-associated bacterial extract recorded a reduction in growth. The *Planomicrobium* culture treated with 25 µg ml^{-1} recorded a growth of 47.66% and 50 µg ml^{-1} exhibited a growth percentage of 39. ANOVA results indicated a significant difference in the growth of *Planomicrobium* sp. treated with different concentrations of coral-associated bacterial extract ($F=162$, $df=2,6$; $P<0.001$). Moreover, the growth of *Planomicrobium* sp. differed significantly between control and extract treated cultures (Tukey test, Table 1).

3.4 Antibiofilm Assay

The antibiofilm assay indicated that the extract of coral-associated bacterial strain inhibited the settlement of biofilm-forming bacteria (Fig. 4). The settlement of *V. harveyi* ($F=93.09$, $df=2, 15$; $P<0.001$) and *Planomicrobium* sp. ($F=77.79$, $df=2, 15$; $P<0.001$) on microtitre plate was reduced significantly (Table 1) due to the treatment of coral-associated bacterial extract.

3.5 Barnacle Larval Settlement Assay

The results of the barnacle larval settlement assay are presented in Fig. 5. In the control well, an average of 84% (21 individuals) settled on the plates after 48 h. However, the settlement of barnacle larva was reduced considerably when treated with the coral-associated bacterial extract. The number of larvae settled on the wells treated with 25 µg ml^{-1} of the extract was 11 (44%) after 48 h of the experiment. Likewise, a low settlement was recorded from the wells treated with 50 µg ml^{-1} of extract (7 individuals, 28%) during the same period. Further, the settlement was very low in the treatment wells during the initial 24 h of the experiment. Two-way ANOVA results showed significant variation in the settlement of barnacle larvae in relation to extract concentration and observation time (Table 2).

3.6 GC-MS Analysis of the Coral-Associated Bacterial Extract

The GC-MS analysis of the coral-associated bacterial extract showed the presence of bioactive metabolites such as hexadecane, octatriacontyl pentafluoropropionate, cis-Z-à-bisabolene epoxide geranyl isovalerate, pentadecane- 7-methyl, tetradecane, 2-Ethylhexyl -2-ethylhexanoate, p-Toluic acid- 2-ethylhexyl ester, 1-hexacosene, 1-chloroeicosane and dibutyl phthalate (Fig. 6, Table 3). Among the compounds, p-Toluic acid- 2-ethylhexyl ester, dibutyl phthalate and Cis-Z-à-bisabolene epoxide were exhibited higher concentrations (based on peak area).

Table 1. Approximate Probabilities for Post Hoc Tukey HSD tests. P<0.05= significant.

Factor 1	Factor 2	Bacterial growth inhibition		Antibiofilm assay	
		V. harveyi	*Planomicrobium* sp.	*V. harveyi*	*Planomicrobium* sp.
25µg	50 µg	0.007	0.029	0.004	<001
	Control	0.006	<001	<001	<001
50 µg	control	<001	<001	<001	<001

Table 2. Two-way ANOVA results for the settlement of barnacle larvae treated with coral-associated bacterial extract. Extract concentration and experiment duration were considered as factors. P<0.05= significant.

	Degrees of Freedom	F	p
Concentration	2	244.884	0.000
Time	3	46.029	0.000
Concentration*Time	6	2.217	0.076
Error	24		
Total	35		

Table 3. The compounds identified from the extract of soft-coral associated bacterium *R. lacuscaerulensis*.

Compound name	**RT**	**Area (%)**
Heptane, 2,2,4,6,6-pentamethyl	4.67	0.16
Tetradecane, 2,6,10-trimethyl	10.95	0.11
Hexadecane	11.08	0.1
3-(2H)-Benzofuranone, 2-methyl	13.91	0.05
Pentacosane, 13-phenyl	14.00	0.06
ert-Hexadecanethiol	16.17	0.19
Octatriacontyl pentafluoropropionate	16.65	0.07
Geranyl isovalerate	18.12	0.30
Methoxyacetic acid, 2-tetradecyl ester	18.95	0.53
17-Pentatriacontene	20.27	0.35
Pentadecane, 7-methyl	21.78	4.57
Tetradecane	22.01	1.31
2-Ethylhexyl 2-ethylhexanoate	22.11	5.65
Benzoic acid, 2-ethylhexyl ester	22.52	3.26
1-Hexacosene	23.11	1.54
1-Chloroeicosane	23.56	0.66
1-Octadecanesulphonyl chloride	24.27	0.30
Heptadecane	24.59	1.50
Dodecane, 2,6,11trimethyl	24.72	1.60
2-Octyl benzoate	24.88	1.96
Cholestan-3-ol, 2-methylene,	25.32	0.39
Cis-1-Chloro-9-octadecene	25.81	0.56
Benzene, (1,3,3trimethylnonyl	26.40	2.23
p-Toluic acid, 2-ethylhexyl ester	27.47	20.61
Cis-13-Eicosenoic acid	28.53	0.13
1,2-Benzenedicarboxylic acid, bis-(2methylpropyl) ester	28.63	1.48
Ethaneperoxoic acid,	29.91	0.06
Ethyl isoallocholate	30.83	0.06
Dibutyl phthalate	31.24	14.08
Fenretinide	32.60	0.14
a-D-Mannofuranoside, farnesyl	35.84	0.08
Trans-Geranylgeraniol	35.98	0.38
6-epishyobunol	36.16	0.17
Cis-Z-à-Bisabolene epoxide	36.46	3.02
4,8,13-Cyclotetradecatriene1,3-diol,	38.73	1.08
Squalene	47.76	0.56

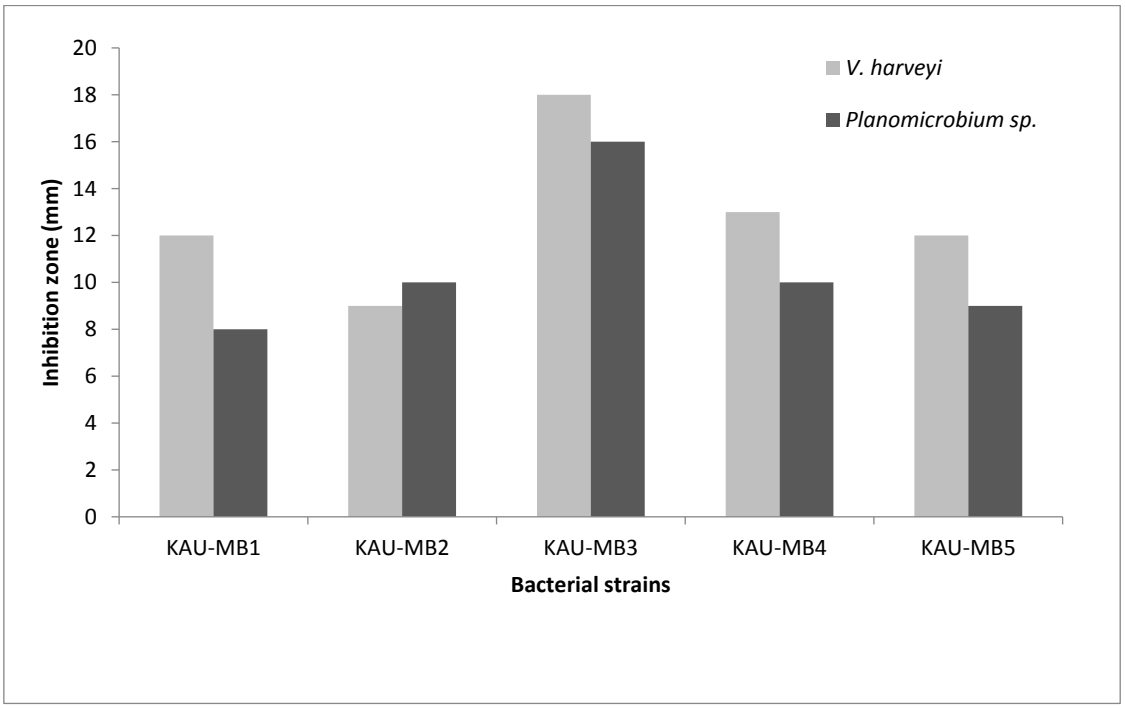

Fig. 1. Antiibacterial activity of coral-associated bacterial extract against biofilm-forming bacteria.

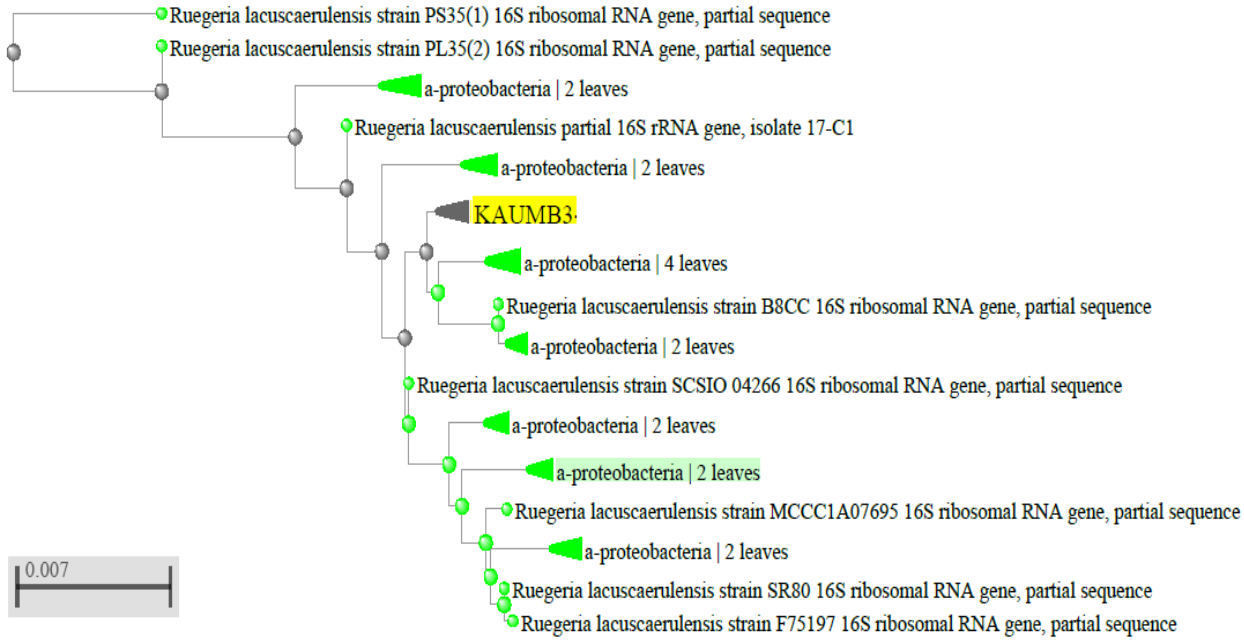

Fig. 2. Phylogenetic tree of coral-associated bacterial strain KAU-MB3. The strain was identified as *Ruegeria lacuscaerulensis.*

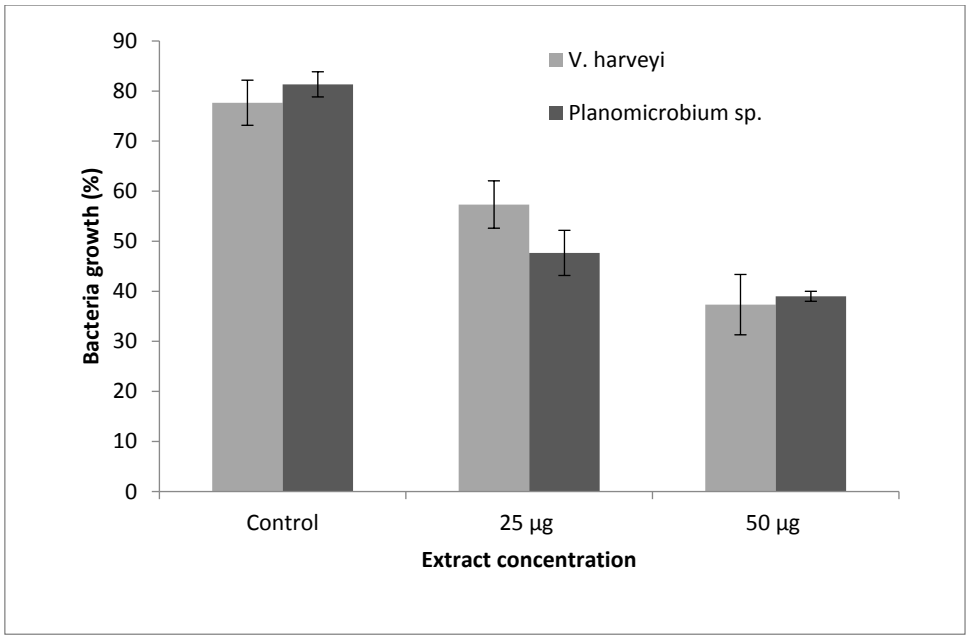

Fig. 3. Growth inhibitory activity of coral-associated bacterial extract against biofilm-forming bacteria.

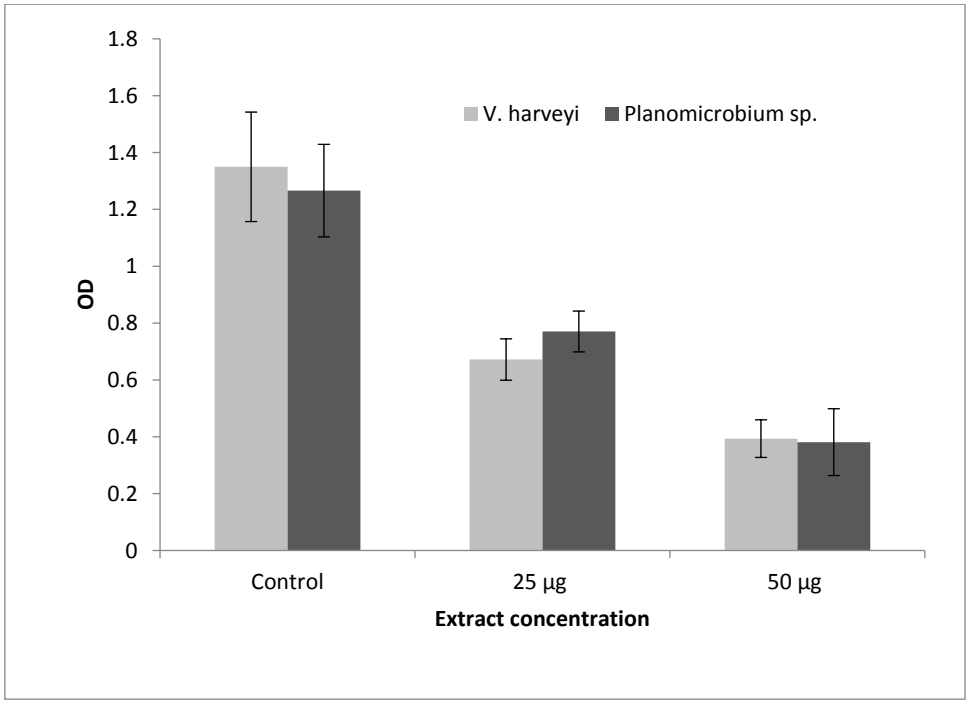

Fig. 4. Antibiofilm activity of coral-associated bacteria against two biofilm-forming bacteria.

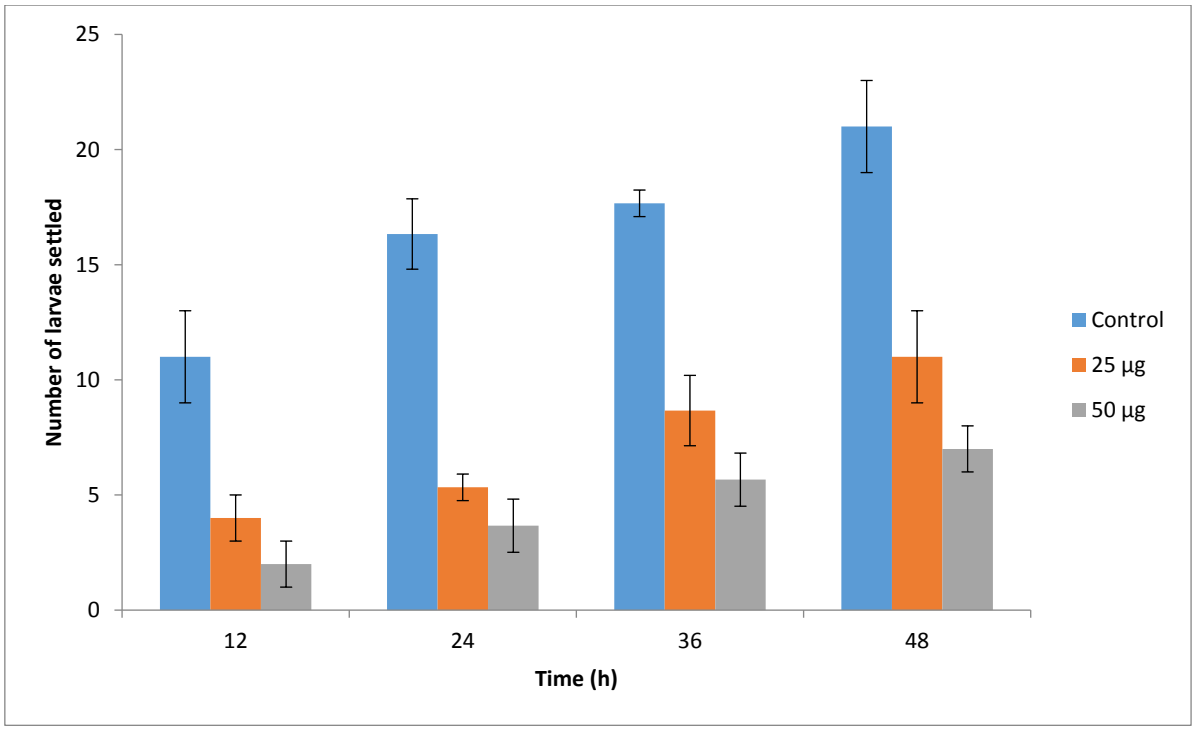

Fig. 5. Barnacle larval settlement inhibitory activity of coral-associated bacterial extract.

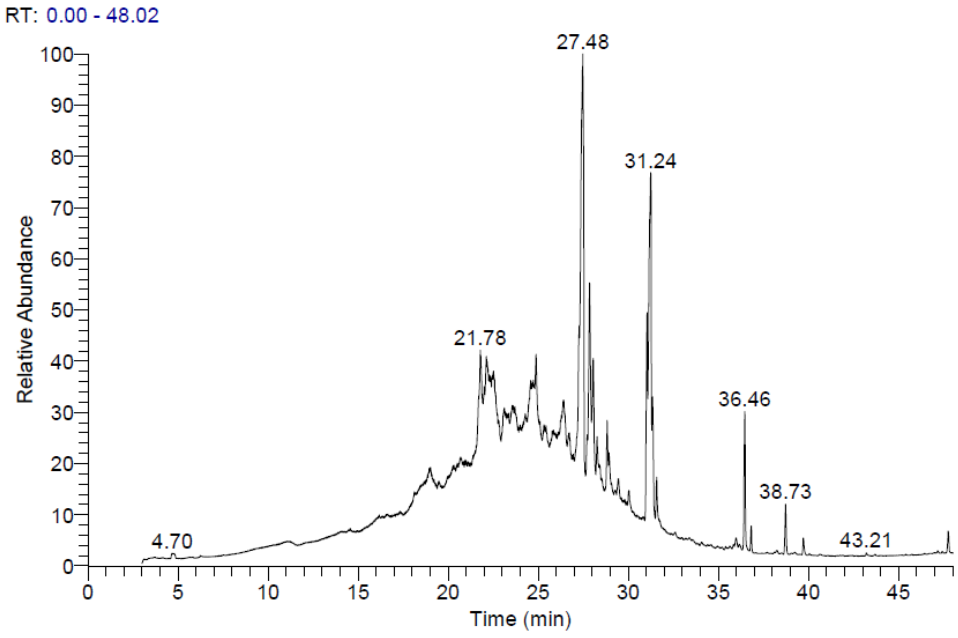

Fig. 6. GC-MS analysis of crude extract of coral-associated bacterium *Ruegeria lacuscaerulensis*.

4. Discussion

The results of the present study indicate that soft corals harbour many bacterial species which may provide protection to the host through the production of secondary metabolites. Many microorganisms including bacteria have a wide range of metabolic and physiological attributes which enable them to grow and survive in a diverse environment. A number of bioactive metabolites have been isolated from soft corals and their associated microbes (Sarma *et al*., 2009; Sang *et al*., 2019). The microbes associated with soft corals have also been reported to possess antifouling activity (Sabdono and Radjasa, 2006; Zhang *et al*. 2019). Previous studies also indicated that the bacteria associated with marine invertebrates could be a potential source for the biologically active compounds (Gil-Turnes and Fenical, 1992, Fenical, 1993). For instance, the bioactive compound Ubiquinone-8 that was isolated from the sponge-associated *Alteromonas* sp. showed strong antifouling activity against the barnacle larvae (Kon-ya *et al.*, 1995).

The genus *Ruegeria* (Roseobacter-clade) is a Gram-negative, aerobic rods mainly reported from the marine waters (Vandecandelaere *et al*., 2008). This genus includes the formerly known marine *Agrobacterium* species. Further, the association of bacteria *R. lacuscaerulensis* with tropical and subtropical coral reefs was previously reported by Gong *et al*., (2020). Previous studies reported the presence of *Pseudomonas Alteromonas, Flavobacterium* and *Vibrio* from the surface of marine invertebrates (Vacelet and Danady, 1997; Santavy and Colwell, 1990; Ward-Rainey *et al*., 1996; Chelossi *et al*., 2004). *Enterobacteriaceae*, *Aeromonas*, *Actinomyces* and *Streptomyces* were rarely reported from the invertebrates (Chelossi *et al*., 2004). *Cornybacteria, Actinomyces* and *Streptomyces* are widely distributed in the marine environment. They are considered as a source of bioactive agents and display competitive biosynthetic capabilities.

The results of this study indicated that bacteria associated with soft corals could be used as a potential source for the isolation of natural product antifouling compounds. Microbes as a source for the extraction of bioactive metabolites have more advantages than using marine invertebrates and algae. Mainly, for extraction of bioactive compounds from marine organisms requires the collection of a large amount of raw material from the natural sources (Satheesh *et al*., 2016). However, microorganisms are cultivable under laboratory conditions and using the modern fermentation methods, the required amount of metabolites can be extracted from the microbial sources (Satheesh *et al*., 2016).

In conclusion, the present study suggests that bacteria associated with the soft corals could serve as a potential source for the searching of new secondary metabolites. Since bacteria multiply quickly and produce large quantities of biomass within a short duration, the microorganisms producing biologically active compounds can be easily obtained on a biotechnological scale without the need for collecting the soft corals from the marine environment. Though laboratory assays of the extract obtained in this study showed strong antifouling activity, further field tests are essential to confirm the activity.

References

Anand, T.P., Bhat, A.W., Shouche, S.Y., Roy, U., Siddharth, J. and **Sarma, S.P.** (2006). Antimicrobial activity of marine bacteria associated with sponges from the waters off the coast of South East India. *Microbiological Research*, **161**: 252-262.

Anthony, K.R.N. and **Fabricius, E.F.** (2000). Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. *Journal of Experimental Marine Biology and Ecology*, 252: 221–253.

Balqadi, A.A., Salama, A.J. and **Satheesh, S.** (2018). Microfouling development on artificial substrates deployed in the central Red Sea. *Oceanologia,* **60**: 219-231.

Ben-Haim, Y., Thompson, F.L., Thompson, C.C., Cnockaert, M.C., Hoste, B., Swings, J. and **Rosenberg, E.** (2003). Vibrio coralliilyticus sp. nov., a temperature-dependent pathogen of the coral Pocillopora damicornis.

International Journal of Systematic and Evolutionary Microbiology, **53**: 309–315.

Bernan, V.S., Greenstein, M. and **Maiese, W.M.** (1997). Marine micro-organisms as a source of new natural products. *Advances in Applied Microbiology*, **43**: 57–89.

Boyd, K. G., Adams, D.R. and **Burgess, J.G.** (1999). Antimicrobial and repellent activities of marine bacteria associated with algal surfaces. *Biofouling*, **14** : 227-236.

Burgess J.G., Jordan, E.M., Bregu, M., Mearns-spragg, A. and **Boyd, K.G.** (1999). Microbial antagonism: a neglected avenue of natural products research, *Journal of Biotechnology*, **1**: 27-32.

Chandramohan, D. (1997). Recent advances in Marine Microbiology: The Indian scenario. *Marine Biotechnology*, **5** :73-81.

Chelossi, E., Milanese, M., Milano, A., Pronzato, R. and **Riccardi, G.,** 2004. Characterization and antimicrobial activity of epibiotic bacteria from Petrosia ficiformis. Journal of Experimental Marine Biology and Ecology 309:21–33.

Devi, P., Wahidullah, S., Rodrigues, C. and **Souza, L.D.,** 2010. The Sponge-associated Bacterium Bacillus licheniformis SAB1: A Source of Antimicrobial Compounds. Marine Drugs 8: 1203-1212.

Fabricius, K.E. and **Alderslade, P.,** 2001. Soft Corals and Sea Fans. Australian Institute of Marine Science, Queensland, Australia.

Fenical, W., 1997. New pharmaceuticals from marine organisms. Trends in Biotechnology 15: 339–341.

Fenical, W. (1993). Chemical studies of marine bacteria; developing a new resource. *Chemical Reviews*, **93**: 1673-168.

Gil-Turnes, M.S. and **Fenical, W.** (1992). Embryos of Homarus americanus are protected by epibiotic bacteria. *The Biological Bulletin*, **182**: 105-108.

Gong, S., Jin, X., Ren, L., Tan, Y. and **Xia, X. (**2020). Unraveling Heterogeneity of Coral Microbiome Assemblages in Tropical and Subtropical Corals in the South China Sea. *Microorganisms*, **8(**4): 604.

Harvell, C.D., Kim, K., Burkholder, J.M. *et al.* (1999). Review: marine ecology-emerging marine diseases–climate links and anthropogenic factors. *Science*, **285**: 1505–1510.

Kennedy, J., Baker, P., Piper, C., Cotter, P.D., Walsh, M., Mooij, M.J., Bourke, M.B., Rea, M.C., O'Connor, P.M., Ross, R.P., Hill, C., O'Gara, F., Marchesi, J.R., and **Dobson, A.D. (**2009). Isolation and analysis of bacteria with antimicrobial activities from the marine sponge Haliclona simulans collected from Irish waters. *Marine Biotechnology*, **11**: 384–396.

Kon-Ya, K., Shimidzu, N., Otaki, N., Yokoyama, A., Adachi, K. and **Miki, W. (**1995). Inhibitory effect of bacterial ubiquinones on the settling of barnacle, Balanus amphitrite. *Experientia*, **51**: 153-155.

Koren, O. and **Rosenberg, E. (**2006). Bacteria associated with mucus and tissues of the coral Oculina patagonica in summer and winter. Applied and Environmental Microbiology 72: 5254–5259.

Kwon, K.K., Lee, H.S., Jung, S.Y., Yim, J.H., Lee, J.H. and **Lee, H.K. (**2002). Isolation and identification of biofilm-forming marine bacteria on glass surfaces in Dae-Ho Dike, Korea. Journal of Microbiology 40: 260-266.

Milinski, M. (1993). Predation risk and feeding behavior. In: T.J. Pitcher (Ed.), Behavior of teleost fishes (pp: 285-305). London, UK : Chapman & Hall.

Nehring, S. (2001). After the TBT era: alternative anti-fouling paints and their ecological risks. *Senckenbergiana maritima*, **31**: 341-351.

O'Toole, G. A. (2011). Microtiter dish biofilm formation assay. *JoVE* (*Journal of Visualized Experiments*) **47**: e2437.

Qi, S.H., Zhang, S., Yang, L.H. and **Qian, P.Y. (**2008). Antifouling and antibacterial compounds from the gorgonians Subergorgia suberosa and Scripearia gracillis. *Natural Product Research*, **22**: 154-166.

Qian, P.Y., Xu, Y. and **Fusetani, N. (**2009). Natural products as antifouling compounds: recent progress and future perspectives. *Biofouling*, **26**: 223-234.

Radjasa, O.K., Salasia, S.I.O., Sabdono, A., Weise, J., Imhoff, J.F., Lammler, C. and **Risk, M.J. (**2007). Antibacterial activity of marine bacterium Pseudomonas sp. associated with soft coral Sinularia polydactyla against Streptococcus equi subsp. zooepidemicus. *International Journal of Pharmacology*, **3**: 170-174.

Reinicke, G.B. (1997). Xeniidae (Coelenterata: Octocorallia) of the Red Sea, with descriptions of six new species of Xenia. *Fauna of Saudi Arabia*, **16**: 5–62.

Ritchie, K.B. and **Smith, W.G. (**1997). Physiological comparison of bacterial communities from various species of scleractinian corals. In: *Proc 8th Int Coral Reef Symp Publ*, **1**: 521–526.

Ritchie, K.B. (2006). Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Marine Ecology Progress Series*, **322**: 1–14.

Rohwer, F., Breitbart, M., Jara, J., Azam, F. and **Knowlton, N. (**2001). Diversity of bacteria associated with the Caribbian coral Montastraea franksi. *Coral Reefs*, **20**: 85–91.

Rosenberg, E., Koren, O., Reshef, L., Efrony, R. and **Zilber-Rosenberg, I.** (2007). The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology*, **5**: 355–362.

Rosenfeld, M., Bresler, V. and **Abelson, A. (**1999). Sediment as a possible source of food for corals. *Ecology Letters*, **2**: 345–348.

Sabdono, A. and **Radjasa, O.K. (**2006). Antifouling activity of bacteria associated with soft coral Sarcophyton sp. Against marine biofilmforming bacteria. J*ournal of Coastal Development*, **10**: 56-62.

Salama, A.J., Satheesh, S. and **Balqadi, A.A. (**2018). Antifouling activities of methanolic extracts of three

macroalgal species from the Red Sea. *Journal of Applied Phycology*, **30**: 1943-1953.

Sang, V.T., Dat, T.T.H., Vinh, L.B., Cuong, L.C.V., Oanh, P.T.T., Ha, H., Kim, Y.H., Anh, H.L.T. and **Yang, S.Y.** (2019). Coral and coral-associated microorganisms: A prolific source of potential bioactive natural products. *Marine Drugs*, **17**: 468.

Santavy, D.L., Willenz, P. and **Colwell, R.R. (**1990). Phenotypic study of bacteria associated with the caribbean scleraponge, Ceratoporella nicholsoni. *Applied and Environmental Microbiology*, **56**: 1750-1762.

Sarma, N.S., Krishna, M.S., Pasha, S.G., Rao, T.S.P., Venkateswarlu, Y. and **Parameswaran, P.S. (**2009). Marine metabolites: The sterols of soft coral. *Chemical Reviews,* **109**: 2803-2828.

Satheesh, S. and **Wesley, S. (**2010). Biofilm development on acrylic coupons during the initial 24 hour period of submersion in a tropical coastal environment. *Oceanological and Hydrobiological Studies*, **39**: 27-38.

Satheesh, S., Ba-akdah, M.A. and **Al-Sofyani, A.A. (**2016). Natural antifouling compound production by microbes associated with marine macroorganisms: A review. *Electronic Journal of Biotechnology*, **19**: 26-35.

Thiel, V. and **Imhoff, J.F. (**2003). Phylogenetic Identification of Bacteria with Antimicrobial activities Isolated from Mediterranean sponges. *Biomolecular Engineering*, **20**: 421-423.

Vacelet, J. and **Danadey, C. (**1977). Electron microscope study of the association between some sponges and bacteria. *Journal of Experimental Marine Biology and Ecology*, **30**: 301-314.

Vandecandelaere, I., Nercessian, O., Segaert, E., Achouak, W., Faimali, M. and **Vandamme, P. (**2008). *Ruegeria scottomollicae* sp. nov., isolated from a marine electroactive biofilm. *International Journal of Systematic and Evolutionary Microbiology*, **58**: 2726-2733.

Viju, N., Satheesh, S. and **Punitha, S.M.J. (**2017). Antifouling activities of antagonistic marine bacterium Pseudomonas putida associated with an octopus. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, **87**: 1113-1124.

Viju, N., Punitha, S.M.J. and **Satheesh, S. (**2020). Antibiofilm activity of symbiotic Bacillus species associated with marine gastropods. *Annals of Microbiology*, **70**: 1-12.

Ward-Rainey, N., Rainey, F. and **Stackebrandt, E. (**1996). A study of the bacterial flora associated with Holoturia atra. *Journal of Experimental Marine Biology and Ecology*, **203**: 11 –26.

Wesley, S.G. and **Satheesh, S. (**2009). Temporal variability of nutrient concentration in marine biofilm developed on acrylic panels. *Journal of Experimental Marine Biology and Ecology*, **379**(1-2): 1-7.

Zhang, X.Y., Hao, H.L., Lau, S.C.K., Wang, H.Y., Han, Y., Dong, L.M. and **Huang, R.M. (**2019). Biodiversity and antifouling activity of fungi associated with two soft corals from the South China Sea. *Archives of Microbiology*, **201**: 757-767.

النشاط المضاد للحشف للبكتيريا Epibiotic المرتبطة بالشعاب المرجانية الرخوة *Sarcophyton* sp. جمعت من وسط البحر الأحمر

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المستخلص. تعتبر المجتمعات الميكروبية المرتبطة باللافقاريات البحرية أنها مصدر غزير من المستقبلات النشطة بيولوجيا. هذه الدراسة تطرقت إلى البكتيريا المصاحبة للشعاب المرجانية الناعمة، والتي تم جمعها من البحر الأحمر بحثًا عن نشاط مضاد للحشف لتحديدها المركبات الطبيعية المضادة للحشف. أظهر مستخلص سلالة بكتيريا (*Ruegeria lacuscaerulensis*) KAU-MB3 المرتبط بالشعاب المرجانية الناعمة نشاطًا قويًا مضادًا للحشف في الاختبارات المعملية، حيث يقلل المستخلص البكتيري الخام من نمو البكتيريا المكونة للغشاء الحيوي ويثبط تشكيل biofilm بشكل ملحوظ. وأشارت مقايسة تسوية يرقات البرنكل إلى انخفاض كبير في تسوية اليرقات المعالجة بالمستخلص الخام. كما تم تحليل المستخلص الخام بواسطة GC- MS لفهم التركيب الكيميائي. وأظهرت النتائج وجود مركبات مثل: hexadecane و octatriacontyl pentafluoropropionate وcis-Z-à-bisabolene epoxide وgeranyl isovalerate. ختامًا أشارت هذه الدراسة إلى أن البكتيريا المصاحبة لشعاب الناعمة يمكن استخدامها كأحد المصادر المحتملة لمضادات التلوث الطبيعية.

الكلمات المفتاحية: الشعاب المرجانية الناعمة، التعايشين البكتيري، الحشف الحيوي، مركبات نشطة بيولوجيا، علم البيئة الميكروبي، البحر الأحمر.

JKAU: Mar. Sci., **Vol. 31** No. 2 pp: 15-29 (1442 A.H. / 2021 A.D.)
DOI :10.4197/Mar.31-2.2

Abundance and Diversity of Early Stages of Shorefishes in Jeddah, Red Sea, Saudi Arabia

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Abstract. The composition of fish larval community was studied in Jeddah coast from January to December 2020. Larvae were collected from Obhur Creek and Shaara Bay on Jeddah coast twice a month using light traps that deployed for 2 hours after sunset at the new moon and the full moon. A total of 40 samples yielding 5717 larvae with an average of 1429 larvae/2h belonging to 32 fish families were collected from the two bays. The highest abundance was recorded in Shaara Bay (3413 larvae forming 59.7% of total larvae), whereas Obhur Creek recorded 2304 larvae (40.3% of total larvae). During the present work, the most abundant fish families were Clupeidae, Gobiidae, Scaridae, Pomacentridae, and Blennidae that collectively constituted about 86% of the total larvae. Family Clupeidae was the most dominant one constituted approximately 57% of total larvae followed by Gobiidae (1093 larvae) that formed 19.1% of the total larvae. Much more larvae and more families were collected during the new moon suggesting that settlement of reef fishes mostly takes place in the darker new moon than full moon periods.

Keywords: Fish larvae, light trap, settlement, Red Sea.

1. Introduction

The Red Sea is unique water, hosting some of the most productive and diverse coral reefs. Coral reefs are one of the world's most diverse ecosystems, with fish as an essential component (Maaty *et al*., 2021). Marine fish stocks are essential in the world food system and are particularly important for many of the poorest people in the globe (Hilborn *et al*., 2020; Maaty *et al*., 2021). The study of coral reef fish reproduction, as well as the ecology and biology of their early life stages, is critical for fish stock management (Fuiman 2002; Abu El-Regal 2013, 2017; Abu El-Regal *et al*., 2019). It is also important to understand population dynamics and ecological functioning (Sale 2004). However, the great biodiversity of coral reef fish and the large number of fish larvae recruited make it a difficult area of research. (Robitzsch and Berumen 2020). The Red Sea is a closed and relatively, isolated tropical sea, with little change in environmental conditions. The Red Sea system has very few atmospheric changes such as rainfall and cloudy days and lacks any significant tides 20 cm at its center (Pugh *et al.*, 2019). Fish eggs and larvae represent the meroplanktonic stages of fishes that are found mainly in the upper 200 meters of the water column and can be collected by planktonic gears. They can be utilized to determine the regional distribution of fishes since they have a wider range than their reef stationary adult stages (Leis 1986; Leis and McCormick 2002; Sale 1980, 2002). They are also used to estimate the commercial fishes' spawning stock, spawning seasons, and spawning sites. Because a simple plankton net may sample numerous species across large distances, determining the quantity of eggs and larvae in an area is generally less expensive

than collecting the adults. Furthermore, the plankton samples contain not only fish larvae but also a portion of their prospective zooplanktonic prey and predator (Smith and Richardson, 1977). Zooplankton is the first food item for virtually all fish larvae, as well as many plankton-eating adult fishes, when they transition from their yolk sacs to external feeding. Natural (e.g., current fluctuations, climate change, *etc*.) and human (e.g., growing pollution, river dams, *etc*.) influences can alter zooplankton, and hence fish larvae survival, and thus fisheries resources. The behavior of reef fish larvae may affect general trends in the dispersal and recruitment of reef fish. The orientation cues of the larvae, the swimming ability, and the vertical distribution of fish larvae in the water column may influence their ability to return to the natal reef. The horizontal distribution of fish larvae has been extensively investigated (Stobutzki and Bellwood 1994, 1997; Leis and Carson-Ewart 1997, 1998; Fisher *et al*., 2000; Fisher and Bellwood 2001). On the other hand, the vertical distribution and migration behavior of reef fish larvae has received less attention (Leis 1991). Assessing the early life cycle requires efficient sampling methods for fish larvae; different gears work for different habitats and often choose different sizes or life stages.

Despite a large number of various plankton nets and trawls, opening-closing mechanisms for discrete depth hauls, and environmental sensing systems, the open water ichthyoplankton methods are poorly suited over complex reef structures because rocks and corals are dangerous for research vessels and dragged sampling gear. Different methods are used for sampling fish larvae around complex reef environments. These methods include diver-steered plankton tows (Marliave 1986), diver-pushed nets (Smith *et al*., 1987), visual censuses (Kingsford and Choat 1989), free-fall nets (Kobayashi 1989), and night-lighting (Dennis *et al*., 1991; Victor 1991). Of these, towing nets and light traps have been used to describe the vertical distribution of reef fish larvae in the field. The study of the vertical distribution of larval fishes around Lizard Island (Leis 1986, 1991) found that reef fish exhibit taxon-specific vertical distribution patterns that can change during ontogenesis. In the Red Sea, towed plankton nets of different mesh size have been extensively used to study the abundance and distribution of fish larvae around reefs (Abu El-Regal *et al*., 2008; Abu El-Regal *et al*., 2019; Abu El-Regal 2013). However, the problem with towed net methods is that most larvae were small and preflexion while the late-stage larvae, most likely to return to reefs, are always under-estimated (Doherty 1987; Choat *et al*., 1993; Abu El-Regal, 2000, 2008; Leis *et al*., 2002). Information about the vertical distribution of postlarvae will lead to a better understanding of the settlement processes and consequently the recruitment success of reef fish.

Night lighting (catching fish larvae by light at night) is an unusual collecting technique that is particularly effective at capturing larvae over the reef right before settlement. Although no convincing explanation exists for this behavior, competent fish larvae are greatly attracted to lights at night, a trait they share with moths and squid (Victor, 1991). Light traps have been extensively used near reefs for sampling larvae reef fish while settling to the reef in the Red Sea (Froukh 2001; Fricke and Abu El-Regal 2017a&b; Abu El-Regal and Kon 2019; Robitzsch and Berumen 2020). They have been used to investigate the vertical distribution of reef fish larvae in the field (Doherty and Carleton 1997; Hendriks *et al*., 2001). These studies have shown that there are clear differences between taxa although many taxa are abundant in the surface. Little is known about the vertical distribution of tropical shore fish larvae (Leis 1978, 1986, 1991; Liew 1983; Robison 1985). These studies all agree that there are taxon-specific vertical distribution patterns that appear to have little spatial or temporal variations, except for day/night fluctuations. Since a few species were studied in more than one study, it is uncertain whether the trends are the same

for related taxa. The purpose of this study is to examine the settlement of larvae of shore fish (mainly reef fish) in the relatively shallow (30 m), sheltered waters of two lagoons during the new and full moons.

2. Material and Methods

2.1 Study Sites

Plankton samples were collected from two coastal bays (Obhur Creek and Shaara Bay) using a light trap on the Jeddah coast, Saudi Arabia, Red Sea. Obhur Creek (21° 42′ 30.8″ N, 39° 05′ 48.3″ E) is a 9.2 km long natural cut in the coralline limestone of the Tihama coastal plain that flows into the Red Sea through a narrow 264 m wide exit at the south-western end (Basaham and El-Sayed 2006). It has a depth of around 50 meters at the mouth, which rapidly diminishes towards the north-eastern tip until it is less than 6 meters deep at the terminus (Basaham and El-Shater 1994). It is a popular tourist destination that attracts a large number of tourists each year and offers mooring services for yachts as well as leisure activities (Fig. 1). The second sampling site is Shaara Bay, which is roughly 100 kilometers south of the first (21° 05′ 28.3″ N and 39° 05′ 48.1″E). This location is a coastal coral reef lagoon with a short fringing reef separating it from the beach (Fig. 2). The lagoon's depth ranges from one to five meters. The lagoon has a soft bottom with a few spots of seagrass and coral.

2.2 Fieldwork

This study was conducted for a year (12 months, 2020), where samples were collected twice a month at the new moon and the full moon from Obhur Creek Bay and Shaara Bay. The net was deployed in the water about two meters from the shoreline at night an hour after the sunset for two hours (6:00 pm) yielding two samples/night. The plankton samples were fixed in 90% ethanol immediately after collection for further examination in the laboratory. Due to the Covid-19 pandemic, it was difficult to take samples in April and May.

2.3 Laboratory Work

Fish larvae were sorted under a stereomicroscope then preserved in 90% ethanol. The sorted larvae were separated into their respective families and were identified to the lowest taxon and the larvae of each taxon were enumerated and then photographed under the microscope using AMOS camera. The identification of larvae was based on the literature and the expertise of other scientists, because the literature on the Red Sea fish larvae is very rare, we had to use the closest Indo-Pacific larval fish guides. The literature used for the identification of larvae in this study were Leis and Rennis (1983), Leis and Trnski (1989), Leis and Carson-Ewart (2002), Abu El-Regal (1999, 2017), Leis and Carson-Ewart (2004) and Froukh (2001).

3. Data Analysis

The monthly abundance of the larvae of each taxon was the mean of the standardized number of larvae at all positive stations for individual species. The univariate statistics were done in SPSS 22, using ANOVA to determine differences in number of individuals and number of species between months and sites. All data were tested for homogeneity of variance. Where the samples were not homogeneous, data were either transformed or the non-parametric Kruskal-Wallis test was used (Zar, 1999; Dytham, 2003). The multivariate technique cluster analysis, to determine similarities between sites and months, and diversity indices (species richness, the evenness and Shannon-Wiener), were calculated using PRIMER (Plymouth Routines in Multivariate Ecological Research) v 5.2. Similarity percentage analysis (SIMPER) was used to determine the dominant taxa in each seasonal grouping. All graphs were illustrated using GraphPad prism 8.

Fig. 1. Obhur Creek where the fish larval samples were taken by light trap.

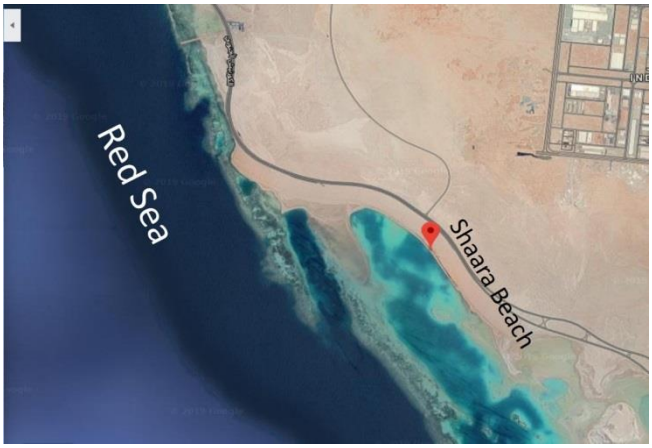

Fig. 2. Shaara Bay where fish larval samples were taken by light trap.

4. Results

4.1 Total Number of Fish Larvae

Throughout a year of sampling, a total of 5717 larvae with an annual average of 1429 larvae/2h belonging to 32 fish families were collected from the two bays. The highest number of fish larvae (3413, 59.7% of total larvae) was recorded from Shaara Bay, whereas Obhur Creek recorded 2304 larvae that constituted 40.3% of all larvae (Table 1). Regarding the monthly distribution in both sites, the highest number of larval fish was taken in July with 2486 larvae followed by September (631), whereas the lowest one was collected in December with 141 larvae (Fig. 3-4). Regardless to site, the highest number of fish larvae from both sites was found during the new moon with 3618 larvae (63.3% from the total fish larvae) compared to 2099 larvae (36.7%) during the full moon (Fig. 5-8). Furthermore, the maximum abundance in a single sample was observed in July at Shaara Bay at the new moon with 1647 larvae/2h (constituted 28.8% of total larvae), followed by Shaara Bay in the same month at the full moon (518 larvae/2h, 9% of all larvae). On the other hand, the minimum abundance of fish larvae was recorded in June at Obhur Creek at the new moon with only three larvae (0.05%). The analysis of variance (one-way ANOVA) showed that there is no significant difference between sites, months, or moon phases regarding the abundance and number of fish families. However, the two-way ANOVA showed that abundance and number of fish families varied significantly at phase moon in certain months. Moreover, abundance and number of fish larval families varied significantly in some sites ate certain months.

In general, the highest number of fish larvae families was collected from Shaara Bay at the new moon (24 families) followed by Obhur Creek Bay at the new moon (20 species). On the other hand, the lowest number of fish larval families was found in Shaara at a full moon and Obhur Creek full moon with only 15 and 16 families respectively (Table 2, Fig. 8). The highest number of fish families was recorded in March with 18 families, whereas the lowest number of families was found in January (six families). Shaara Bay harbored the highest number of families in March at the new moon (13 families) and in August at the full moon (10 species) (Table 2).

4.2 Diversity Indices

The highest richness of species fish larvae was observed in Shaara Bay (3.0) at the new moon, whereas the lowest richness of species fish larvae was recorded in Shaara Bay (2.0) at the full moon. The evenness reached its maximum in Shaara Bay (0.6) at the full moon, and its minimum value in Shaara Bay (0.3) at the new moon. The highest Shannon-Wiener diversity index was recorded in Shaara Bay (1.5) at the full moon, followed by Obhur Creek (1.4) at the new and full moon (Fig. 9). On the other hand, the Shannon index attained its lowest value in Shaara new moon (0.9). Richness values varied from 0.95 in January to 2.82 in March and the evenness varied between the minimum value in September (0.27) to the maximum value in December (0.75). The Shannon diversity index attained its maximum in August and December (1.81), and its lowest value in September (0.60) (Fig. 10).

4.3 Species Composition

The fish larval community collected during the present study was dominated by larvae of the family Clupeidae where they of constituted approximately 57% of all larvae. The most abundant five fish families, Clupeidae, Gobiidae, Scaridae, Pomacentridae, and Blennidae constituted about 86% of all larval fish collected during the present work. Gobiidae was the second most abundant family with 1093 constituting 19.1% of all larvae. With 249 larvae, family Scaridae was the third most abundant group (4.4%). On the other hand, larvae of the family Bythitidae showed the lowest abundance with two larvae (0.03%) (Tables 3 & 4; Fig. 11-13).

Table 1. Monthly variation in total number of fish larvae at different sites.

Month	Shaara New Moon	Shaara Full Moon	Obhur New Moon	Obhur Full Moon	Total
Jan	58	10	81	43	192
Feb	16	173	8	19	216
Mar	253	20	116	19	408
Jun	45	0	305	3	353
Jul	1647	518	267	54	2486
Aug	19	116	185	97	417
Sep	14	7	61	549	631
Oct	97	94	63	51	305
Nov	174	37	92	265	568
Dec	101	14	16	10	141
Total	**2424**	**989**	**1194**	**1110**	**5717**

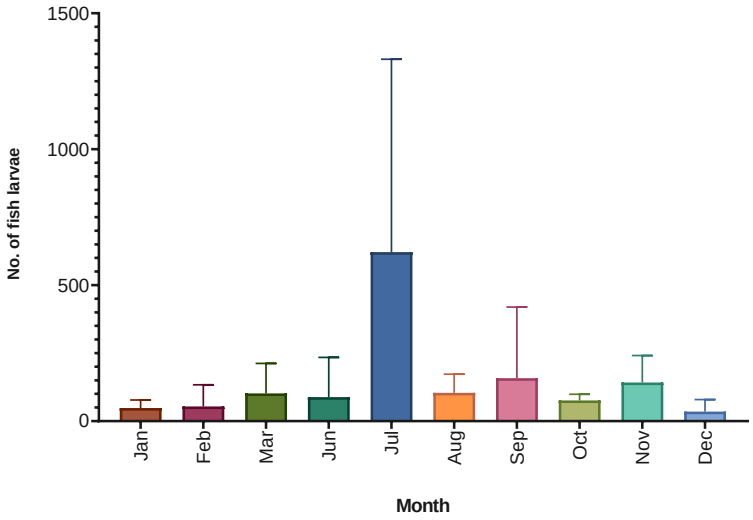

Fig. 3. Monthly average number of fish larvae collected by light trap from both studied sites.

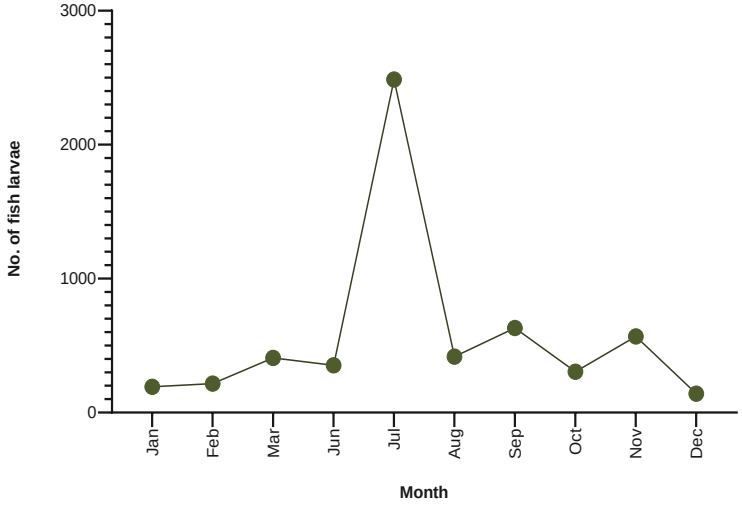

Fig. 4. Average of monthly number of fish larvae collected from both studied sites.

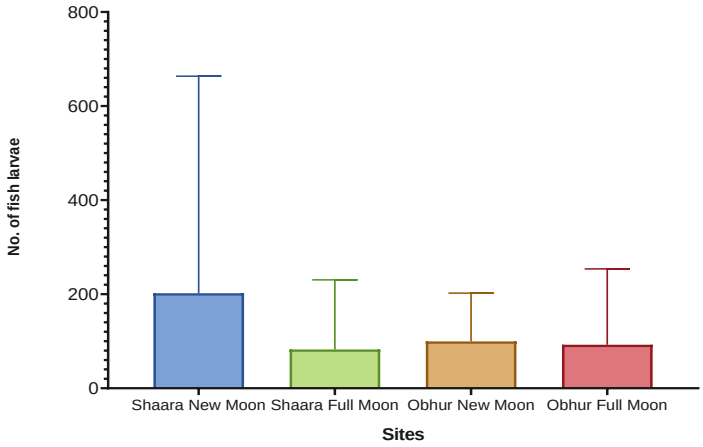

Fig. 5. Average number of fish larvae collected by light trap from both studied sites.

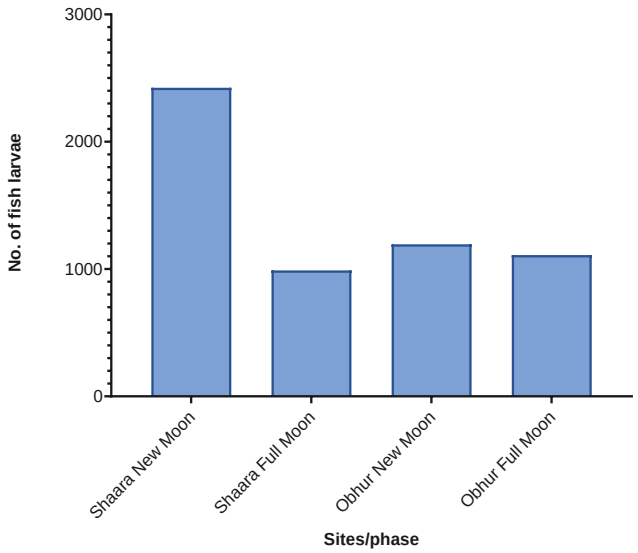

Fig. 6. Total number of fish larvae collected from different sampling sites.

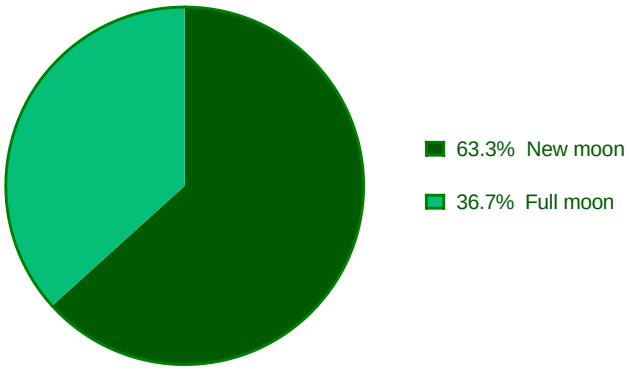

Fig. 7. Percentage contribution of fish larvae in both new and full moon.

Table 2. Total number of families of fish larvae at different sites and month.

Month	Shaara New Moon	Shaara Full Moon	Obhur Creek New Moon	Obhur Creek Full Moon
Jan	4	2	2	4
Feb	3	2	3	4
Mar	13	5	6	2
Jun	5	0	8	1
Jul	2	5	6	5
Aug	4	10	6	6
Sep	2	3	4	5
Oct	8	4	3	6
Nov	9	3	8	9
Dec	7	3	5	4

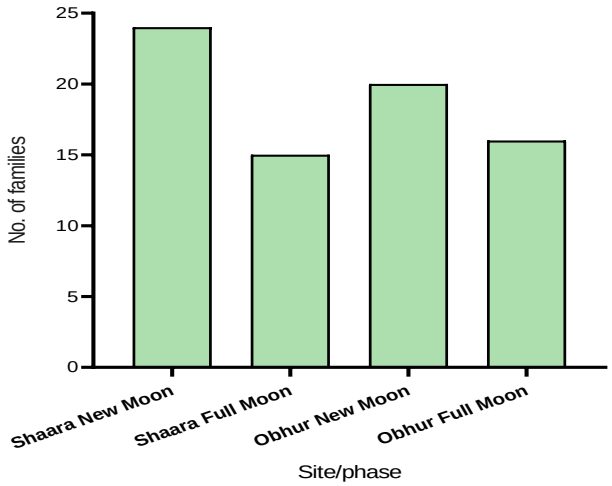

Fig. 8. Number of families in the different sites and moon phases.

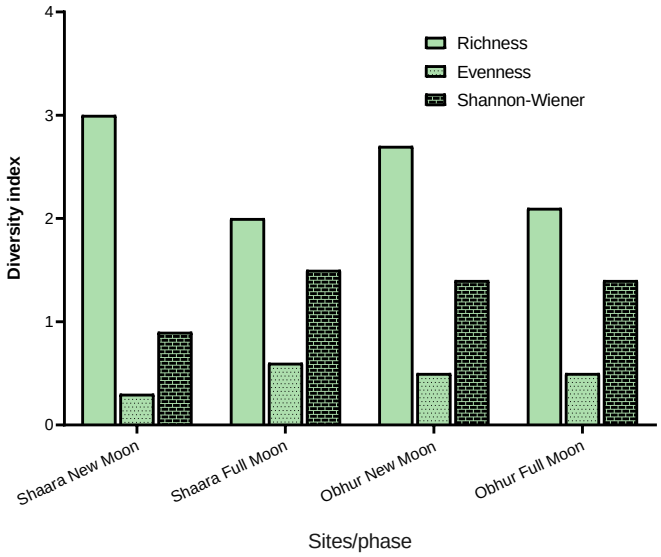

Fig. 9. Variations of diversity index; richness, evenness and Shannon-Wiener at different sites in Jeddah, Red Sea.

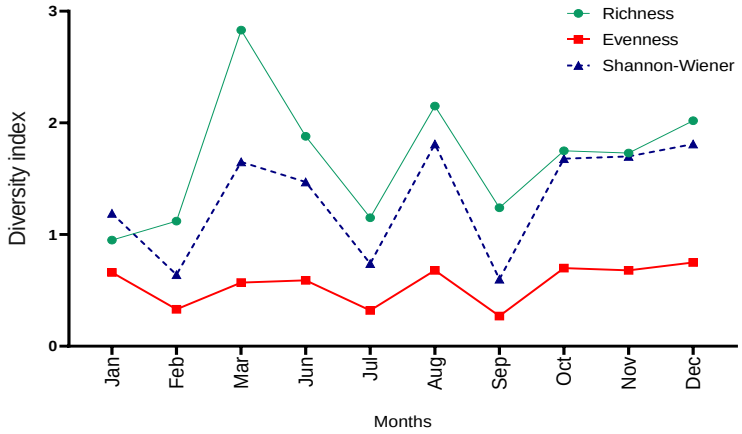

Fig. 10. Monthly variation of diversity index; richness, evenness and Shannon-Wiener of fish larvae.

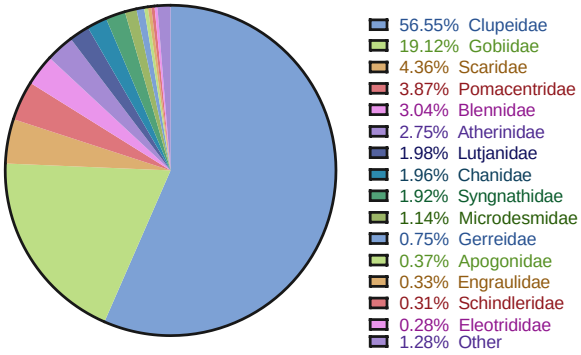

Fig. 11. Percentage contribution of fish families to the larval fish community in Jeddah.

Table 3. Monthly number of fish larvae recorded at different sites in the area of study.

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Clupeidae	83	178	158	-	-	61	1984	111	512	44	62	40	3233
Gobiidae	80	27	142	-	-	134	260	98	104	90	130	28	1093
Scaridae	3	2	1	-	-	11	0	7	0	8	217	0	249
Pomacentridae	4	5	5	-	-	120	4	0	2	3	78	0	221
Blennidae	6	1	7	-	-	1	135	12	4	0	6	2	174
Atherinidae	0	0	12	-	-	0	2	53	1	84	5	0	157
Lutjanidae	0	0	2	-	-	0	3	0	5	61	42	0	113
Chanidae	0	0	0	-	-	0	0	105	0	3	4	0	112
Syngnathidae	0	2	2	-	-	10	91	4	0	0	1	0	110
Microdesmidae	0	0	0	-	-	1	0	3	0	2	19	40	65
Gerreidae	0	0	43	-	-	0	0	0	0	0	0	0	43
Apogonidae	0	1	7	-	-	1	2	2	0	3	0	5	21
Engraulidae	0	0	9	-	-	0	1	6	0	3	0	0	19
Schindleriidae	16	0	2	-	-	0	0	0	0	0	0	0	18
Eleotrididae	0	0	7	-	-	0	0	0	0	4	0	5	16
Mugilidae	0	0	0	-	-	0	0	0	0	0	0	10	10
Haemulidae	0	0	1	-	-	0	0	8	0	0	0	0	9
Soleidae	0	0	0	-	-	8	0	0	0	0	0	0	8
Albulidae	0	0	2	-	-	1	4	0	0	0	0	0	7
Pempheridae	0	0	5	-	-	0	0	0	0	0	0	1	6
Belonidae	0	0	0	-	-	0	0	0	0	0	0	6	6
Mullidae	0	0	0	-	-	0	0	0	0	0	3	0	3
Labridae	0	0	1	-	-	0	0	1	1	0	0	0	3
Carangidae	0	0	2	-	-	0	0	0	0	0	0	0	2
Acanthuridae	0	0	0	-	-	0	0	0	1	0	0	0	1
Synodontidae	0	0	0	-	-	0	0	0	0	0	1	0	1
Gobiesocidae	0	0	0	-	-	0	0	0	0	0	0	1	1
Hemiramphidae	0	0	0	-	-	0	0	1	0	0	0	0	1
Scorpaenidae	0	0	0	-	-	0	0	0	1	0	0	0	1
Sphyraenidae	0	0	0	-	-	0	0	6	0	0	0	0	6
Tripterygiidae	0	0	0	-	-	3	0	0	0	0	0	3	6
Bythitidae	0	0	0	-	-	2	0	0	0	0	0	0	2
Total	**192**	**216**	**408**	**-**	**-**	**353**	**2486**	**417**	**631**	**305**	**568**	**141**	**5717**

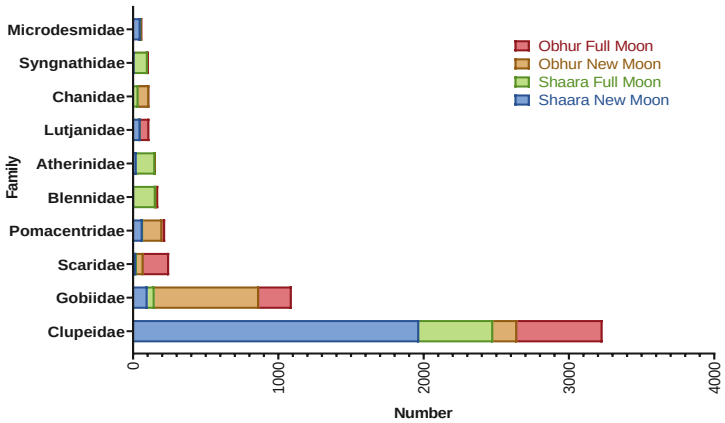

Fig. 12. Abundance and distribution of the most abundant fish families at sites and moon phase.

Table 4. Total number of different families of fish larvae at studied sites.

Family	Shaara New Moon	Shaara Full Moon	Obhur New Moon	Obhur Full Moon	Total
Clupeidae	1970	510	165	588	3233
Gobiidae	101	49	718	225	1093
Scaridae	22	5	47	175	249
Pomacentridae	67	3	132	19	221
Blennidae	8	149	6	11	174
Atherinidae	26	128	2	1	157
Lutjanidae	52	0	3	58	113
Chanidae	5	34	73	0	112
Syngnathidae	11	92	0	7	110
Microdesmidae	53	4	3	5	65
Gerreidae	43	0	0	0	43
Apogonidae	9	0	9	3	21
Engraulidae	12	6	1	0	19
Schindleridae	1	0	17	0	18
Eleotrididae	5	0	7	4	16
Mugilidae	10	0	0	0	10
Haemulidae	0	2	2	5	9
Soliedae	8	0	0	0	8
Albulidae	2	2	2	1	7
Pempheridae	3	3	0	0	6
Belonidae	6	0	0	0	6
Mullidae	2	0	1	0	3
Labridae	0	0	2	1	3
Carangidae	2	0	0	0	2
Acanthuridae	0	0	1	0	1
Synodontidae	1	0	0	0	1
Gobiesocidae	0	0	1	0	1
Hemirhamphidae	0	1	0	0	1
Scorpaenidae	0	0	0	1	1
Sphyraenidae	5	1	0	0	6
Tripterygiidae	0	0	0	6	6
Bythitidae	0	0	2	0	2
Total	**2424**	**989**	**1194**	**1110**	**5717**

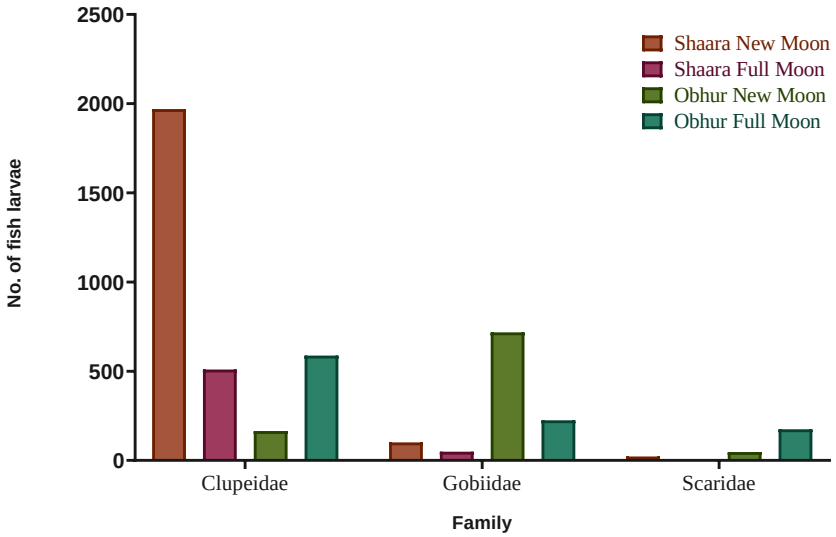

Fig. 13. Distribution of the most abundant fish families in different sites and moon phases.

5. Discussion

Most marine fishes have a planktonic larval stage (Llopiz and Cowen 2009), which is commonly referred to as ichthyoplankton and can be sampled quantitatively with plankton gear. While there is a variety of active and passive sampling equipment available (Neal *et al*., 2012), the applicability of each is debatable. Ichthyoplankton of reef fishes in the Red Sea is poorly studied compared to that in other Indo-Pacific areas such as Great Barrier Reef (Abu El-Regal *et al.*, 2014 a, b).

The identification of the mechanisms that determine patterns of distribution and abundance of early stages of fish is an essential goal of ecology and conservation biology. In species that experience ontogenetic changes in habitat utilization, patterns of early life-stage dispersal, return (i.e. settlement), and persistence in adult habitats (i.e. recruitment) can have an impact on population dynamics and connectivity (Besson *et al.*, 2017). To maximize output that ensure sustainability and fishery management requires an understanding of diversity and distribution of ichthyoplankton. The larval source, supply, and recruitment patterns of reef-dependent fish can considerably influence the adult population dynamics of reef-dependent fish on both natural (Sponaugle and Shaw 2003) and artificial reefs (Hernandez and Shaw 2003).

The vertical distribution of late stages of reef fish larvae may potentially influence their dispersal, recruitment success, and energetic expenditure during the recruitment process. To date, methods of examining the vertical distribution of reef fish larvae either under-sample late-stage individuals or are incapable of discretely sampling the water column (Fisher and Bellwood 2002). The majority of fish species' pelagic phase is poorly understood. Many species' larvae have been taken close to shore, while others have been recovered from large distances offshore (Abu El-Regal *et al*., 2014a, b; Maaty 2015). Some studies have been carried out on the species composition of larval fish inshore and offshore (Clark 1991; Leis 1986, 1991; Maaty 2015; Abu El-Regal 2017). Despite their relevance in fisheries management, there are very few research on Red Sea reef fish larvae (Abu El-Regal *et al.*, 2014b; Maaty 2015).Because of its intimate ties to fisheries, ichthyoplankton research has been more relevant since the turn of the century. Studies on fish population dynamics and the reasons of big variations in

fish stock production have aided in establishing a better knowledge of fish population dynamics and pinpointing the causes of major fluctuations in fish stock production (Fuiman 2002).

Studies on ichthyoplankton have become important since the beginning of last century in view of its close relationship with fisheries. Studies on the early life history of fish have been useful in developing a better understanding of fish population dynamics and determining the causes of major fluctuations in fish stock production (Fuiman 2002). Data about where and when larvae of these commercial fish could be very helpful in the determination of their spawning seasons and grounds and hence management of their fisheries of the Red Sea (Abu El-Regal *et al.*, 2014 a, b). Larvae of many commercial fishes were collected during the present study. Families such as Mullidae, Lutjanidae, Scaridae, Carangidae, Sphyraenidae, Gerridae and Serranidae are represented in the current samples by larvae indicating nearby spawning grounds. This study presents important information on the spawning seasons and spawning grounds of these fishes that form baseline data concerning the larvae of commercial fishes as an essential part in fisheries management. However, the larvae of some fishes whose adults are important constituents of Jeddah fisheries were rare or even absent in the collection. This may be due to the adult or the larvae behavior (Leis 1991; Montgomery *et al*., 2001). Larvae of lethrinid fishes were absent in the ichthyoplankton samples during the period of study and other studies in the Red Sea (Abu El-Regal 1999, 2008, 2017; Maaty 2015; Abu El-Regal *et al.*, 2014 a, b; 2019). Lunar periodicities were investigated because there are many hypotheses on lunar reproductive patterns pertaining to propagule dispersal and predation rates that occur both at the beginning (spawning) and end (settlement) of the planktonic phase (Robertson, 1991). Many reef fish appear to time their spawning events with different lunar cycles (Thresher, 1984). Higher rates of fish settlement often occur during darker, new moon periods than full moon periods (Victor 1986; Rooker *et al*., 1996), presumably a response to mortality associated with visual predators. These patterns of spawning, transport, recruitment, and settlement in association with the local physical oceanographic regime, often result in variable larval supply and settlement patterns with distinct lunar periodicities.

In the current study, most of the larvae were collected during the new moon nights in comparison to the full nights. Approximately, two-thirds (63%) of all larvae were collected in the new moon nights. Furthermore, most of the larvae were recorded in Shaara Bay (about 75%). Species composition varied significantly among Shaara, Obhur in the new and full moon nights. The number of clupeid larvae in the full moon nights is almost half that of the clupeid collected in the new moon nights. Hemirhamphidae, Tripterygiidae, and Scorpaenidae are absent in the new moon and occurred in the full moon. On the other hand, larvae of 11 fish families occurred only dark in the new moon nights. These include Acanthuridae, Synodontidae, Gobiesocidae, Carangidae, Bythitidae, Mullidae, Belonidae, Soliedae, Mugilidae, Schindleridae and Gerreidae. Settlement of reef fishes mostly takes place in the darker new moon than full moon periods.

References

Abu El-Regal, M. A. (1999). Some biological and ecological studies on the larvae of coral reef fishes in Sharm El-Sheikh (Gulf of Aqaba-Red Sea) *M. Sc. Thesis*. Marine Science Department, Faculty of Science Suez Canal University). 200 p.

Abu El-Regal, M. A. (2013). Spawning seasons, spawning grounds and nursery grounds of some Red Sea fishes. *The Global Journal of Fisheries and Aqua*, **6**(6): 105-125.

Abu El-Regal, M. A. (2017). Spatial and temporal distribution of larvae of coral reef fishes in northern Red Sea, Egypt. *Iranian Journal of Fisheries Sciences*, **16**(3): 1043-1062.

Abu El-Regal, M. A. and **Kon, T.** (2019). First record of the Schindler's fish, Schindleria praematura (Actinopterygii: Perciforms: Schindleriidae), from the Red Sea. *Acta Ichthyologica et Piscatoria*, **49**(1).

Abu El-Regal, M. A., Ahmed, A. I., El-Etreby, S. G., El-Komi, M. and **Elliott, M.** (2008). Abundance and diversity of coral reef fish larvae at Hurghada, Egyptian Red Sea. *Egyptian Journal of Aquatic Biology and Fisheries*, **12**(2): 17-33.

Abu El -Regal, M. A., Abu Zeid, M. M., Hilal, A. and **Maaty, M. M.** (2014a). Abundance and diversity of reef fish larvae in Mabahiss Bay, on the Egyptian Red Sea coast. *Egyptian Journal of Aquatic Biology and Fisheries*, **18**: 61 -77.

Abu El-Regal, M. A., Abu Zeid, M.M., Hilal, A. and **Maaty, M. M.** (2014b). Adult and larval fish assemblages in Mabahis Bay, northern Red Sea, Egypt. *International Journal of Environmental Science and Engineering*, **5**: 111 -126.

Abu El-Regal, M. A., Maaly, M. A. and **Abd EL-Naby, A. S.** (2019). Investigations on larvae of commercial fish from Hurghada, Red Sea with notes on the spawning seasons and grounds of some species. *Iranian Journal of Fisheries Sciences*, **18**(3): 476-496.

Basaham, A. S. and **El-Sayed, M. A.** (2006). Sharm Obhur: environmental consequences of 20 years of uncontrolled coastal urbanization. *Marine Scienes*, **17**(1).

Basaham, A. S. and **El-Shater, A.** (1994). Textural and mineralogical characteristics of the surficial sediments of Sharm Obhur, Red Sea coast of Saudi Arabia. *Marine Scienes*, **5**(1).

Besson, M., Gache, C., Brooker, R. M., Moussa, R. M., Waqalevu, V. P., LeRohellec, M. and **Lecchini, D.** (2017). Consistency in the supply of larval fishes among coral reefs in French Polynesia. *PLoS One*, **12**(6): e0178795.

Choat, J.H., Doherty, P.J., Kerrigan B.A. and **Leis, J.M.** (1993). A comparison of towed nets, purse seine, and light-aggregation devices for sampling larvae and pelagic juveniles of coral reef fishes. *Fish. Bull.* (US), **91**: 195-209.

Clark, W. G. (1991). Groundfish exploitation rates based on life history parameters. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**(5): 734-750.

Dennis, G.D., Goulet, D. and **Rooker, J.R.** (1991). Ichthyoplankton assemblages sampled by night lighting in nearshore habitats of southwestern Puerto Rico. In: R.D. Hoyt (Editor), *Larval Fish Recruitment and Research in the Americas*. NOAA Technical Report NMFS, **95**: 89-97.

Doherty, P. J. (1987). Light-traps: Selective but useful devices for quantifying the distributions and abundances of larval fishes. *Bulletin of Marine Science*, **41**(2): 423-431.

Doherty, P. P. and **Carleton, J. J.** (1997). The distribution and abundance of pelagic juvenile fish near Grub Reef, central Great Barrier Reef. In: *Proceedings of the 8th International Coral Reef Symposium*, Panama, 24-29 June 1996-pages: 2: 1155-1160. Smithsonian Tropical Research Institute.

Fisher, R. and **Bellwood, D.** (2001). Effects of feeding on the sustained swimming abilities of late-stage larval Amphiprion melanopus. *Coral Reefs*, **20**(2): 151-154.

Fisher, R. and **Bellwood, D.** (2002). A light trap design for stratum-specific sampling of reef fish larvae. *Journal of Experimental Marine Biology and Ecology*. **296** (1): 27-37

Fisher, R., Bellwood, D. R. and **Job, S. D.** (2000). Development of swimming abilities in reef fish larvae. *Marine Ecology Progress Series*, **202** : 163-173.

Fricke, R. and **Abu El-Regal, M. A.** (2017a). *Schindleria nigropunctata, a new species of paedomorphic goby from the Red Sea* (Teleostei: Schindleriidae). Marine Biodiversity. https://doi.org/10.1007/s12526-017-0831-z. 1-5.

Fricke, R. and **Abu El-Regal, M. A.** (2017b). Schindleria elongata, a new species of paedomorphic goby from the Red Sea (Teleostei: Schindleriidae). *Journal of Fish Biology*. 1-8 doi:10.1111/jfb.13280.

Froukh, T.J. (2001). Studies on taxonomy and ecology of some fish larvae from the Gulf of Aqaba. *M.Sc. Thesis*. Faculty of Graduate Studies. University of Jordan. 103 pp.

Fuiman, L. A. (2002). *Special consideration on fish eggs and larvae*. Fishery science: The unique contributions of early life stages, 1-32.

Hendriks, I., Wilson, D. and **Meekan, M.** (2001). Vertical distributions of late stage larval fishes in the nearshore waters of the San Blas Archipelago, *Caribbean Panama. Coral Reefs*, **20**(1): 77-84.

Hernandez, F. J. and **Shaw, R. F.** (2003). Comparison of plankton net and light trap methodologies for sampling larval and juvenile fishes at offshore petroleum platforms and a coastal jetty off Louisiana. In : *American Fisheries Society Symposium* (pp. 15-38). American Fisheries Society.

Kingsford, M. J. and **Choat, J. H.** (1989). Horizontal distribution patterns of presettlement reef fish: are they influenced by the proximity of reefs? *Marine Biology*, **101**(3): 285-297.

Kobayashi, D. R. (1989). Fine-scale distribution of larval fishes: patterns and processes adjacent to coral reefs in Kaneohe Bay, *Hawaii*. *Marine Biology*, **100**(3): 285-293.

Leis, J. M. (1978). Systematics and zoogeography of the porcupinefishes (Diodon, Diodontidae, Tetraodontiformes), with comments on egg and larval development. *Fish. Bull*, **76**(3) : 535-567.

Leis, J. M. (1986). Vertical and horizontal distribution of fish larvae near coral reefs at Lizard Island, Great Barrier Reef. *Marine Biology*, **90**(4): 505-516.

Leis, J. M. (1991). Vertical distribution of fish larvae in the Great Barrier Reef Lagoon, Australia. *Marine Biology*, **109**(1): 157-166.

Leis, J. M. and **Carson-Ewart, B. M.** (1997). In situ swimming speeds of the late pelagic larvae of some Indo-Pacific coral-reef fishes. *Marine Ecology Progress Series*, **159**: 165-174.

Leis, J. M. and **Carson-Ewart, B. M.** (1998). Complex behaviour by coral-reef fish larvae in open-water and near-reef pelagic environments. *Environmental Biology of Fishes*, **53**(3): 259-266.

Leis, J. M. and **Carson-Ewart, B. M.** (2002). In situ settlement behaviour of damselfish (Pomacentridae) larvae. *Journal of Fish Biology*, **61**(2): 325-346.

Leis, J. M. and **Carson-Ewart, B. M.** (2004). The larvae of Indo-Pacific coastal fish. *Fauna Malesiana handbook*, 2.

Leis, J. M. and **McCormick, M. I.** (2002). *The biology, behavior, and ecology of the pelagic, larval stage of coral reef fishes.* Coral reef fishes: Dynamics and diversity in a complex ecosystem, 171-199.

Leis, J. M. and **Rennis, D. S.** (1983). *The larvae of Indo-Pacific coral reef fishes*. University Press Sydney and university Hawaii press Honolulu. 269p.

Leis, J. M. and **Trnski, T.** (1989). *The larvae of Indo-Pacific shorefishes.* University Press Sydney and university Hawaii press Honolulu in association with Australian Museum. 371p.

Leis, J. M., Carson-Ewart, B. M. and **Cato, D. H.** (2002). Sound detection in situ by the larvae of a coral-reef damselfish (Pomacentridae). *Marine Ecology Progress Series*, **232**: 259-268.

Liew, H. C. (1983). Studies on Flatfish Larvae (fam. Psettodidae and Bothide, Pleuronectiformes) in the Shelf Waters of the Central Great Barrier Reef, Australia, *Doctoral Dissertation*, James Cook University, North Queensland).

Llopiz, J. K. and **Cowen, R. K.** (2009). Variability in the trophic role of coral reef fish larvae in the oceanic plankton. *Marine Ecology Progress Series*, **381**: 259-272.

Maaty, M.M. (2015) Ecological studies on early stages of coral reef fishes and their movement in-between major habitats in Hurghada, Red Sea. *PhD Thesis*. Al-Azhar University. Cairo.

Maaty, M. M., Azab, A. M. and **Desouky, M. G.** (2021). Distribution, abundance and diversity of reef fishes in waters of some cities along the northern Egyptian Red Sea coast, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*, **25**(5): 901 – 918.

Marliave, J. B. (1986). Lack of planktonic dispersal of rocky intertidal fish larvae. *Transactions of the American Fisheries Society*, **115**(1): 149-154.

Montgomery, J. C., Tolimieri, N. and **Haine, O. S.** (2001). Active habitat selection by pre-settlement reef fishes. *Fish and Fisheries*, **2**(3): 261-277.

Neal, J. W., Adelsberger, C. M. and **Lochmann, S. E.** (2012). A comparison of larval fish sampling methods for tropical streams. *Marine and Coastal Fisheries*, **4**(1): 23-29.

Pugh, D. T., Abualnaja, Y. and **Jarosz, E.** (2019). The tides of the Red Sea. In : *Oceanographic and Biological Aspects of the Red Sea* (pp. 11-40). Springer, Cham.

Richards, A. (2005). Big fish in small ponds: The trading behavior and price impact of foreign investors in Asian emerging equity markets. *Journal of Financial and quantitative Analysis*, **40**(1): 1-27.

Robison, R. A. (1985). Affinities of Aysheaia (Onychophora), with description of a new Cambrian species. *Journal of Paleontology*, 226-235.

Robitzsch, V. and **Berumen, M. L**. (2020). Recruitment of coral reef fishes along a cross-shelf gradient in the Red Sea peaks outside the hottest season. *Coral Reefs*, **39**(6): 1565-1579.

Rooker, J. R., Dennis, G. D. and **Goulet, D.** (1996). Sampling larval fishes with a nightlight lift-net in tropical inshore waters. *Fisheries Research*, **26**(1-2): 1-15.

Robertson, D. R. (1991). *The Role of Adult Biology in the Timing of Spawning of Tropical Reef Fishes*. The ecology of fishes on coral reefs, 356-386.

Sale, P. F. (1980). Assemblages of fish on patch reefs-predictable or unpredictable? *Environmental Biology of Fishes*, **5**(3): 243-249.

Sale, P. F. (2002). The science we need to develop for more effective management. *Coral reef fishes: Dynamics and Diversity in a Complex Ecosystem*, 361-376.

Sale, P. F. (2004). Connectivity, recruitment variation, and the structure of reef fish communities. *Integrative and Comparative Biology*, **44**(5): 390-399.

Smith, C. L., Tyler, J. C. and **Stillman, L.** (1987). Inshore ichthyoplankton: A distinctive assemblage. *Bulletin of Marine Science*, **41**(2): 432-440.

Smith, P. E. and **Richardson, S.** (1977). Standard techniques for pelagic. *FAO Fisheries Techniques Paper*, **175** : 27-73.

Stobutzki, I. C. and **Bellwood, D. R.** (1994). An analysis of the sustained swimming abilities of pre-and post-settlement coral reef fishes. *Journal of Experimental Marine Biology and Ecology*, **175**(2): 275-286.

Stobutzki, I. C. and **Bellwood, D. R.** (1997). Sustained swimming abilities of the late pelagic stages of coral reef fishes. *Marine Ecology Progress Series*, **149**: 35-41.

Victor, B. C. (1991). *Settlement strategies and biogeography of reef fishes*. The ecology of fishes on coral reefs, 231-260.

Victor, B. C. (1986). Larval settlement and juvenile mortality in a recruitment-limited coral reef fish population. *Ecological Monographs*, **56**(2), 145-160.

Thresher, R. E. (1984). *Reproduction in Reef Fishes*. TFH Publications, Inc New Jersey. 399 p.

كثافة وتنوع الأطوار المبكرة للأسماك الشاطئية في جدة، البحر الأحمر، المملكة العربية السعودية

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المستخلص. تمت دراسة تكوين مجتمع يرقات الأسماك في ساحل جدة من يناير إلى ديسمبر ٢٠٢٠. حيث جمعت اليرقات من خور أبحر وخليج شعارة على امتداد ساحل جدة مرتان شهريًا باستخدام المصيدة الضوئية لمدة ساعتين بعد غروب الشمس عند القمر الجديد والقمر المكتمل (البدر). تم جمع ٤٠ عينة تضم ٥٧١٧ يرقة بمتوسط ١٤٢٩ يرقة / ساعتين تنتمي لـ ٣٢ عائلة من الأسماك. سجلت أعلى وفرة في خليج شعارة (٣٤١٣ يرقة تشكل ٥٩,٧٪ من مجموع اليرقات)، بينما سجل خور أبحر ٢٣٠٤ يرقة (٤٠,٣٪ من مجموع اليرقات). خلال العمل الحالي، كانت أكثر عوائل الأسماك وفرة، هي: السردين (Clupeidae)، الجوبى (Gobiidae) والحريد (Scaridae)، أبو دفدف (Pomacentridae)، والبلينى (Blennidae) والتي شكلت مجتمعة حوالي ٨٦٪ من إجمالي اليرقات. كانت عائلة السردين (Clupeidae) هي الأكثر تواجدًا بين جميع العوائل حيث شكلت حوالي ٥٧٪ من إجمالي اليرقات تليها عائلة الجوبى (Gobiidae) (1093 يرقة)، والتي شكلت ١٩,١٪ من مجموع اليرقات. تم جمع أكثر اليرقات وأكثر الأنواع خلال القمر الجديد، مما يشير إلى أن استقرار أسماك الشعاب المرجانية يحدث في الغالب في القمر الجديد الأكثر عتمة من فترات اكتمال القمر.

الكلمات المفتاحية: يرقات الأسماك، المصيدة الضوئية، مستوطنة، البحر الأحمر.

JKAU: Mar. Sci., **Vol. 31** No. 2 pp: 31- 43 (1442 A.H. / 2021 A.D.)
DOI :10.4197/Mar.31-2.3

Factors Controlling Seasonal Variability of Sea Level in the Western Gulf of Aden

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Abstract. Daily means sea level has been computed from hourly observations at Aden and Djibouti for the period of 2011–2014 to study seasonal variability in sea level in the western Gulf of Aden. This variability is investigated against the following controlling factors: sea level pressure, wind, and steric sea level. Variability in sea level at Aden and Djibouti shows strong seasonality with higher sea level in winter and lower sea level in summer with a range up to about 35 cm. The high-frequency variability in sea level agrees with variability in pressure, especially during winter following normal inverse barometric relation. Cross-shore wind affects sea level variability in Djibouti more than that in Aden, while along-shore wind plays a significant role in sea level variability at Aden. Daily steric sea level for both stations play a significant role in seasonal sea level anomaly (SLA) variability. Both stations shows that strong signals are the annual and semiannual, while small frequencies are negligible in comparison. For Aden and Djibouti stations, steric sea level is the dominant factor with a determination coefficient (DC) of 0.73, and 0.71, respectively. Along-shore wind has the second higher contribution for Aden with (DC) of about 0.3. The cross-shore wind component has the second highest contribution on Djibouti SLA with a DC of about 0.3, while for Aden, this factor has no effect on SLA. Sea level pressure (SLP) contribution is clearly seen in short-period variability for both stations.

Keywords: Gulf of Aden, Mean Sea Level, Steric Sea Level, Wavelet, Atmospheric forcing.

1. Introduction

The Gulf of Aden is a strategic water body that connects the Red Sea with the Indian Ocean, and it is distinguished by the complexity and variability of its climate and oceanography. As part of the northwestern Indian Ocean, it falls under the control of the monsoon reversal wind regime, which contributes to the formation of various water circulation patterns such as water exchange with the Red Sea and the Arabian Sea, eddy formation, and coastal upwelling. In the winter NE monsoon, winds push the gulf's surface water westward along the axis of the gulf and continue to flow NNW into the Red Sea through the strait of Bab-el-Mandab. During the summer SW monsoon, winds are relatively stronger and reach 20–40 km/h (Sultan and Ahmed, 1997). The surface water of the gulf is pushed eastward under the effect of the SW wind along the Arabian coast and deflected SE; this type of steering causes some eddies to form along the center of the gulf (Alsaafani *et al*., 2007). Other eddies propagate to the gulf from the Arabian Sea (Alsaafani *et al*., 2007; Fratantoni, *et al*., 2006). Due to these eddies, some weak and scattered upwellings occur in a few spots along the Somali coast. Along the Arabian coast, strong upwelling occurs due to the Ekman transport, which pushes surface water offshore and is replaced with subsurface waters; in this season, upwelled waters come from much greater depth (Currie *et al*., 1973). The Gulf of Aden experiences strong evaporation; Ahmad and Sultan (1989)

investigated the evaporation of the southern part of the Red Sea, including Bab-el-Mandab, and found that evaporation is higher in summer and lower in winter. Their annual average of evaporative heat flux in this region was 152 Wm^{-2}. The western Gulf of Aden has a mixed type of tide with similar tidal amplitudes and phases at both stations (Madah, 2020).

Sea level variability is affected by many factors, which operate at different temporal and spatial scales (Bergant, *et al*., 2005). The main variability in sea level in seasonal and interannual variability is related to meteorological and oceanographic forces (Woodworth *et al*., 1999; Tsimplis and Woodworth, 1994). The contribution of the gravitational forces of annual and semiannual frequencies present in the tide-generating potential can be neglected in comparison with meteorological and oceanographic forcing (Bergant, *et al*., 2005; Woodworth *et al*., 1999; Fukumori, *et al*., 1998; Pugh, 1987; Shankar, 2000). Oceanographic factors include circulation, changes in temperature, and salinity and eddies, while atmospheric factors include changes in average air pressure and wind fields. The impact of wind and atmospheric pressure on sea level was extensively globally studied in many areas (Tsimplis and Vlahakis, 1994; Garcia-Lafunte, *et al*., 2004). The most obvious contribution of oceanic factors is the thermosteric effect associated with heat fluxes at the surface layer of the ocean (Cheney *et al*., 1994; Tsimplis and Woodworth, 1994). Low-frequency variations in air pressure and wind fields associated with the seasonal pattern of atmospheric circulation contribute to the variability of sea level through the in-verted barometer response and wind setup, particularly in coastal areas. They also contribute to sea level variability by inducing fluctuations in the mean ocean circulation at both the regional and global scales (Wunsch, 1991).

Studies of sea level changes in the Gulf of Aden are scarce. Most recent studies related to sea level in the Gulf of Aden investigate the sea level rise in the gulf and its relation to global warming (Unnikrishnan and Shankar 2007; Woodworth *et al*. 2009) and climate modes (Alawad *et al*., 2019), or to study the impact of sea level rise on coastal areas (Dasgupta *et al*., 2009; Alsaafani, *et al*. 2015). The study by Patzert (1972) showed that sea level is higher in winter and low-er in summer, with a range up to 33 cm due to the steric effect and along-shore wind stress based on 1879–1893 and 1937–1946 sea level data at the Port of Aden. The study conducted by Cromwell and Smeed (1998) for sea level fluctuations based on altimetric data from TOPEX/Poseidon from 11 November 1993 to 16 January 1997 shows that the dominant cycle is annual with an amplitude of 13 cm and a secondary semiannual cycle with an amplitude of 4–8 cm. They believe that the annual cycle is due to wind forcing, while the semiannual cycle is due to evaporation. The nearest region to the Gulf of Aden is the southern part of the Red Sea, where fluctuations of MSL at Gizan (Jazan) are investigated by (Abdelrahman, 1997). The author reported that MSL was high in winter and low in summer with a range up to 40 cm, and its seasonal changes are affected by steric effects, evaporation rates, and along-shore wind.

However, since no previous studies investigated seasonal variability at Djibouti station, this study used four-year sea level data along with sea level pressure (SLP), wind stress, and the monthly climatology of steric sea level to investigate the annual and short-er-period variability in SLA, and its relation to the controlling parameters at both Aden and Djibouti.

2. Materials and Methods

Time series of sea level from two tide gauge stations; Aden and Djibouti, located in the western Gulf of Aden were used to investigate seasonal variations of sea level in

the western part of the gulf associated with atmospheric forcing and steric sea level. (See Fig. 1 for locations). The daily and monthly mean sea levels were computed from hourly observations that spanned the period of 2011–2014. Tide-gauge data are collected by the Permanent Service for Mean Sea Level (PSMSL) (Holgate *et al*., 2013), and retrieved from http://www.psmsl.org/data/obtaining/.

Hourly sea level data usually have a spectrum that covers all frequencies. Since our interest is in seasonal variability, we followed (Goden, 1972; Alsaafani, *et al*., 2017) to smooth hourly data to eliminate the tidal effect by applying a filter of type $\frac{\propto_{24}^{2} \propto_{25}}{24^{2}25}$, which entails a loss of 70 h of smoothed observations. A sequence of means is first computed for 25 observations; then, a series of means for 24 of these means is repeated twice, and the mean of this last series gives the smoothed values. Daily and monthly sea level values are estimated from the smoothed one.

Sea level pressure (SLP) and wind stress components covering four years from 2011 to 2014 were provided by European Centre for Medium-Range Weather Forecasts (ECMWF) Reanalysis (ERA) (Uppala *et al*., 2005; Dee *et al*., 2011).

The monthly steric sea level has been estimated for Djibouti (43.15 E and 11.61 N) and Aden (44.97 E and 12.79 N) using WOA18 climatology (Abdullah *et al.*, 2019) for the water column from surface till 700 m based on formula of (Gill, 1982), the daily climatology has been interpolated from the monthly climatology. Since the steric sea level has no noticed interannual variability, therefore the daily climatology of steric sea level is repeated for the four years to get similar time series length as the sea level for the wavelet analysis.

For wavelet analysis the data should be continuous, for that the gaps in Djibouti SLA data are filled and interpolated. As Aden and Djibouti stations show similar variability with (CC of 96% and DC of 92%), Aden SLA is used in interpolation. In this the interpolated value is set as an average value of the previous time step of Djibouti SLA and SLA of Aden from the same time step multiplied with its DC. The interpolated time series match will with AVISO (figure not shown) for the nearest grid to Djibouti station.

The seasonal relationships of sea level elevations to winds, SLP, and steric sea level were examined in both time and frequency domains; multiple linear regression techniques were used to quantify the relations between sea level and different forcing.

2.1. *Wavelet Analysis Approach*

The wavelet analysis transforms any time series to time-frequency space (two-dimensional). Torrence & Compo, (1998) defines the Morlet wavelet as

$$\omega_o(\eta) = \pi^{-¼}\, e^{i\omega_\circ \eta}\, e^{-\eta^2/2} \qquad (1)$$

where η and ω_o are the dimensionless time and frequency, respectively. Frequency $\omega_o = 6$ represents a good balance between time and frequency localization. The continuous wavelet transform (CWT) of the time series X_n, as define by Torrence & Compo, (1998), is

$$W_n(s) = \sum_{n'=0}^{N-1} X_{n'}\, \Psi^* \left[\frac{(n'-n)\delta t}{s}\right] \qquad (2)$$

where, n = one…, N with a uniform time interval, while Ψ^* is the complex conjugate of Ψ. The cone of influence (COI) was used to avoid edging error; therefore, it is defined as the region of which the edge effects become important by distorting the feature either in the beginning or end of the wavelet power spectrum. Thus, the edge dropped by a factor of e^{-2}, where it is negligible here (Grinsted *et al*., 2004; Torrence & Compo, 1998). The red noise is assessed using the first order autoregression model (AR1), while the Monte Carlo method was used to generate the 5% statistical significance level. To measure the intensity of the covariance of two time series X and Y, we followed the approach of

Torrence & Webster, (1999); the cross-wavelet spectrum was used and is defined as

$$W_{XY}(s,t) = W_X(s,t)W_Y^*(s,t) \quad (3)$$

where (*) indicates the complex conjugate.

From the cross-wavelet spectrum, wavelet transform coherence (WTC) can be determined. WTC is a useful method for discovering the coherence and phase lag between two time series as a function of both time and frequency using the following approach (Grinsted *et al*., 2004; Torrence & Webster, 1999)

$$R_n^2(s) = \frac{\left|S\left(s^{-1}W_n^{XY}(s)\right)\right|^2}{S(s^{-1}|W_n^X(s)|^2) \cdot S(s^{-1}|W_n^Y(s)|^2)} \quad (4)$$

Where *S* is the smoothing operator.

3. Results and Discussions

Figure 2 shows the time series of daily mean sea level at Aden and Djibouti as well as the time series of sea level pressure, and along- and cross-shore wind stress components. For clarity in comparing the data, a value of 100 kPa (1000 mb) was subtracted from the sea level pressure time series. Wind stresses were calculated using the formula $(\tau_x\ \tau_y) = \rho C_d|V|(u\ v)$, where ρ is the air density (1.25 kg m^{-3}), C_d is the drag coefficient, and |V|, u, and v are the magnitude, and along- and cross-shore components of wind speed (ms^{-1}), respectively.

Sea level variability at Aden and Djibouti reveals a similar pattern with strong seasonality of higher sea level in winter and lower sea level in summer (Fig. 2a). Superimposed on the seasonal signal is a high-frequency variability of time scale ranging from few days to 30 days, as they were smoothed out in the monthly average. The range in sea level variability is up to about 35 cm at both locations, which is similar to that of (Patzert, 1972). The pat-tern of variability is similar for the four years. Sea level data in Djibouti had more gaps during 2012.

Since Aden and Djibouti are located at the western part of the Gulf of Aden, the atmospheric forcing conditions are similar over the two locations, especially atmospheric pressure, which is high during winter and low during summer at the two stations. High-frequency variability is the same at both stations, with some variability during summer (Fig. 2b). The comparison between variability in daily sea level (Fig. 2a) and daily atmospheric pressure (Fig. 2b) shows high sea level during winter with high pressure and vice versa for summer; thus, the annual cycle of sea level is not the result of an inverted barometric response to atmospheric pressure.

The along-shore wind component also shows strong seasonality at both locations, with stronger positive stress during the southwestern monsoon (June–August) and weaker negative stress during winter, with higher values at Aden compared with those of Djibouti (Fig. 2c). This variability is in the same phase with sea level, with a low sea level lag with about one month with the along-shore wind. Like daily sea level and atmospheric pressure, high-frequency variability of the time scale of the along-shore wind ranges from few days to 30 days is superimposed on the seasonal signal.

The cross-shore component of wind stress is weak at Aden station compared with the along-shore component, with higher values during the southwestern monsoon at Djibouti station (Fig. 2d).

For a clear idea regarding the seasonal variability in sea level and its relation to the controlling forcing, the daily averaged time series for the no-gap year (2014) is selected for all the parameters at Aden and Djibouti. Figure 3 shows the time series of those parameters in addition to daily steric sea level interpolated from monthly climatology. For both stations, sea level variability shows a clear annual cycle with high sea level during winter and low during summer. High-frequency variability in sea level agreed with variability in pressure, especially during winter, where low sea level

coincides with high atmospheric pressure, indicating a normal inverse barometric relation (Fig. 3a, b). The phase difference in low-frequency variability (more than 30 days) show that SLA precedes SLP with 3-4 months; these findings agree with those for the Mediterranean Sea (Le Traon and Gauzelin, 1997), where it is attributed to friction at the strait of Gibraltar. The seasonal cycle of cross-shore wind shows more variability, with a clear cycle for Djibouti, compared with Aden, while it is the oppo-site for the along-shore component (Fig. 3c, d). The along-shore wind is out of phase with SLA at Aden, with negative wind stress during low sea level (summer), and positive stress during winter. This indicates that along-shore wind plays a significant role in sea level variability at Aden. Fig. 3e shows the daily steric sea level for both stations, with a clear high-level annual cycle during winter, and a low-level during summer, which is in phase with sea level variability in both stations, which indicates that steric sea level plays a major role in seasonal SLA variability. The low steric level at summer can be associated with coastal upwelling at both stations.

Since Fig. 2 and 3 (a and b) do not show a clear high frequency relation between SLA and SLP signals; a high-pass filter has been applied for the SLA and SLP time series to make them more pronounced; Fig. 4a and b shows the comparison for the high frequency variability of SLA and SLP for Aden and Djibouti, respectively. The figure shows that the SLA has a prompt inverse parametric response to the SLP all over the year.

To clearly identify signals in all time series, spectral analysis has been conducted as shown in Fig. 5. Regarding the spectral SLA for both stations, Aden and Djibouti shows the most obvious annual and semiannual signals, while the small frequencies were negligible in comparison. For SLP, the annual signal is the most dominant for both stations, while the semiannual in Aden had more of a presence than that in Djibouti. The seasonal (quarter-annual) and semiannual signals are comparable. The along-shore wind signals are clear and strong in Aden and appears to be annual, semiannual, and seasonal. In Djibouti, the along-shore wind shows only the annual signal. The cross-shore wind shows the opposite situation for Aden and Djibouti, where all signals in Aden are weak. Steric spectral signals are almost identical for the SLA in both stations.

To clearly identify signals in all time series, spectral analysis has been conducted as shown in Fig. 5. Regarding the spectral SLA for both stations, Aden and Djibouti shows the most obvious annual and semiannual signals, while the small frequencies were negligible in comparison. For SLP, the annual signal is the most dominant for both stations, while the semiannual in Aden had more of a presence than that in Djibouti. The seasonal (quarter-annual) and semiannual signals are comparable. The along-shore wind signals are clear and strong in Aden and appears to be annual, semiannual, and seasonal. In Djibouti, the along-shore wind shows only the annual signal. The cross-shore wind shows the opposite situation for Aden and Djibouti, where all signals in Aden are weak. Steric spectral signals are almost identical for the SLA in both stations.

3.1. Correlation and Determination Coefficient

As seen from Fig. 6, the sea level is in the same phase with steric sea level and along-shore wind component for Djibouti station, while for Aden station, it is in the same phase with cross-shore wind and steric level. To understand the contribution of each of those factors on seasonal sea level variability, the correlation coefficient (CC) and determination coefficient (DC) were estimated and are summarized in Table 1. For both stations, steric level is the dominant factor with a CC (DC) of 0.86 and 0.84 (0.73, 0.71) for Aden and Djibouti, respectively. This result agrees with that of (Patzert, 1972) for Aden station. The along-shore wind had the second higher contribution for Aden, and the third highest for

Djibouti, with DCs of about 0.3 and 0.21, respectively. The cross-shore wind component had the second highest contribution on Djibouti SLA with DC about 0.3, while for Aden, this factor had no effect on SLA. SLP similarly contributed for both stations.

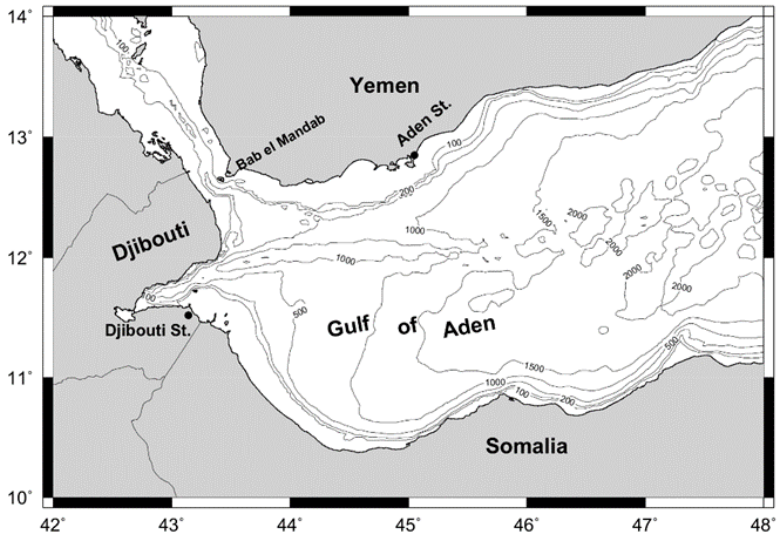

Fig. 1. Study area and tide-gauge stations.

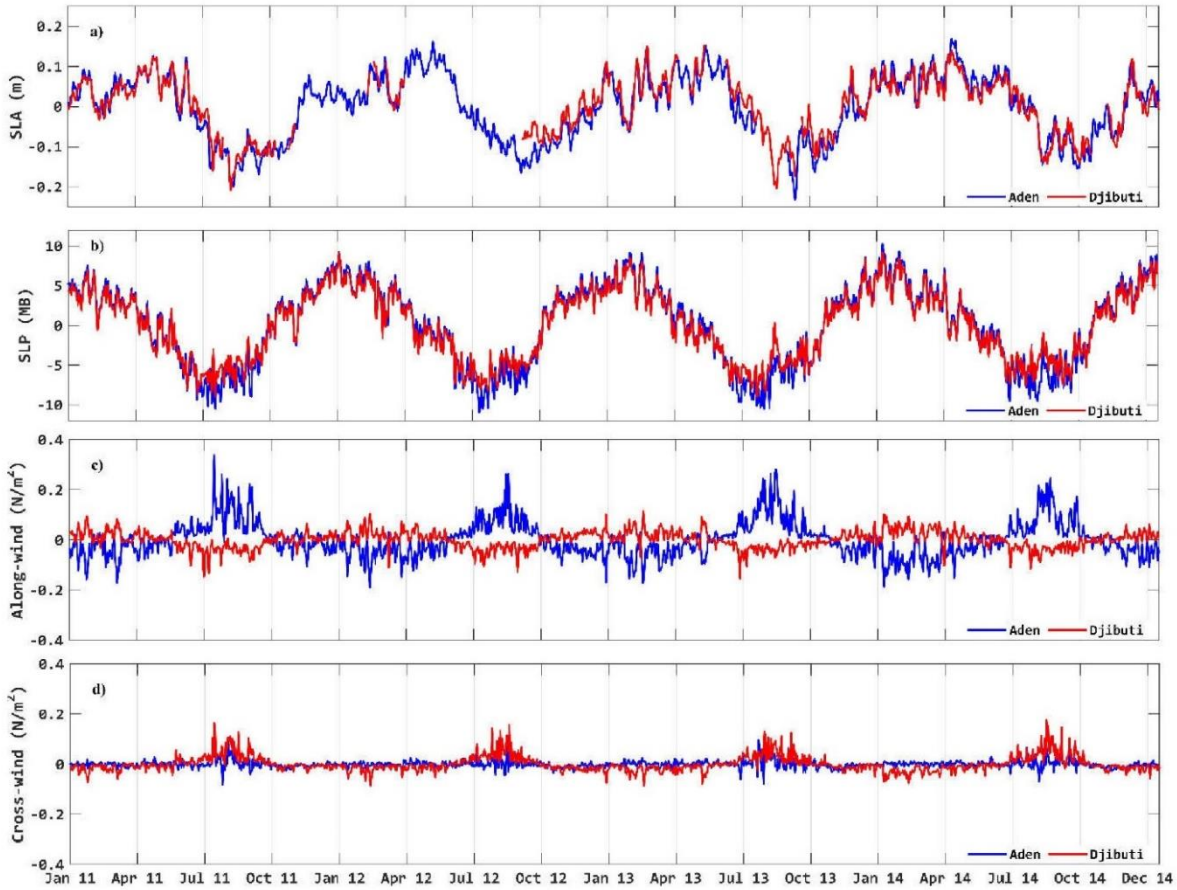

Fig. 2. Time series of daily mean SLA (a) at Aden and Djibouti, SLP (b), and along- and cross-shore wind stress components (c, d). For Aden (Djibouti) station, the cross-shore wind is positive (negative) inshore and negative (positive) offshore while the along-shore wind is positive eastward (northward) and negative westward (southward).

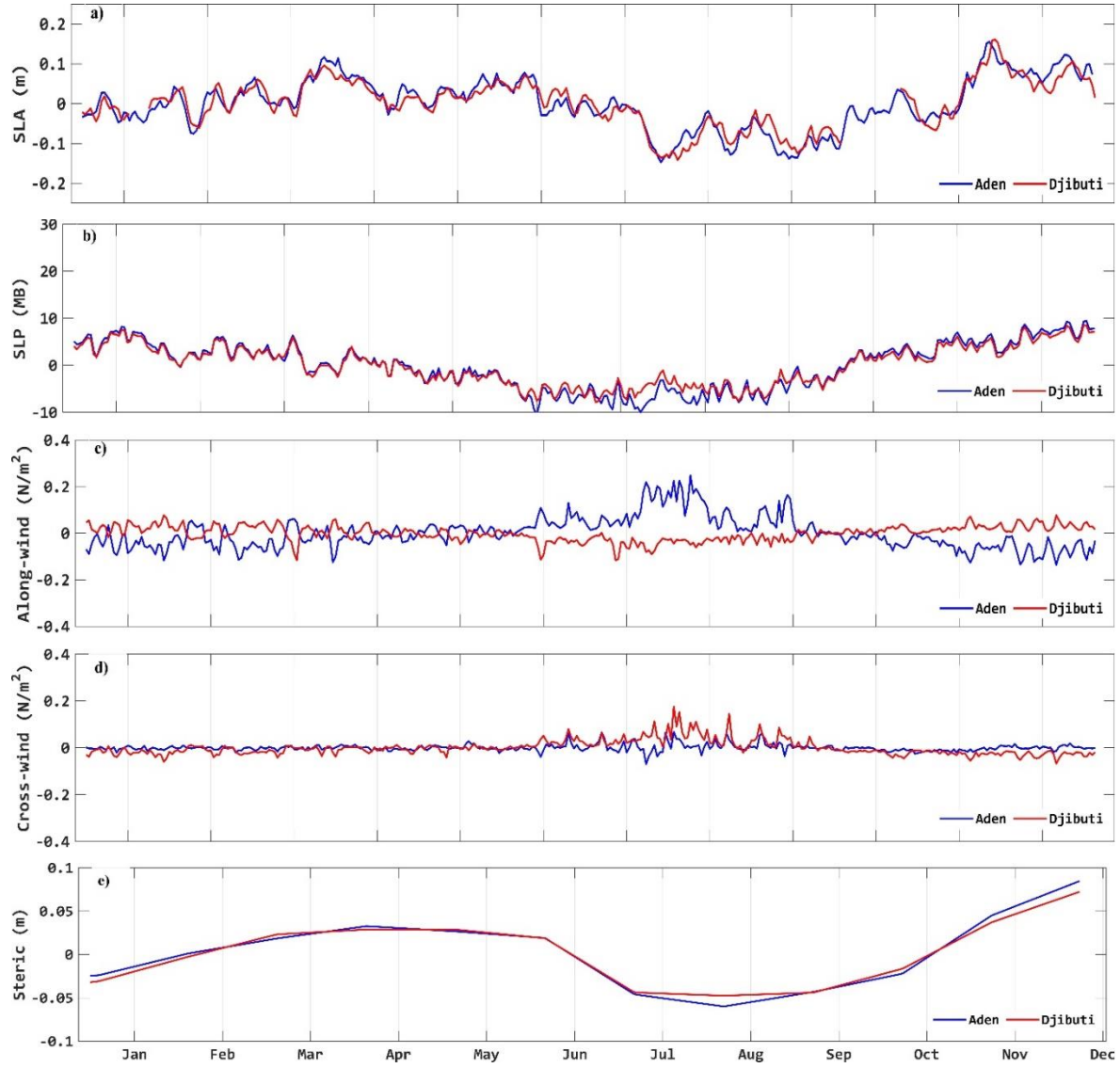

Fig. 3. Time series of daily average of 2014 SLA (a), SLP (b), along-shore (c), cross-shore (d), and steric sea level (e).

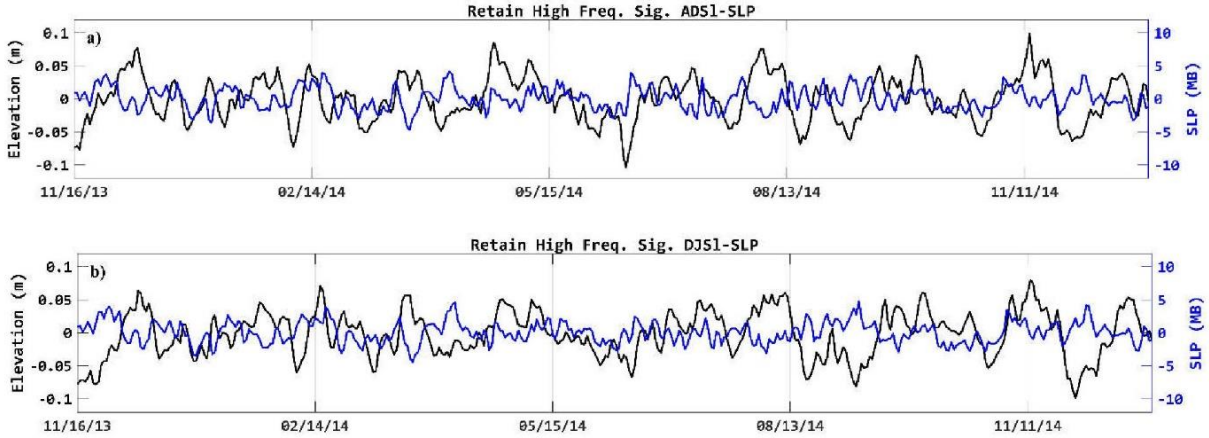

Fig. 4. The SLA and SLP high frequency variability at Aden (a) and Djibouti (b) after applying the high-pass filter.

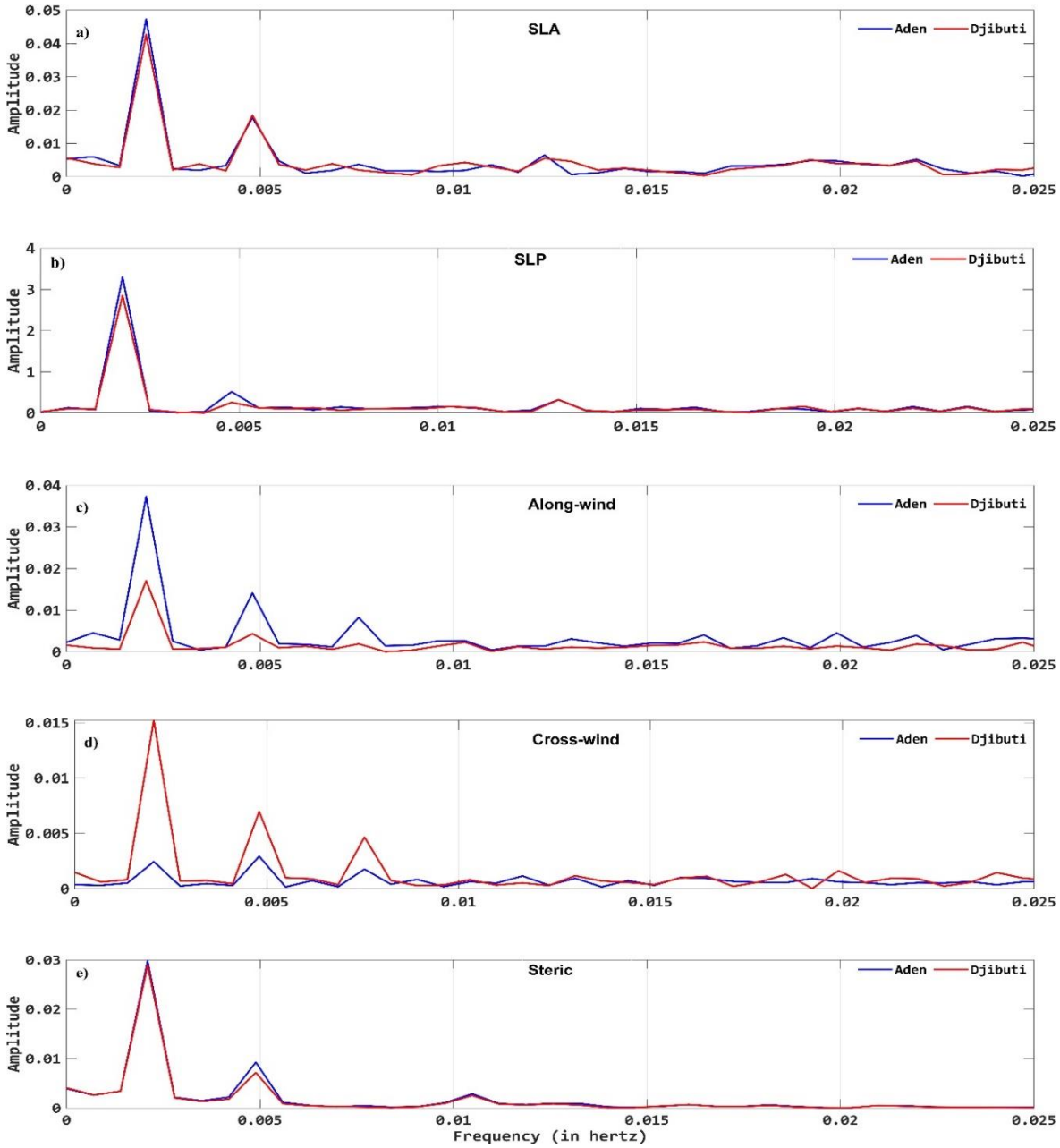

Fig. 5. Power spectrum of SLA, SLP, along-shore, cross-shore, and steric SL.

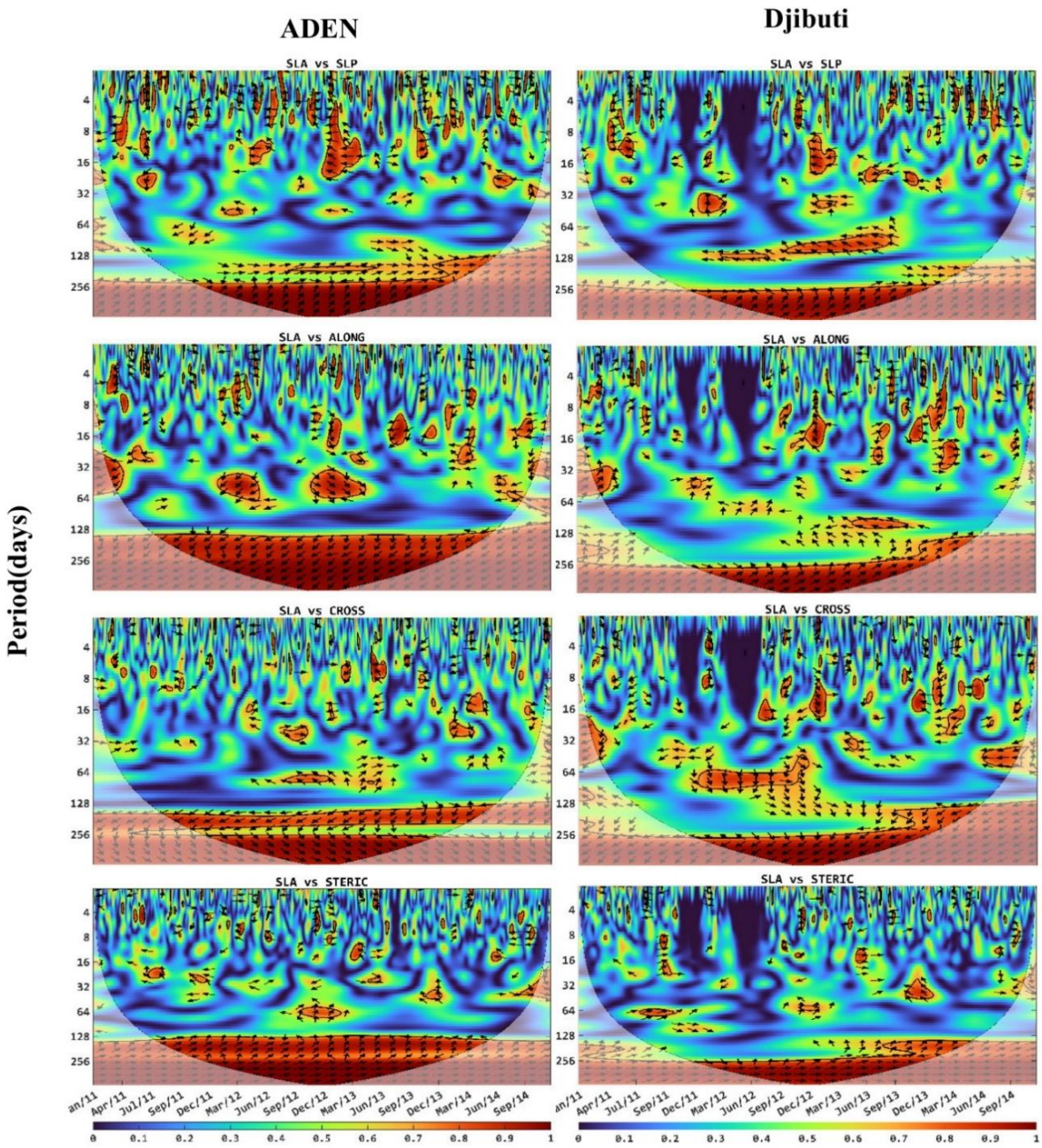

Fig. 6. Wavelet transform coherence (WTC) between SLA time series at (left) Aden and (right) Djibouti with SLP, steric SL, cross-shore wind, and along-shore wind for the same period. The relative phase relationship is shown as arrows (with in-phase pointing right, anti-phase pointing left, and SLA leading by 90◦ pointing straight down). The direction of arrows in the period of 6 month and above (seasonal period) indicates inphase relations among SLA and SLP, steric SL, and cross-shore wind, while it is off-phase with along-shore wind.

Table 1. Correlation and determination coefficients for SLA at Aden and Djibouti, and other controlling parameters.

Variables	Aden Station		Djibouti Station	
	CC (%)	DC (%)	CC (%)	DC (%)
SLP	0.42	0.175	0.37	0.14
Along-shore wind	−0.55	0.30	0.46	0.21
Cross-shore wind	−0.014	0.0002	−0.55	0.30
Strict	0.86	0.73	0.84	0.71

4. Conclusions

Variability in sea level at western Gulf of Aden shows strong seasonality of higher sea level in winter and lower in summer with a range up to about 35 cm which agrees with (Patzert, 1972). The comparison between the variability in daily sea level and daily atmospheric pressure indicates agreement in high sea level during winter with high pressure, and vice versa for summer; thus, the annual cycle of sea level is not a result of an inverted barometric response to atmospheric pressure. However, after applying high-pass filter for the high frequency variability of SLA and SLP for both stations; their SLA show a prompt inverse parametric response to the SLP all over the year. Moreover, the phase difference in low-frequency variability (more than 30 days) show that SLA precedes SLP with 3-4 months, which agrees with those of the Mediterranean Sea (Le Traon and Gauzelin, 1997). Seasonal cycle of the cross-shore wind reveals more variability with SLA in Djibouti compared with that for Aden, while it is the opposite for the along-shore component. The along-shore wind is out of phase with SLA at Aden, with negative wind stress during low sea level (summer) and positive stress during winter. This indicate that along-shore wind plays a significant role in sea level variability at Aden. Daily steric sea level for both stations exhibit an annual cycle with a high level during winter and low during summer, which is in phase with sea level variability in both stations.

Spectral analysis for SLA shows that the most obvious signals are the annual and semiannual, while the small frequencies (less than two months) are negligible in comparison. For SLP, the annual signal is the most dominant for both stations, while the semi-annual in Aden has more of a presence than that in Djibouti.

The seasonal and semiannual signals are comparable, though. Along-shore wind signals are strong in Aden and shows to be annual, semiannual, and seasonal. In Djibouti, only the annual signal could be observed. Cross-shore wind reveals the opposite situation for Aden and Djibouti, where all signals in Aden are weak. Steric spectral signals are almost identical for the SLA in both stations. The wavelet transform coherence (WTC) between SLA and SLP in both stations for short periods is in antiphase, which is the normal inverse barometric relation. For Aden station, the SLA is leading for periods from one season and longer, while the Djibouti SLA shows an antiphase for one season, and SLA is leading for the rest. This indicates that the SLP is not the major contributor to SLA fluctuation in the periods of one season and longer. Aden SLA shows the importance of along-shore wind for all periods with anti-phase relation in general; this effect is normal since Aden station is located along the northern coast of the gulf. The cross-shore wind effect is weak; however, it shows variable relation for the short period, and antiphase for one season, and SLA is almost inphase with short time lagging. Wind components play the opposite role at Djibouti station, where cross-shore wind is the most dominant component, which shows an anti-phase for one season and longer. For both stations, steric sea level shows an inphase with SLA, indicating that it plays a major role in sea level variability for seasonal and annual periods.

To summarize, the SLA shows strong seasonal variability with high values during winter and the opposite in summer. The major contributor for this variability is steric SL with CCs (DCs) of 0.86 and 0.84 (0.73 and 0.71) for Aden and Djibouti, respectively. The second contributor at Aden is along-shore wind with DCs of about 0.3 and 0.21, respectively, while it is cross-shore wind for Djibouti with a DC of about 0.3. The SLP similarly contributes for both stations, and only plays a major role in short SLA variability.

Acknowledgments

The author thanks the PSMSL data repository for providing a comprehensive

access to GPS and tide gauge records. Special thanks also for Dr. Sameer Gharbi for preparing figures.

References

Abdelrahman, **S.M.** (1997). Seasonal fluctuations of mean sea level at Gizan, Red Sea. *Journal of Coastal Research*, **13**(4): 1166-1172.

Abdulla C.P., Alsaafani, M.A., Alraddadi, T.M. and **Albarakati, A.M.** (2019), Climatology of mixed layer depth in the Gulf of Aden derived from in situ temperature profiles. *J. Oceanogr*, **75**: 335–347.

Ahmad, **F.** and **Sultan, S.A.R.** (1989), Surface Heat Fluxes and their Comparison with the Oceanic Heat Flow in the Red Sea. *Oceanology Acta*, **12**: 33-36.

Alawad, K.A., Al-Subhi, A.M., Alsaafani, M.A., Alraddadi, T.M., Ionita, M. and **Lohmann, G.** (2019), Large-Scale Mode Impacts on the Sea Level over the Red Sea and Gulf of Aden. *Remote Sensing*, **11**(19): 2224.

Alsaafani, **M.A., Alraddadi, T.M.** and **Albarakati, A.M.** (2017), Seasonal variability of hydrographic structure in Sharm Obhur and water exchange with the Red Sea. *Arab J. Geo. Sci.*

Alsaafani, **M. A., Shenoi, S. S. C., Shankar, D., Aparna, M., Kurian, J., Durand, F.** and **Vinayachandran, P**. (2007)**,** Westward movement of eddies into the Gulf of Aden from the Arabian Sea, *J. Geophys. Res.*, **112**.

Alsaafani, **M.A., Nagi, H.M., Alhababy, A.M., Hajer, A.** and **Abubakr, M.M.** (2015), Impact of Sea Level Rise and Climate Change on the Coastal Zone of Aden Governorate, Republic of Yemen, Sana'a University, *Faculty of Science Bulletin*, **27**: 15-32.

Bergant, **K., Sušnik, M., Strojan, I.** and **Shaw, A. G. P**. (1997–2010), Sea level variability at Adriatic coast and its relationship to atmospheric forcing, *Ann. Geophys*., **23**.

Cheney, **R., Miller, L., Agreen, R., Doyle, N.** and **J. Lillibridge** (1994), Topex/Poseidon: The 2-cm solution, *J. Geophys. Res.*, **99**, 24555±24563.

Cromwell, **D.** and **Smeed, D.A.** (1998), Altimetric observations of sea level cycles near the strait of Bab al Mandab, *International Journal of Remote Sensing*, **19**(8): 1561-1578.

Currie, R. I., Fisher, A. E. and **Hargreaves, P. M.** (1973), Arabian Sea Upwelling, in: *The Biology of the Indian Ocean*, Edited by: B. Zeitschel, pp. 37-52, Springer-Verlag, Berlin.

Dasgupta, S., Laplante, B., Murray, S. and **Wheeler, D.** (2009), *Climate Change and the Future Impacts of Storm-Surge Disasters in Developing Countries*. Technical Report, Center for Global Development, Working Paper 182. 1800 Massachusetts Ave., NW, Washington, D.C.: http://www.cgdev.org/content/publications/detail/ 1422836.

Dee D.P., Uppala, S.M., Simmons, A.J., Berrisford, P., Poli, P., Kobayashi, S., Andrae, U., Balmaseda, M.A., Balsamo, G., Bauer, P., Bechtold, P., Beljaars, A.C.M., van de Berg, L., Bidlot, J., Bormann, N., Delsol, C., Dragani, R., Fuentes, M., Geer, A.J., Haimberger, L., Healy, S.B., Hersbach, H., Hólm, E.V., Isaksen, L., Kållberg, P., Köhler, M., Matricardi, M., McNally, A.P., Monge-Sanz, B.M., Morcrette, J.J., Park, B.K., Peubey, C., de Rosnay, P., Tavolato, C., Thépaut, J.N. and **Vitart, F.** (2011), The ERA-Interim reanalysis: Configuration and performance of the data as-similation system. *Q. J. R. Meteorol. Soc.*, **137**: 553–597.

Fratantoni, **D. M., Bower, A. S., Johns, W. E.** and **Peters, H.** (2006), Somali Current rings in the eastern Gulf of Aden, *J. Geophys. Res.*, **111**.

Fukumori, **I., Raghunath, R.** and **Fu, L. L.** (1998), The nature of global large-scale sea level variability in relation to atmospheric forcing: A modeling study. J. *Geophys. Res.*, **103** : 5493–5512.

Garcı´a-Lafuente, J., Del Rı´o, J., Alvarez Fanjul, E., Gomis, D. and **Delgado, J.** (2004) Some aspects of the seasonal sea level variations around Spain, *J. Geophys. Res*., **109**.

Gill, **A. E.** (1982), *Atmosphere-Ocean Dynamics. International Geophysics Series*, Academic Press, New-York.

Grinsted, A., Jevrejeva, S. and **Moore, J.** (2004), "Application of the cross wavelet transform and wavelet coherence to geophysical time-series". Submitted to a special issue of : *Nonlinear Process in Geophysics*, Nonlinear analysis of multivariate geoscientific data - advanced methods, theory and application.

Holgate, **S., Matthews, A., Woodworth, P.L., Rickards, L.J., Tamisiea, M.E., Bradshaw, E., Foden, P., Gordon, K.M., Jevrejeva, S.** and **Pugh, J.** (2013), New data systems and products at the permanent service for mean sea level. *Journal of Coastal Research*, **29**: 493–504.

Le Traon, P. Y., and **Gauzelin, P.** (1997), Response of the Mediterranean mean sea level to atmospheric pressure forcing, *J. Geophys. Res*., **102**: 973–984.

Madah, F.A. (2020), The amplitudes and phases of tidal constituents from Harmonic Analysis at two stations in the Gulf of Aden. *Earth Syst Environ*, **4**: 321–328.

Patzert, W. C. (1972), *Seasonal variations of structure and circulation in the Red Sea*. Report of Hawaii Institute of Geophysics. (HIG-72-13), 58p.

Pugh, D. T. (1987), *Tides, Surges, and Mean Sea Level*, John Wiley & Sons, pp. 472.

Shankar, **D.** (2000), Seasonal cycle of sea level and currents along the coast of India. *Current Science*, **78**: 279-288.

Sultan, S.A.R. and **Ahmad, F.** (1997), Heat budget of the Gulf of Aden: surface, advective and upwelling heat fluxes. *Oceanologica Acta*, **20**(5): 665-672.

Torrence, **C.** and **Compo, G.P.** (1998), A practical guide to wavelet analysis, *Bulletin of American Metrological Society*, **79** : 61-78.

Torrence, C. and **Webster, P.** (1999), Interdecadal Changes in the ESNO-Monsoon System, *Journal of Climate*, **12**: 2679-2690.

Tsimplis, M. N. and **Vlahakis, G. N.** (1994), Meteorological forcing and sea level variability in the Aegean Sea, *J. Geophys. Res.*, **99**: 9879-9890.

Tsimplis, M. N. and **Woodworthm, P. L.** (1994), The global distribution of the seasonal sea level cycle calculated from coastal tide gauge data, *J. Geophys. Res.*, **99**: 16, 031–16, 039.

Unnikrishnan, A. and **Shankar, D.** (2007), Are Sea-Level-Rise Trends along the Coasts of North Indian Ocean Coasts Consistent with Global Estimates? *Global and Planetary Change*, **57**: 301–307.

Uppala, **S. M., KÅllberg, P. W., Simmons, A. J., Andrae, U., Bechtold, V. D. C., Fiorino, M., Gibson, J. K., Haseler, J., Hernandez, A., Kelly, G. A., Li, X., Onogi, K., Saarinen, S., Sokka, N., Allan, R. P., Andersson, E., Arpe, K., Balmaseda, M. A., Beljaars, A. C. M., Berg, L. V. D., Bidlot, J., Bormann, N., Caires, S., Chevallier, F., Dethof, A., Dragosavac, M., Fisher, M., Fuentes, M., Hagemann, S., Hólm, E., Hoskins, B. J., Isaksen, L., Janssen, P. A. E. M., Jenne, R., Mcnally, A. P., Mahfouf, J.-F., Morcrette, J. J., Rayner, N. A., Saunders, R. W., Simon, P., Sterl, A., Trenberth, K. E., Untch, A., Vasiljevic, D. and Viterbo, P.** and **Woollen, J.** (2005), The ERA-40 reanalysis, Q. J. Roy. *Meteor. Soc.*, **131**: 2961– 3012, https://doi.org/10.1256/qj.04.176.

Woodworth, P.L., Tsimplis, M.N., Flather, R.A. and **Shennan, I.** (1999), A review of the trends observed in British Isles mean sea level data measured by tide gauges. *Geophysical Journal International*, **136**: 651-670.

Woodworth, **P., Foden, P., Pugh, J., Mathews, A., Aarup, T., Aman, A., Nkebi, E., Odametey, J., Facey, R., Esmail, M. Y. A.** and **Ashraf, M.** (2009), Insight Into. Long Term Sea Level Change Based on New Tide Gauge Installations at Takoradi, Aden and Karachi. *International Hydrographic Review*, **1**(May): 18-23.

Wunsch, **C.** (1991), Large-scale response of the ocean to atmospheric forcing at low frequencies, *J. Geophys. Res.*, **96**: 15,083-15, 092.

العوامل التي تتحكم في التقلبات الموسمية لمنسوب سطح البحر في غرب خليج عدن

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المستخلص. تم حساب المتوسطات اليومية لمنسوب سطح البحر من البيانات الساعية في محطتي عدن وجيبوتي، خلال الفترة ٢٠١١–٢٠١٤م، وذلك لدراسة التغيرات الموسمية في منسوب سطح البحر (SLA) في غرب خليج عدن. تم التحقيق في هذه التغيرات مقابل عوامل التحكم التالية: الضغط الجوي عند سطح البحر، والرياح، ومنسوب البحر الستيريكي (الحراري). يظهر في (SLA) في عدن وجيبوتي تغيرات موسمية قوية مع ارتفاع في الشتاء وانخفاض في الصيف بنطاق يصل إلى حوالي ٣٥ سم. ويتوافق التغير عالي التردد في منسوب سطح البحر مع التغير في الضغط الجوي، خاصة خلال فصل الشتاء بعلاقة بارومترية عكسية طبيعية. تؤثر الرياح العابرة للساحل على تقلب منسوب سطح البحر في جيبوتي أكثر من تأثيرها في عدن، بينما تلعب الرياح الموازية للساحل دورًا مهمًا في تقلب منسوب سطح البحر في عدن. ويلعب منسوب سطح الستيريكي لكلتا المحطتين دورًا مهمًا في التقلب الموسمي لـ (SLA). وتظهر كلتا المحطتين أن أقوى التذبذبات في (SLA) هي السنوية ثم نصف السنوية، في حين أن الترددات الصغيرة لا تكاد تذكر بالمقارنة. وبالنسبة لمحطتي عدن وجيبوتي، فإن منسوب سطح البحر الستيريكي هو العامل المهيمن بمعامل تحديد (DC) يبلغ ٠,٧٣ و٠,٧١ على التوالي. وتعتبر الرياح الموازية للساحل ثاني أعلى مساهمة في عدن بحوالي ٠,٣. في حين تمثل الرياح الموازية للساحل ثاني أعلى مساهمة في جيبوتي بمعامل تحديد يبلغ حوالي ٠,٣، بينما في عدن، لا يؤثر هذا العامل على (SLA). وتظهر مساهمة الضغط الجوي بوضوح في التذبذبات القصيرة لكلتا المحطتين.

الكلمات المفتاحية: خليج عدن، متوسط منسوب سطح البحر، منسوب سطح البحر الحراري، نظرية المويجات، العوامل الجوية.

JKAU: Mar. Sci., **Vol. 31** No. 2 pp: 45-54 (1442 A.H. / 2021 A.D.)
DOI :10.4197/Mar.31-2.4

Isolation and Identification of Biofilm Bacteria from Microfouling Assemblage Developed on Artificial Materials Submerged in the Red Sea

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Abstract. Biofilm bacteria are primary surface colonizers in marine biofouling assemblage on submerged surfaces and dominate the early microfouling phase. They are an important target in the design of antifouling treatment due to their ability to initiate biofouling and support of the subsequent macrofouling colonization. In this study, several biofilm bacteria were isolated from Petri dish and polyvinyl chloride (PVC) pipes submerged in the Red Sea coast for a week. The biofilm-forming bacteria were isolated by spread plate method under standard conditions and identified by 16S rRNA sequencing method. Each of the isolates was evaluated for biofilm formation qualitatively using the tube assay. Microtiter plate assay was used to quantify the biofilm produced by the selected organisms. A total of 11 out of 21 isolated bacteria were able to form biofilm under laboratory conditions. Most of the isolates (7 out of 11) are from the genus *Pseudoalteromonas* and one isolate each from the genera *Halomonas*, *Marinomonas*, *Psychrobacter* and *Vibrio*. This study indicated that the bacterial community forming the biofilms on hard substrates in the Red Sea are diverse and capable of forming biofilms on surfaces under laboratory conditions. These isolates could be used as target microorganisms for antifouling screening assays.

Keywords: Biofilm; bacteria; biofouling; antifouling; *Pseudoalteromonas*; Red Sea.

1. Introduction

Marine biofouling occurs on all submerged surfaces following colonization by living organisms in marine environment. It is an undesirable process that has economic and environmental consequences (Cruz, 2020). It is impacting negatively on the finances of maritime, naval, tourism, aquaculture, and fisheries industries due to operational and maintenance cost to prevent the attachment of the organisms through the application of protective coatings or in their labour-intensive removal and increase in fuel consumption (Mathew *et al*., 2021). To the environment, fuel increase causes increase burning of greenhouse gases for hull and propeller machines and several coatings applied to prevent biofouling causes water pollution and alters the marine ecological setup (Farkas *et al*., 2021). The consequence of biofouling is increase in transportation cost. It also affects other services such as service delivery and quality of the products.

Biofouling is a sequential process that involves the attachment of different organisms at different stages at distinct time interval. It begins with the conditioning of the surface by organic matter followed by development of microfouling assemblage dominated by extracellular polymeric substances (EPS)

producing bacteria and diatoms. The microfouling phase marks the initial stage of the colonization process by living organisms leading to microbial biofouling (biofilm) formation (Grasland *et al*., 2003). The biofilm communities colonize the surfaces rapidly in the multistage process less than one hour of the surface contact with water to alter its chemistry due to their attachment strength (Salta *et al*., 2013). Bacteria dominate in the biofilm among surface associated microbes with its high rate of occurrence by attaching to the surface faster than other organisms (Zobell and Allen, 1935; Papadatou *et al*., 2021). They colonize both living and non-living surfaces and the biofilm is held firmly by EPS secreted by the bacteria that enable their attachment and modification of the submerged surface (Renner and Weibel, 2011). This modification enables the biofilm bacteria to colonize surfaces quickly and further provide access for the subsequent colonization by macrofouling organisms (Lorite *et al*., 2011). The EPS form a matrix to hold the individual organisms together which allow the bacteria to withstand harsh environmental conditions (Flemming *et al*., 2007). The biofilm is a highly dynamic, stable, heterogeneous structure that can survive for longer period (Flemming, 2008; Salta *et al*., 2013). The structure is difficult to remove by cleaning when it matures on surfaces (Flemming, 2008) and is affected by site and type of substrates (Briand *et al*., 2012). Despite protective coatings applied on surfaces to prevent attachment of fouling organisms, microfouling assemblage still occurs due to adhesion strength of the biofilm forming organisms (Oliveira and Granhag, 2016). This stage can have impact on the performance of ships as such, it is important in determining the efficacy of antifouling treatments (Hearin *et al*., 2016). To improve the effectiveness of antifouling strategies, the strength, nutritional requirement, and physico-chemical properties of the biofilm bacteria is very relevant.

Different biofilm bacteria are involved in the assemblage making them an important target in preventing and controlling biofouling. The composition of the bacterial communities' changes with time and seasonal variation making the communities highly variable and complex (Sawant *et al*., 1995). All bacterial phyla are involved but proteobacteria often dominates the stage which can be attributed to their success in the competition for space and nutrients during the biofilm development stages and production of inhibitory substances (Burchard and Sorongon, 1998; Matz *et al*., 2004). The biofilm influences the metamorphosis of the larvae of benthic invertebrates (Hadfield, 2010) and release of macroalgal spores (Goecke *et al*., 2010). The larval settlement is dictated by the nature and type of the biofilm communities (Dobretsov and Qian, 2006) which serve as natural settlement cues for the larvae to choose the right settlement site (Qian *et al*., 2007). The settlement is also enhanced or inhibited by the properties of the bacteria forming the biofilm in the environment (Dobretsov *et al*., 2006).

Understanding the early stage of biofouling (microfouling) is important for the development of non-toxic techniques to control biofouling on surfaces (Qian *et al*., 2007). This depends on the identification of the different biofilm bacteria involved and their physico-chemical properties (Grasland *et al*., 2003; Afonso *et al*., 2021). Microfouling assemblage can be estimated based on the bacterial cell density forming the biofilm and relate directly with the rate of attachment of subsequent colonizers (Dang and Lovell, 2015). Biofilm is detrimental to all submerged surface in seawater. Understanding the diversity of the biofilm bacteria which are the dominant cause of microfouling assemblage and factors relevant in biofilm formation are important in combating biofouling. This is an important consideration in antifouling technologies to minimize or prevent the problem and it completes eradication.

In this study, biofilm bacteria from two substrates (Petri dish and PVC pipes) submerged in the Red Sea coast of Central Red

Sea were isolated and identified with morphological and 16S rRNA features. This will have significant contribution in the development of control strategies against the occurrence of microfouling on submerged surfaces in marine environments.

2. Materials and Method

2.1 Isolation and Identification of Microfouling Bacteria

The formation of microfouling communities was studied by submerging polyvinyl chloride (PVC) pipes and sterile Petri dishes (SaudiPlast, Saudi Arabia) at 2m depth in the Obhur creek of the Red Sea (N21°42.562′ E39°05.764′) in six replicates each. The PVC pipes are white in colour, cut to 15cm each per piece in length and a rope was tight to one end of the pipe and then submerged in the sea by hanging the rope tightened to the Jetty stationed at Obhur creek. The petri dish which is made up of polystyrene is 90 mm in diameter. A hole is made at a side of the plate to hang the rope before submerging it in the sea in the same way as PVC. This was carried out at the beginning of fall in September 2021. The method of Balqadi *et al*. (2018) was adopted and modified for the development of biofilm on the submerged surfaces. In brief, prior to submersion in water, the PVCs and Petri dishes were cleaned, dried, and sterilized with 99 % ethanol. They were allowed to stay for at least 48 hours after exposure in the sea water after which they were removed, rinsed in sterilized filtered sea water (SFSW) and immersed in a sterilized container containing SFSW before transporting it to the laboratory. At the laboratory, microfouling bacteria were isolated from the microfouling assemblage using traditional culture method with Zobell marine agar (ZMA) after vigorous agitation of the container to release the attached biofilm bacteria. Serial dilution was carried out using SFSW using ten-fold dilution, inoculated in ZMA and incubated at 28 ^{o}C for 48 hours. Individual distinct colonies were subcultured on fresh ZMA plates and incubated as before. Unique organisms identified based on colony morphology; microscopic features were screened for biofilm formation ability. Morphologically distinct bacterial strains were selected, re-streaked, purified and tested for its biofilm forming ability through crystal violet assay.

2.2 Assessment of Biofilm Formation Ability

To assess the ability of the strains to form biofilm, crystal violet (CV) assay described by Haney *et al*. (2018) was followed with some modifications. Briefly, to prepare the inoculum, one colony of each of the bacterial isolate was inoculated in 10 ml Zobell marine broth (ZMB) and incubated with shaking at 150 rpm overnight at 28 ^{o}C. Fresh overnight culture of each of the organism (10%) was inoculated in borosilicate glass tubes containing 5ml of ZMB and incubated for 24 hours (incubated for 12 hours with shaking in a shaker incubator at 150 rpm and 12 hours under static condition) at 28 ^{o}C. The planktonic cells were removed gently, and the tubes were washed with phosphate buffered saline (PBS) twice and allowed to dry. To the attached cells, crystal violet at 0.2% was added and the tubes were incubated for 20 mins at room temperature without shaking. The tubes were then washed again with PBS after discarding the CV as described before and allowed to dry. The biofilm formation was observed directly with the eyes and in most cases a visible coloured ring formed at the interface in the tube or at the bottom is interpreted as a sign of biofilm formation.

2.3 Microtiter Biofilm Quantification

The biofilm bacteria screened were subjected further for biofilm quantification using the microtiter quantification assay. The procedure of O'Toole (2011) was adopted and modified. In brief, the 96-well round bottom microplates previously sterilized with 95% ethanol were inoculated with 200 µl of autoclaved FSW for 1 h prior to condition the wells. Overnight culture of each biofilm bacteria was diluted in fresh media (1:100) and 200 µl aliquot was suspended into the microtiter wells

(n=12). Control contains 200 µl media only without bacterial culture to detect contamination. The plates were incubated statically for 48 h at 28 °C. The planktonic suspension was carefully removed with a multichannel pipette into a new 96 well plates. The absorbance was read at an optical density of 570 nm in a microplate reader. For the attached cells in the plates, 300 µl of PBS was suspended into each well using a multichannel pipette to wash the plates. This was repeated 3 times and dried in inverted position. The attached cells were stained using 200 µl of 0.2% crystal violet (CV) in water and incubated at room temperature for 20 minutes after which the excess CV stain was removed by washing the plates twice as described above. The leftover of the CV was solubilized with 200 µL of 96 % ethanol to destain the wells and incubated with shaking for 15 min at room temperature and then transferred to fresh 96 well plates. The plates were read with the microplate reader (Synergy, Biotek) at 570 nm (OD_{570}). Biofilm formation ability was recorded as highly positive, low grade positive or negative when OD_{570} is ≥ 1, 0.1 to < 1 or < 0.1 respectively (Lagha *et al*., 2019).

2.4 Identification of Biofilm Bacteria by 16s rRNA Method

The promising biofilm bacterial strains were then identified up to species level through 16S rRNA gene sequencing. Genomic DNA was extracted from each of the isolate and polymerase chain reaction (PCR) was carried to amplify the 16S rRNA gene sequence with universal primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (AAGGAGGTGATCCACCC). The 16S rRNA gene was sequenced at Macrogen inc. and sequences obtained were analyzed and compared with closely related sequences available in NCBI using BLAST limiting the search to sequences from type materials (Zhang *et al*., 2000). The processed sequences were submitted to NCBI GenBank and their respective accession numbers were obtained. This was followed by phylogenetic tree construction using MEGA neighbour joining method to determine the phylogenetic position of the strains and their evolutionary relatedness (Kumar *et al*., 2018).

3. Results

3.1 Identification and Confirmation of Biofilm Bacteria

A total of 11 strains of microfouling bacteria isolated from the PVCs and Petri plates were identified and confirmed as biofilm bacteria from 21 different isolates. The biofilm forming strains were labelled as IMB1, IMB2, IMB8, IMB10, IMB11 IMB12, IMB13, IMB14, IMB15, IMB16 and IMB17. All the strains were Gram-negative and have mucoid colonies except IMB2 and IMB8. From PVC, IMB12 - 17 were confirmed to be biofilm-forming bacteria while IMB 1, IMB2, IMB8, IMB10 and IMB 11 isolated from Petri dish plates were having the ability for biofilm formation. Based on 16S rRNA gene sequences and similarity search, 7 of the 11 microfouling bacteria isolated were identified as members of the *Pseudoalteromonas* (IMB1, IMB10, IMB12, IMB13, IMB14, IMB15, and IMB17). The other bacterial strains identified include *Halomonas* (IMB2), *Psychrophile* (IMB8), *Vibrio* (IMB11) and *Marinomonas* (IMB16) genera. They all belong to the proteobacteria phylum and all except *Marinomonas* (an alphaproteobacteria) are gammaproteobacteria. Details of each isolate with the closest strain are provided in Table 1. Based on phylogenetic relatedness, all the isolates are closely related to each other as shown in Fig. 1.

3.2 Biofilm Quantification

The result of biofilm quantification is shown in Fig. 2. The value which represents an average of 12 replicates ranges from 0.36 to 1.57. The categorization of the biofilm bacteria as low grade positive and highly positive is presented in Table 2 as quantified based on the OD_{570} absorbance values. Apart from two isolates that were classified as highly positive, all the remaining 9 isolates are low grade biofilm formers.

Table 1. Identification of biofilm-forming bacteria isolated from microfouling assemblage formed on PVC pipes and Petri dish.

Isolates	Substrate	Identification (closest NCBI relative)	Gram reaction
IMB1	**Petri Dish**	*Pseudoalteromonas sp.* (97.93%)	Gram negative
IMB2	**Petri Dish**	*Halomonas sp.* (81.54%)	Gram negative
IMB8	**Petri Dish**	*Psychrobacter sp.* (97.16%)	Gram negative
IMB10	**Petri Dish**	*Pseudoalteromonas sp.* (95.36%)	Gram negative
IMB11	**Petri Dish**	*Vibrio alginolyticus* (96.67%)	Gram negative
IMB12	**PVC**	*Pseudoalteromonas issachenkonii* (97.73%)	Gram negative
IMB13	**PVC**	*Pseudoalteromonas shioyasakiensis* (97.67%)	Gram negative
IMB14	**PVC**	*Pseudoalteromonas gelatinilytica* (97.59%)	Gram negative
IMB15	**PVC**	*Pseudoalteromonas gelatinilytica* (99.01%)	Gram negative
IMB16	**PVC**	*Marinomonas aquiplantarum* (96.71%)	Gram negative
IMB17	**PVC**	*Pseudoalteromonas sp.* (97.26%)	Gram negative

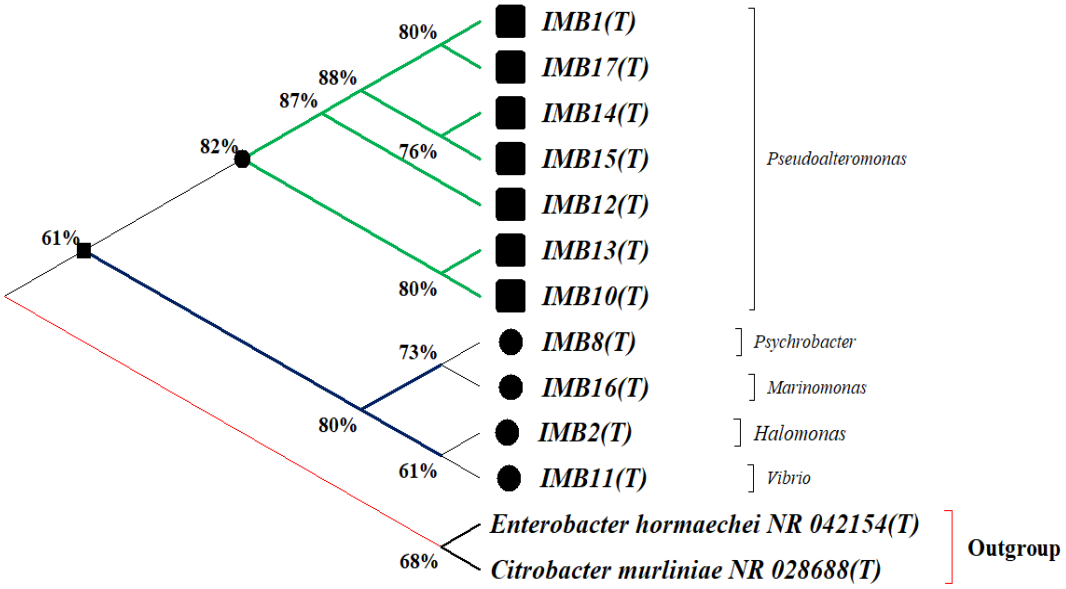

Fig. 1. Phylogenetic tree showing evolutionary relationship among the biofilm bacteria identified from submerged substrates of PVC and Petri dish.

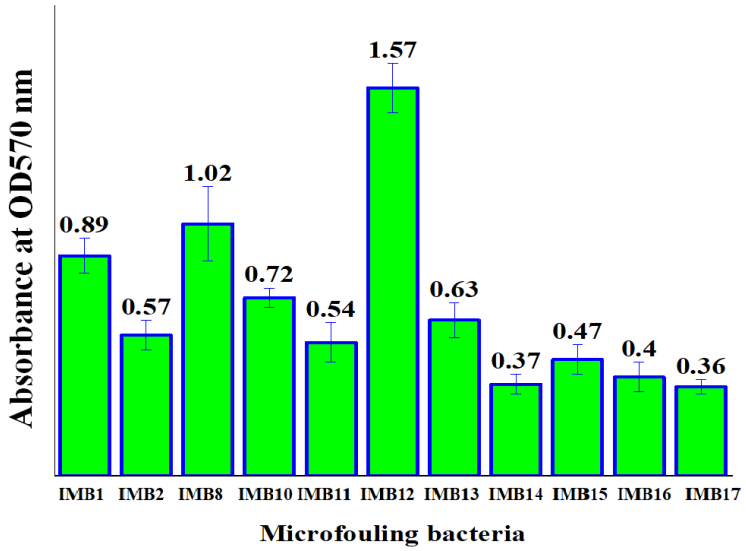

Fig. 2. Biofilm quantification of microfouling bacteria isolated from submerged PVC pipes and Petri dish at OD_{570}. Results presented is an average of 12 replicates with standard deviation.

Table 2. Categorization of biofilm formation by biofilm bacteria isolated from submerged PVC pipes and Petri dish.

Isolate code	Substrate	Biofilm categorization
IMB1	**Petri Dish**	low-grade positive
IMB2	**Petri Dish**	low-grade positive
IMB8	**Petri Dish**	Highly positive
IMB10	**Petri Dish**	low-grade positive
IMB11	**Petri Dish**	low-grade positive
IMB12	**PVC**	Highly positive
IMB13	**PVC**	low-grade positive
IMB14	**PVC**	low-grade positive
IMB15	**PVC**	low-grade positive
IMB16	**PVC**	low-grade positive
IMB17	**PVC**	low-grade positive

4. Discussion

Biofilm formation by bacteria is a necessary requirement for the development of microfouling assemblage on surfaces. The control of biofilm formation by bacteria has great potential in the prevention or minimization of biofouling on surfaces (Flemming, 2011). In the biofilm development, different surfaces attract diverse bacteria (Kerr *et al*., 1998) because the surface properties have effect on the development of microfouling (Dobretsov *et al*., 2013). Diverse bacteria are involved in biofilm formation on submerged surfaces during initial biofouling forming stage, the microfouling stage (Chen *et al*., 2013). In this study, we used two different surfaces to study the diversity of biofilm bacteria capable of biofilm formation. A diverse group of biofilm bacteria were reported in this study from the PVC and Petri Dish surfaces with the genus *Pseudoalteromonas* dominating among all the genera identified. The bacteria isolated from PVC are less diverse than those isolated from Petri dish. All of the isolates from PVCs are members of the genus *Pseudoalteromonas* except one organism that belong to the genus *Marinomonas*. The Petri dish comprises of four different genera from 5 bacterial isolates which are *Vibrio, Halomonas, Psychrobacter* and *Pseudoalteromonas*. The difference in species composition may be due to the composition and colour of the substrates used in this study. The Petri dish is made up of polystyrene and is transparent while the PVC is made up of polyvinyl chloride and white in colour. These differences can affect the attachment of the different biofilm bacteria due to different properties and structure of the surface (Camps *et al*., 2014). Colour of the substrate is important in determining the attachment and colonization of surfaces by biofilm bacteria (Dobretsov *et al*., 2013; Balqadi *et al*., 2018). Surfaces that differ in hydrophobicity, hydrophilicity, roughness, or topography will be colonized differently (Kim *et al*., 2022). Different biofilm bacteria will attach differently on the same surfaces based on their lifestyle due to their properties such as motility, cell-cell communication (Vance, 2019; Zheng *et al*., 2021). This is an indication on how the type and nature of surfaces dictate the type of biofilm bacteria that will colonize the surfaces and its diversity.

The bacterial biofilm is an important target of antifouling compounds, and their diversity is relevant in the design and implementation of antifouling technologies (Qian *et al*., 2007). Attachment to surfaces by biofilm bacteria determines the success of the initial colonization process and subsequent colonizations (Slightom and Buchan, 2009). Once the diverse bacteria colonize the surfaces, they accumulate forming films on surfaces which aggregate and begin the process of the microfouling. The microfouling assemblage is held firmly by a matrix of EPS which comprises of majorly polysaccharides,

proteins, as slimy layer which allow the biofilm bacteria to colonize many surfaces. The bacterial cells are closely associated with each other in high densities (Sutherland, 1999). Other factors that support the aggregation of the biofilm structure, shape and functions comprises of intra and inter species interaction among the species and ability to survive the competition (Dang and Lovell, 2015).

The domination of *Pseudoalteromonas* is reflected on its ability to outcompete other species in the biofilm formation process. *Pseudoalteromonas* is well known to be significant biofilm forming bacteria in biofouling process for it out competition of other species in biofilm communities and in inducing metamorphosis and settlement of marine invertebrates (Bowman, 2007). The organism is known to grow rapidly and form biofilm at a faster rate couple with the production of inhibitory compounds against other bacteria (Rao *et al*., 2005). This gives the organism greater advantage than other bacteria forming the multispecies biofilm on surfaces in natural biofilm formation (Rao *et al*., 2010). Multispecies biofilm is evidence of inter/intra specific association among the bacteria with benefits or inhibition through their metabolic products and it's the survival of the fittest through competition (Guillonneau *et al*., 2018).

The biofilm formation observed in the tubes and in the microtiter plates is an indication of the strains ability to form biofilm. Formation of biofilm in a ring form on the side or bottom of the well in the tubes via the CV stain is an evidence of biofilm formation (O'Toole, 2011). The evidence of biofilm formation is necessary to determine the ability to form microfouling assemblage by the bacteria. Members of the genera identified in this study *Halomonas Marinomonas, vibrio, Psychrobacter* and *Pseudoalteromonas* were reported to produce biofilm on surfaces (Heyrman *et al*., 2002; Rao *et al*., 2010; Bernbom *et al*., 2013; Brian-Jaisson *et al*., 2016; Balqadi *et al*., 2018; Aykin *et al*., 2019; Delacuvellerie *et al*., 2021). While biofilm formation gives the bacteria the ability to overcome many stresses in the marine environment, but its deleterious effect on surfaces leading to microfouling development causes damage to marine structures and installations (de Carvalho, 2018).

5. Conclusion

In conclusion, several biofilm bacteria from different genera are involved in the formation of microfouling assemblage on surfaces through their ability to form biofilm structure. The organisms form multispecies biofilm and most of the biofilm bacteria are Gram-negative bacteria. The organisms are important consideration in antifouling studies and can be good candidates for used as target in antibiofilm assays.

References

Afonso, A. C., Gomes, I. B., Saavedra, M. J., Giaouris, E., Simoes, L. C. and **Simoes, M.** (2021). Bacterial coaggregation in aquatic systems. *Water Research,* **196**. doi:10.1016/j.watres.2021.117037

Aykin, E., Omuzbuken, B. and **Kacar, A.** (2019). Microfouling bacteria and the use of enzymes in eco-friendly antifouling technology. *Journal of Coatings Technology and Research,* **16**(3): 847-856. doi:10.1007/s11998-018-00161-7

Balqadi, A. A., Salama, A. J. and **Satheesh, S.** (2018). Microfouling development on artificial substrates deployed in the central Red Sea. *Oceanologia,* **60**(2): 219-231. doi:https://doi.org/10.1016/j.oceano.2017.10.006

Bernbom, N., Ng, Y. Y., Olsen, S. M. and **Gram, L.** (2013). Pseudoalteromonas spp. serve as initial bacterial attractants in mesocosms of coastal waters but have subsequent antifouling capacity in mesocosms and when embedded in paint. *Applied and Environmental Microbiology,* **79**(22): 6885-6893. doi:10.1128/AEM.01987-13

Bowman, J. P. (2007). Bioactive Compound Synthetic Capacity and Ecological Significance of Marine Bacterial Genus Pseudoalteromonas. *Marine Drugs,* **5**(4): 220-241.

Brian-Jaisson, F., Molmeret, M., Fahs, A., Guentas-Dombrowsky, L., Culioli, G., Blache, Y., . . . Ortalo-Magné, A. (2016). Characterization and anti-biofilm activity of extracellular polymeric substances produced by the marine biofilm-forming bacterium Pseudoalteromonas ulvae strain TC14. *Biofouling,* **32**(5): 547-560. doi:10.1080/08927014.2016.1164845

Briand, J.-F., Djeridi, I., Jamet, D., Coupé, S., Bressy, C., Molmeret, M., . . . Blache, Y. (2012). Pioneer marine

biofilms on artificial surfaces including antifouling coatings immersed in two contrasting French Mediterranean coast sites. *Biofouling*, **28**(5): 453-463. doi:10.1080/08927014.2012.688957

Burchard, R. P. and **Sorongon, M. L.** (1998). A gliding bacterium strain inhibits adhesion and motility of another gliding bacterium strain in a marine biofilm. *Appl Environ Microbiol*, **64**(10): 4079-4083. doi:10.1128/aem.64.10.4079-4083.1998

Camps, M., Barani, A., Gregori, G., Bouchez, A., Berre, B. L., Bressy, C., . . . Lovell, C. R. (2014). Antifouling Coatings Influence both Abundance and Community Structure of Colonizing Biofilms: a Case Study in the Northwestern Mediterranean Sea. *Applied and Environmental Microbiology*, *80*(16): 4821-4831. doi:doi:10.1128/AEM.00948-14

Chen, C.-L., Maki, J. S., Rittschof, D. and **Teo, S. L.-M.** (2013). Early marine bacterial biofilm on a copper-based antifouling paint. *International Biodeterioration and Biodegradation*, *83*, 71-76. doi:https://doi.org/10.1016/j.ibiod.2013.04.012

Cruz, H. (2020). *Biofouling Challenges and Possible Solutions, 1.20.003 – Final Project Report.* Retrieved from Launceston, Australia.

Dang, H. and **Lovell, C. R.** (2015). Microbial Surface Colonization and Biofilm Development in Marine Environments. *Microbiology and molecular biology reviews : MMBR*, **80**(1): 91-138. doi:10.1128/MMBR.00037-15

de Carvalho, C. C. (2018). Marine Biofilms: A Successful Microbial Strategy With Economic Implications. *Frontiers in Marine Science*, **5**: 126.

Delacuvellerie, A., Benali, S., Cyriaque, V., Moins, S., Raquez, J.-M., Gobert, S. and **Wattiez, R.** (2021). Microbial biofilm composition and polymer degradation of compostable and non-compostable plastics immersed in the marine environment. *Journal of Hazardous Materials*, **419**: 126526. doi:https://doi.org/10.1016/j.jhazmat.2021.126526

Dobretsov, S., Dahms, H.-U. and **Qian, P.-Y.** (2006). Inhibition of biofouling by marine microorganisms and their metabolites. *Biofouling*, **22**(1): 43-54. doi:10.1080/08927010500504784

Dobretsov, S. and **Qian, P.-Y.** (2006). Facilitation and inhibition of larval attachment of the bryozoan Bugula neritina in association with mono-species and multi-species biofilms. *Journal of Experimental Marine Biology and Ecology*, **333**(2): 263-274. doi:https://doi.org/10.1016/j.jembe.2006.01.019

Dobretsov, S., Abed, R. M. M. and **Voolstra, C. R.** (2013). The effect of surface colour on the formation of marine micro and macrofouling communities AU - Dobretsov, Sergey. *Biofouling*, **29**(6): 617-627. doi:10.1080/08927014.2013.784279

Farkas, A., Degiuli, N. and **Martić, I.** (2021). The impact of biofouling on the propeller performance. *Ocean Engineering*, **219**: 108376. doi:https://doi.org/10.1016/j.oceaneng.2020.108376

Flemming, H.-C., Neu, T. R. and **Wozniak, D. J.** (2007). The EPS matrix: the "house of biofilm cells". *Journal of Bacteriology*, **189**(22): 7945-7947. doi:10.1128/JB.00858-07

Flemming, H.-C. (2008). Why Microorganisms Live in Biofilms and the Problem of Biofouling. In *Springer Series on Biofilms* (pp. 1-10). Berlin, Heidelberg: Springer Berlin Heidelberg.

Flemming, H.-C. (2011). Microbial Biofouling: Unsolved Problems, Insufficient Approaches, and Possible Solutions. In H.-C. Flemming, J. Wingender, and U. Szewzyk (Eds.), *Biofilm Highlights* (pp. 81-109). Berlin, Heidelberg: Springer Berlin Heidelberg.

Goecke, F., Labes, A., Wiese, J. and **Imhoff, J. F.** (2010). Chemical interactions between marine macroalgae and bacteria. *Marine Ecology Progress Series*, **409**: 267-299. doi:https://doi.org/10.3354/meps08607

Grasland, B., Mitalane, J., Briandet, R., Quemener, E., Meylheuc, T., Linossier, I., . . . Haras, D. (2003). Bacterial biofilm in seawater: Cell surface properties of early-attached marine bacteria. *Biofouling*, **19**(5): 307-313. doi:10.1080/0892701031000121041

Guillonneau, R., Baraquet, C., Bazire, A. and **Molmeret, M.** (2018). Multispecies Biofilm Development of Marine Bacteria Implies Complex Relationships Through Competition and Synergy and Modification of Matrix Components. *Frontiers in Microbiology*, **9**. doi:10.3389/fmicb.2018.01960

Hadfield, M. G. (2010). Biofilms and Marine Invertebrate Larvae: What Bacteria Produce That Larvae Use to Choose Settlement Sites. *Annual Review of Marine Science*, **3**(1): 453-470. doi:10.1146/annurev-marine-120709-142753

Haney, E. F., Trimble, M. J., Cheng, J. T., Vallé, Q. and **Hancock, R. E. W.** (2018). Critical Assessment of Methods to Quantify Biofilm Growth and Evaluate Antibiofilm Activity of Host Defence Peptides. *Biomolecules*, **8**(2): 29. doi:10.3390/biom8020029

Hearin, J., Hunsucker, K. Z., Swain, G., Gardner, H., Stephens, A. and **Lieberman, K.** (2016). Analysis of mechanical grooming at various frequencies on a large scale test panel coated with a fouling-release coating. *Biofouling*, **32**(5): 561-569. doi:10.1080/08927014.2016.1167880

Heyrman, J., Balcaen, A., De Vos, P. and **Swings, J.** (2002). Halomonas muralis sp. nov., isolated from microbial biofilms colonizing the walls and murals of the Saint-Catherine chapel (Castle Herberstein, Austria). *Int J Syst Evol Microbiol*, **52**(Pt 6): 2049-2054. doi:10.1099/00207713-52-6-2049

Kerr, A., Cowling, M. J., Beveridge, C. M., Smith, M. J., Parr, A. C. S., Head, R. M., . . . Hodgkiess, T. (1998). The early stages of marine biofouling and its effect on two types of optical sensors. *Environment International*, **24**(3): 331-343. doi:https://doi.org/10.1016/S0160-4120(98)00011-7

Kim, T., Kwon, S., Lee, J., Lee, J. S. and **Kang, S.** (2022). A metallic anti-biofouling surface with a hierarchical

topography containing nanostructures on curved micro-riblets. *Microsystems and Nanoengineering,* **8**(1): 6, doi:10.1038/s41378-021-00341-3

Kumar, S., Stecher, G., Li, M., Knyaz, C. and **Tamura, K.** (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution,* **35**(6): 1547-1549.

Lagha, R., Ben Abdallah, F., AL-Sarhan, B. O. and **Al-Sodany, Y.** (2019). Antibacterial and Biofilm Inhibitory Activity of Medicinal Plant Essential Oils Against Escherichia coli Isolated from UTI Patients. *Molecules,* **24**(6): 1161.

Lorite, G. S., Rodrigues, C. M., de Souza, A. A., Kranz, C., Mizaikoff, B. and **Cotta, M. A.** (2011). The role of conditioning film formation and surface chemical changes on Xylella fastidiosa adhesion and biofilm evolution. *Journal of Colloid and Interface Science,* **359**(1): 289-295. doi:https://doi.org/10.1016/j.jcis.2011.03.066

Mathew, N. T., Kronholm, J., Bertilsson, K., Despeisse, M. and **Johansson, B.** (2021). Environmental and Economic Impacts of Biofouling on Marine and Coastal Heat Exchangers. In Y. Kishita, M. Matsumoto, M. Inoue, and S. Fukushige (Eds.), *EcoDesign and Sustainability II: Social Perspectives and Sustainability Assessment* (pp. 385-398). Singapore: Springer Singapore.

Matz, C., Bergfeld, T., Rice, S. A. and **Kjelleberg, S.** (2004). Microcolonies, quorum sensing and cytotoxicity determine the survival of Pseudomonas aeruginosa biofilms exposed to protozoan grazing. *Environ Microbiol,* **6**(3): 218-226. doi:10.1111/j.1462-2920.2004.00556.x

O'Toole, G. A. (2011). Microtiter dish biofilm formation assay. *JoVE (Journal of Visualized Experiments)*(47), e2437.

Oliveira, D. and **Granhag, L.** (2016). Matching Forces Applied in Underwater Hull Cleaning with Adhesion Strength of Marine Organisms. *Journal of marine science and engineering,* **4**(4): 66.

Papadatou, M., Robson, S. C., Dobretsov, S., Watts, J. E. M., Longyear, J. and **Salta, M.** (2021). Marine biofilms on different fouling control coating types reveal differences in microbial community composition and abundance. *MicrobiologyOpen,* **10**(4): e1231-e1231. doi:10.1002/mbo3.1231

Qian, P. Y., Lau, S. C. K., Dahms, H. U., Dobretsov, S. and **Harder, T.** (2007). Marine Biofilms as Mediators of Colonization by Marine Macroorganisms: Implications for Antifouling and Aquaculture. *Marine Biotechnology,* **9**(4): 399-410. doi:10.1007/s10126-007-9001-9

Rao, D., Webb, J. S. and **Kjelleberg, S.** (2005). Competitive interactions in mixed-species biofilms containing the marine bacterium Pseudoalteromonas tunicata. *Applied and Environmental Microbiology,* **71**(4): 1729-1736. doi:10.1128/AEM.71.4.1729-1736.2005

Rao, D., Webb, J. S. and **Kjelleberg, S.** (2006). Microbial colonization and competition on the marine alga Ulva australis. *Appl Environ Microbiol,* **72**(8): 5547-5555. doi:10.1128/aem.00449-06

Rao, D., Skovhus, T., Tujula, N., Holmström, C., Dahllöf, I., Webb, J. S. and **Kjelleberg, S.** (2010). Ability of Pseudoalteromonas tunicata to colonize natural biofilms and its effect on microbial community structure. *FEMS Microbiol Ecol,* **73**(3): 450-457. doi:10.1111/j.1574-6941.2010.00917.x

Renner, L. D. and **Weibel, D. B.** (2011). Physicochemical regulation of biofilm formation. *MRS Bull,* **36**(5): 347-355. doi:10.1557/mrs.2011.65

Salta, M., Wharton, J. A., Blache, Y., Stokes, K. R. and **Briand, J. F.** (2013). Marine biofilms on artificial surfaces: structure and dynamics. *Environ Microbiol,* **15**(11): 2879-2893. doi:10.1111/1462-2920.12186

Sawant, S. S., Khandeparker, D., Tulaskar, A., Venkat, K. and **Garg, A.** (1995). Corrosion and microfouling of copper and its alloys in a tropical marine waters of India (Mangalore). *Indian Journal of Chemical Technology,* **2**(6): 322-326.

Slightom, R. N. and **Buchan, A.** (2009). Surface Colonization by Marine Roseobacters: Integrating Genotype and Phenotype. *Applied and Environmental Microbiology,* **75**(19): 6027-6037. doi:doi:10.1128/AEM.01508-09

Sutherland, I. W. (1999). Biofilm Exopolysaccharides. In J. Wingender, T. R. Neu, and H.-C. Flemming (Eds.), *Microbial Extracellular Polymeric Substances: Characterization, Structure and Function* (pp. 73-92). Berlin, Heidelberg: Springer Berlin Heidelberg.

Vance, T. D. (2019). *Adhesion Proteins: Keeping Bacteria in Their Place.* Queen's University (Canada).

Zhang, Z., Schwartz, S., Wagner, L. and **Miller, W.** (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational biology: A Journal of Computational Molecular Cell Biology,* **7**(1-2): 203-214. doi:10.1089/10665270050081478

Zheng, S., Bawazir, M., Dhall, A., Kim, H.-E., He, L., Heo, J. and **Hwang, G.** (2021). Implication of Surface Properties, Bacterial Motility, and Hydrodynamic Conditions on Bacterial Surface Sensing and Their Initial Adhesion. *Frontiers in Bioengineering and Biotechnology,* **9**. doi:10.3389/fbioe.2021.643722

Zobell, C. E. and **Allen, E. C.** (1935). The Significance of Marine Bacteria in the Fouling of Submerged Surfaces. *Journal of Bacteriology,* **29**(3): 239-251. doi:10.1128/jb.29.3.239-251.1935

عزل والتعرف على بكتيريا الأغشية الحيوية من تجمع الحشف الدقيق المطوَّر على مواد اصطناعية مغمورة في البحر الأحمر

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المستخلص. تعتبر بكتيريا الأغشية الحيوية الرقيقة مستعمرات سطحية أولية تقوم بتجميع الحشف الحيوي البحري على الأسطح المغمورة، وتعتبر في المرحلة المبكرة من الحشف الدقيق. وحيث الحاجة المكتسبة في تصميم العلاج المضاد للحشف، نظرًا لقدرتها على بدء الحشف الحيوي، ودعم الاستعمار اللاحق للحشف الكبير. في هذه الدراسة، تم عزل العديد من بكتيريا الأغشية الحيوية من طبق بتري وأنابيب البولي فينيل كلوريد (PVC) المغمورة في ساحل البحر الأحمر لمدة أسبوع، كما تم عزل البكتيريا المكونة للغشاء الحيوي عن طريقة لوحة الانتشار تحت ظروف قياسية، وتحديدها بواسطة طريقة تسلسل الرئا الريباسي ٥١٦. وتم تقييم كل من العزلات من حيث تكوين الأغشية الحيوية نوعيًا باستخدام مقايسة الأنبوب، ثم استخدام فحص لوحة Microtiter لتحديد البيوفيلم الذي تتجه الكائنات الحية المختارة، وتمكن ١١ نوعًا من البكتيريا المعزولة من أصل ٢١ من تكوين غشاء حيوي تحت ظروف المختبر. ومعظم العزلات (٧ من ١١) من جنس *Pseudoalteromonas* وعزلة واحدة من *Halomonas*، و*Marinomonas*، و*Vibrio*، و*Psychrobacter*. وأوضحت الدراسة أن المجتمع البكتيري المكون للأغشية الحيوية على ركائز صلبة في البحر الأحمر متنوع وقادر على تكوين أغشية حيوية على الأسطح في الظروف المعملية. ويمكن استخدام هذه العزلات ككائنات دقيقة مستهدفة لفحوصات إيجاد مضادات للحشف.

الكلمات المفتاحية: بيوفيلم، بكتيريا، الحشف الحيوي، البحر الأحمر.

JKAU: Mar. Sci., **Vol. 31** No. 2 pp: 55-65 (1442 A.H. / 2021 A.D.)
DOI :10.4197/Mar.31-2.5

Effect of Disinfectants on Sea Cucumber Juveniles (*Holothuria scabra*) in Farming Practices

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Abstract. The rapid development of the intensive sea cucumber industry poses the appearance of potential threats, which makes it necessary to develop safe concentrations of common disinfectants used in aquaculture for controlling pathogens or infestation of predators. In this study, the effect of dip immersion and prolonged exposure of disinfectants on sea cucumber was investigated in order to formulate the optimum treatment concentrations. Acetic acid, formalin-F^{TM}, copper sulphate, methylene blue, trichlorfon, potassium permanganate and dechlorinated freshwater were used as disinfectants. The effects such as evisceration, skin lesion and mortality were determined during exposure and post-exposure. The 1-min dip immersion of all chemicals used in the study was safe for disinfectant treatments and did not show any negative impact on sea cucumbers. The effect of prolonged exposure varied based on chemical toxicity, chemical concentration, and duration of exposure. Sea cucumber was less sensitive to acetic acid and trichlorfon for prolonged treatment and tolerates a wide range of safe concentrations up to 100 mg l^{-1} for acetic acid and 4 mg l^{-1} for trichlorfon. Sea cucumber was more sensitive to prolonged exposure to formalin, copper sulphate and methylene blue which caused severe effects. The upper-level concentrations which considered safe and did not show significant effect were 1 mg l^{-1}, 0.05 mg l^{-1} and 4 mg l^{-1} for formalin, copper sulphate and methylene blue respectively. Potassium permanganate at 2 mg l^{-1} was very toxic to the sea cucumber. Freshwater bath for 1 and 10 minutes considered safe treatments for sea cucumber and no negative impact was observed. The study concluded that short dip immersion is a potential treatment for sea cucumber and considered safe to control the external pathogens and predators. Prolonged treatment with freshwater, acetic acid and trichlorfon could also be positively considered for sea cucumber treatment due to low sensitivity. Even though some of the disinfectants used in this study were toxic, they can be used at lower concentrations.

Keywords: Aquaculture, Sea cucumbers, Echinoderms, Disease control, Pond management.

1. Introduction

The populations of sea cucumber (*Holothuria scabra*) are threatened by the depletion by artisanal fisheries due to the high price for human consumption and their precious nature (Choo, 2008). Therefore, since the first application of artificial sea cucumber breeding techniques in the 1980s, attempts have made to establish and refine the breeding protocols (Wang *et al*., 2004). The massive development and entrenchment of sea cucumber cultivation have resulted in the emergence of multiple diseases, causing significant economic losses for aquaculture and being one of the constraints of the industry's sustainable production (Michael and Kelly 2015). Furthermore, many parasites such as protozoans, turbellarians and gastropods besides to pathogenic bacteria and fungi emerge in the system when growing the sea cucumber in outdoor ponds and cause ulcers, skin erosion, and many diseases that contribute to high mortality, which can exceed 80 percentage of the yield (Jangoux, 1987, 1990;

Smith, 1984; Wang *et al*., 2004). Snails can attack sea cucumbers causing death with eviscerated animals. Depending on the severity of the injury, the healing process might take one to several weeks (Tresnati *et al*., 2019). Evisceration is a type of autotomy performed by many holothurian echinoderms which include ejection of the digestive tract and other internal organs (Emson and Wilkie 1980). Evisceration was found to be a response to artificial stimuli such as body injury, overcrowding or water pollution (Wilkie, 2001).

Many disinfectant compounds are used in fish culture ponds to reduce disease-causing parasites and pathogen bacteria (Jo and Andrew, 2007). In fish farming, formalin is used as disinfection to destroy parasites such as monogeneans and ciliated protozoans or it may be used for ammonia elimination and plankton killing at 25-40 ppm (Leal *et al*., 2018). On the other hand, potassium permanganate ($KMnO_4$) at 5 ppm is the most widely used inorganic compounds globally for the treatment of external parasites and bacterial disease which helps the healing of wounds (Duncan, 1978). Moreover, some compounds such as methylene blue, trichlorfon and potassium nitrate, etc., are also used in disinfection and cause considerable toxicity when their concentrations rise (Claude and Laurence, 1999; Tonguthai, 2000; Park *et al*., 2019). The attack of predators and disease outbreaks were one of the main issues of sea cucumber *Holothuria scabra* production in the outdoor ponds (Tresnati *et al*., 2019).

Previous studies discussed the effect of different chemical disinfectants such as acetic acid (Forrest *et al*., 2007; Locke *et al*., 2009; Meira-Filho *et al*., 2017), copper sulphate (Bambang *et al*., 1995; Chen and Lin, 2001), formalin (Speare *et al*., 1997; Buchmann *et al*., 2004; Lamela *et al*., 2008), methylene blue (Turnipseed *et al*., 1997; Park *et al*., 2019), potassium permanganate (Duncan, 1978; Mischke *et al*., 2014) and trichlorfon (Meyer 1966; Flores-Nava and Vizcarra-Quiroz, 1988) on aquatic organisms. Therefore, the main objective of this study was to determine the safe concentration of different common disinfectant used in aquaculture on farmed sea cucumber *Holothuria scabra*.

2. Material and Methods

2.1 Animal Source and Acclimatization

The experiment was conducted in the fish farm at the Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia under lab-controlled conditions. Sea cucumber juveniles (average body weight: 35±5g) were obtained from the National Aquaculture Group, Saudi Arabia. Animals were transported in a 300L tub to the experimental site with filtered (10 microns) seawater (40 psu salinity, temperature 26°C) with oxygen supply. Animals were acclimatized in a fiberglass tank of 1.9 m diameter for one-week period (40 psu salinity, 7.9 pH and temperature 29°C). The bottom of the tank was covered with a substrate of 5 cm dune sand. Water exchange was carried out daily at 100% during the acclimatization period and animals were fed with a compound diet (14.5% protein, 4.3% lipid, 38% ash and 2395 kcal kg^{-1} energy) at the ratio of 2% of body weight.

2.2 Setup of the Experiment

The effect of seven treatments on sea cucumber was investigated. Each treatment was conducted in triplicates using a 250L fiberglass tank (Fig.1). All buckets were stocked with four sea cucumbers each with an average body weight of 35±5g. The treatments tested in the experiment were Acetic acid (CH_3COOH- 99.8%), Formalin-F^{TM} (37%), Copper Sulphate ($CuSO_4.5H_2O$- 25%), Methylene blue ($C_{16}H_{18}ClN_3S.xH_2O$- 85%), Trichlorfon (2, 2, 2 trichloro-1-hydroxyethylphosphonate- 94%), Potassium permanganate ($KMnO_4$- 97%) and dechlorinated freshwater. Each chemical treatment was examined in dip immersion for a minute of exposure and prolonged exposure for 48 hours except the freshwater treatment

which was conducted for a dip immersion of one and ten minutes. Animals were starved for 24 hours prior to conducting the treatments exposure. Upon the completion of each treatment, survived treated animals from both dip and prolonged exposure were washed and transferred to post-exposure observation tanks of 500L covered with 5cm dune sand for a period of 168 hours (7 days). Mortality, skin lesion and evisceration were observed during the exposure and post-exposure period. No feeding was given to animals during the exposure period whereas animals were fed in recovery post-exposure tanks at the rate of 2% after 24 hours of transfer. Observations in the post-exposure tanks were recorded every 24 hours and Dead animals were removed. The observation factors as mortality, skin lesion and evisceration were converted to percentage from the total number of animals in the tank. The selected chemicals are usually used in the aquaculture sector for pathogen removal at the concentrations reported by Noga (2010). However, higher chemical concentrations were tested in this study to determine the toxicity on sea cucumbers.

3. Results

Short dip immersion of studied treatments showed no mortality, skin lesion and evisceration on sea cucumber during exposure period or during the post-exposure period (Table 1). The results of prolonged chemical toxicity on sea cucumber showed various abnormal behaviours during exposure as a sign of stress such as rolling motion, size shrinking and at severely affected stage evisceration of intense and various levels of skin lesion (light to high) on the dorsal and ventral side of Sandfish were observed. Acetic acid did not cause any harmful effect on sea cucumber up to 75 mg l^{-1} whereas a noticeable increase of skin lesion was observed with a rise of concentrations at 100, 125 and 150 mg l^{-1} which recorded 10, 50, 100%, respectively. Mass mortality of 100% was noticed in sea cucumber when exposed to 150 mg l^{-1} acetic for 48 hours.

The formalin has shown varied toxicity effects on the sea cucumber. The formalin at 1 mg l^{-1} was very safe whereas 5 mg l^{-1} showed 100% skin lesion (12 animals) and 50% evisceration (6 animals). The concentration of 10 and 20 mg l^{-1} formalin was very toxic and recorded 100% (12 animals) mortality with 10 mg l^{-1} at 48 hours and with 20 mg l^{-1} at 24 hours of exposure. The high concentration of formalin showed skin burn after 8 hours of exposure (Fig. 2). Copper sulphate of 0.05 mg l^{-1} did not show any effect on sea cucumber whereas 0.06 mg l^{-1} resulted in 50% mortality during the post-exposure period. Effects on sea cucumber appeared to increase with a rise of copper sulphate concentration. Copper sulphate 0.08 mg l^{-1} showed 25% mortality at 48 hours of exposure and rose to 100% during the post-exposure period. The concentrations of 0.1 and 0.2 mg l^{-1} resulted in 100% mortality in sea cucumber at 24 and 48 hours, respectively.

Methylene blue at 2 mg l^{-1} was safe and did not show any effect on sea cucumber whereas 4 mg l^{-1} showed 25% evisceration at 48 hours of exposure without any mortality during exposure or post-exposure. The concentrations of 5 and 6 ppm copper sulphate were highly toxic and showed a severe effect of skin lesion and evisceration and resulted in 50% and 100% at 48 hours of exposure with 5 and 6 mg l^{-1}. Trichlorfon concentrations at 2, 3 and 4 mg l^{-1} did not record any mortality during treatment or post-exposure period. A slight effect was noticed in 4 mg l^{-1} resulted in 11% evisceration during the post-exposure period whereas potassium permanganate at 2 mg l^{-1} was very toxic and showed 100% mortality, skin lesion and evisceration at 48 hours exposure period. Further, the freshwater treatment bath in 1 and 10 minutes did not show any effect on sea cucumber health or survival during exposure or post- treatment.

4. Discussion

The global rapid growth of sea cucumber intensive farming necessitated to investigate about the toxicity of some common chemical

disinfectants and to develop safe concentrations for use to control pathogens or infestation of predators. Previous studies reported the outbreak of diseases on sea cucumbers resulted in significant mortality such as viral diseases (Yin-Geng *et al*., 2005; Wang *et al*., 2007; Deng *et al*., 2008), bacterial diseases (Ma *et al*., 2006; Deng *et al*., 2009; Lu *et al*., 2015; Zhang *et al*., 2015), fungal diseases (Yin-Geng *et al*., 2005) predatory of copepods and snails (Liu *et al*., 2002; Tresnati *et al*., 2019).

Sea cucumber exposed to chemical disinfectants showed extreme mortality. However, the mortality occurred within the groups exposed to the high concentration and this could be due to the ability of acclimation reaction in sea cucumber under the chronic metal exposure (Li *et al*., 2016). It was reported by Tresnati *et al*. (2019) that the small skin lesions due to the effect of predators on sea cucumber *Holothuria scabra* can be healed in a week and larger could take longer period (2-3 weeks) for complete healing whereas eviscerated sea cucumber was unable to accumulate the energy needs for regenerating the internal organs and healing the body lesions. It was revealed that eviscerated sandfish *Holothuria scabra* can regenerate internal organs after viscera ejection and the development could take 30 days (Dolmatov *et al*., 2012).

In the current study, acetic acid for prolonged bath up to 125 mg l^{-1} for 48 hours exposure period was safe for treating the sea cucumber. Previous studies reported the use of acetic acid as anti-parasitic using different protocols of concentrations and exposure time. Harms (1996) recommend the concentration of 500 mg l^{-1} for 30 seconds for the elimination of protozoa. It was concluded by Meira-Filho (2017) that a bath of glacial acetic acid for one hour at 238 and 467 mg l^{-1} is effective against Ciliophora protozoans and the registered mortality in exposed fish juveniles *Mugil liza* was 0 and 5%, respectively. Another study reported that acute toxicity (96 hours) in sand shrimp showed no mortality up to the exposure of 50 mg l^{-1} acetic acid and 100% mortality was recorded at 100 mg l^{-1} whereas chronic toxicity (14 days) showed 15% and 100% mortality in sand shrimp when exposed to 100 mg l^{-1} and 320 mg l^{-1} acetic acid (Locke *et al*., 2009).

The result of the current study indicates that sea cucumber is sensitive to formalin. Although dip immersion at 250 mg l^{-1} for 10 min did not affect the sea cucumber, a high effect has appeared on sea cucumber when exposed to formalin for 48 hours resulting in 100% evisceration at exposure of 5 mg l^{-1} and 100% mortality at 10 mg l^{-1}. It was revealed that formalin is used in aquaculture for water treatment and to kill the infectious agents as prophylactic or with therapeutic measure and found to be highly successful against most protozoan parasites and monogenetic trematodes (FDA, 1995; Francis-Floyd, 1996; Shao, 2001; Leal *et al*., 2018). The recommended dose of formalin for short dip treatment is up to 250 mg l^{-1} for 1-hour bath (Scott, 1993; Francis Floyd, 1996; Shepherd and Bromage, 2001). Previous studies on olive flounder *Paralichthys olivaceus* fingerlings registered the lethal concentration (LC_{50}) of 182 mg l^{-1} at 48 hours and 141 mg l^{-1} at 96 hours (Jung and Kim 1998). The mean lethal concentration reported in adult shrimp *Streptocephalus seali* was between 15 and 25 mg l^{-1} (Moss 1978).

Copper sulphate is commonly used in shrimp ponds for the elimination of filamentous algae and reducing the abundance of phytoplankton, and in the fish, culture ponds, for treatment of protozoan ectoparasites (Chen and Lin, 2001; Noga, 2010). The dip immersion as well as the prolonged exposure at 0.05 mg l^{-1} for 48 hours of copper sulphate in the current study did not show any harmful effect on sea cucumber, whereas higher concentrations were very toxic to sea cucumbers and caused 25% and 100% mortality when exposed to for 48 hours at 0.08 and 0.1 mg l^{-1}, respectively. It is worth noting

that to kill parasites, free copper ion levels must be held between 0.15 and 0.20 ppm (Noga, 2010). It was reported by Chen and Lin (2001) that copper sulphate has a greater lethal effect at lower salinity and the mean lethal LC_{50} at 48 hours exposure for shrimp juvenile *Penaeus monodon* was 6.92 and 13.15 mg l^{-1} at salinity 15 and 25 psu, respectively.

The methylene blue is applied in aquaculture to control protozoan parasites and could be used as antifungal and to reduce the incidence of bacterial and water mold infection (Turnipseed *et al*., 1997; Noga, 2010). The doses of dip immersion used in the current study were safe for sea cucumber. The 48 hours prolonged exposure up to 4 mg l^{-1} had no effect on sea cucumber while 50% and 100% mortality was recorded at the exposure of 5 and 6 mg l^{-1}, respectively. Noga (2010) recommend using 1-3 mg l^{-1} of methylene blue as prolonged immersion for treatment of ectoparasite. A previous study on the toxicity of methylene blue determined the 48 hours LC_{50} for rainbow trout was 16 ppm (Willford, 1967).

Trichlorfon is useful in eradicating ectoparasites such as trematodes, water lice and for the elimination of undesirable zooplanktonic crustaceans such as copepods (Flores-Nava and Vizcarra-Quiroz, 1988; Wang *et al*., 2005). The current study revealed that sea cucumber can tolerate trichlorfon as no mortality was detected in dip immersion as well as 48 hours of prolonged exposure up to 5 mg l^{-1}. The recommended dose of trichlorfon for dip immersion was between 2 and 5 mg l^{-1} and between 0.5 and 1 mg l^{-1} for marine organisms (Noga, 2010). The lethal toxicity of trichlorfon (48 hours LC_{50}) to fry of *Cichlasoma uwphthalmus* Giinther was 23.71 mg l^{-1} (Flores-Nava and Vizcarra-Quiroz, 1988).

Potassium permanganate is an effective external parasiticide and bactericide, particularly, for protozoan parasites and crustaceans. It is also effective for *Trichodina* or *Ambiphrya* infestations, as well as for external columnaris infections (Tucker and Robinson, 1990; Stoskopf, 1993; Noga, 2010). The current study indicated that dip immersion at 100 mg l^{-1} caused 10% mortality whereas 48 hours prolonged treatment at 2 mg l^{-1} resulted in 100% mortality. Concentration of potassium permanganate recommended is 100 mg l^{-1} for 5-10 minutes as dip immersion and 2 mg l^{-1} for prolonged treatment (Noga, 2010).

The freshwater treatment bath is used for marine ectoparasites and that can be useful with the therapy of clinical case of marine *Trichodina*, some crustaceans and other protozoans Monogenea (Langdon, 1992). The current study showed that freshwater dip had no negative effects on the sea cucumber. Further, previously Noga (2010) recommend the freshwater bath for 3-15 minutes.

Fig. 1. Setup of experimental tanks.

Fig. 2. Skin burn after 8 hours of 20 ppm formalin exposure.

Table 1. Toxicity of dip immersion (1 minute) of chemical treatments.

Treatment	Dosage (mg l^{-1})	Observation (%)	While exposure 1 minute	Post exposure 168 hours
Formalin -FTM	250	Mortality	0	0
		Skin lesion	0	0
		Evisceration	0	0
Copper Sulphate	2	Mortality	0	0
		Skin lesion	0	0
		Evisceration	0	0
	1	Mortality	0	0
		Skin lesion	0	0
		Evisceration	0	0
	0.5	Mortality	0	0
		Skin lesion	0	0
		Evisceration	0	0
Methylene blue	10	Mortality	0	0
		Skin lesion	0	0
		Evisceration	0	0
Trichlorfon	2	Mortality	0	0
		Skin lesion	0	0
		Evisceration	0	0
Potassium permanganate	100	Mortality	10	10
		Skin lesion	10	10
		Evisceration	0	0

Table 2. Toxicity of prolonged exposure (48 hours) of chemical treatments.

Treatment	Dosage (mg l^{-1})	Observation (%)	While exposure		Post exposure
			24 hours	48 hours	168 hours
Acetic acid	75	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	0
	100	Mortality	0	0	0
		Skin lesion	0	10	0
		Evisceration	0	0	0
	125	Mortality	0	0	0
		Skin lesion	0	50	0
		Evisceration	0	75	0
	150	Mortality	0	100	-
		Skin lesion	0	100	-
		Evisceration	50	75	-
Formalin -^{FTM}	1	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	0
	5	Mortality	0	0	0
		Skin lesion	0	100	0
		Evisceration	0	50	0
	10	Mortality	0	100	-
		Skin lesion	0	50	-
		Evisceration	0	100	-
	20	Mortality	100	-	-
		Skin lesion	80	-	-
		Evisceration	100	-	-
Copper sulphate	0.05	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	0
	0.06	Mortality	0	0	50
		Skin lesion	0	50	0
		Evisceration	0	0	50
	0.08	Mortality	0	25	100
		Skin lesion	0	75	-
		Evisceration	0	25	-
	0.1	Mortality	60	100	-
		Skin lesion	30	70	-
		Evisceration	40	100	-
	0.2	Mortality	100	-	-
		Skin lesion	50	-	-
		Evisceration	100	-	-
Methylene blue	2	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	0
	4	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	25	0
	5	Mortality	25	50	0
		Skin lesion	0	0	25
		Evisceration	25	75	0
	6	Mortality	0	100	-
		Skin lesion	0	50	-
		Evisceration	25	100	-
Trichlorfon 92%	2	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	0
	3	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	0
	4	Mortality	0	0	0
		Skin lesion	0	0	0
		Evisceration	0	0	11
Potassium permanganate	2	Mortality	11	100	-
		Skin lesion	55	100	-
		Evisceration	66	100	-

5. Conclusion

Short dip immersion is a potential treatment for sea cucumber and considered safe to control the external pathogens and predators as no obvious negative impact was determined. Prolonged treatment with freshwater, acetic acid and trichlorfon could also be positively considered for sea cucumber treatment due to low sensitivity. Even though some of the disinfectants used in this study were toxic, they can be used at lower concentrations. It is recommended to develop higher concentrations for safe short dip immersion treatment and to determine the efficacy in eliminating the potential predators and pathogens.

Acknowledgements

The authors would like to express the gratitude to the Managing Director of National Aquaculture Group for the support extended. We also thank the Dean, Faculty of Marine Sciences, King Abdulaziz University, Jeddah for the facilities provided at KAU Fish Farm, Obhur.

References

Bambang, Y., Thuet, P., Charmantier-Daures, M., Trilles, J.P. and **Charmantier, G.** (1995) Effect of copper on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* Bate Crustacea, Decapod. *Aquat. Toxicol*., **33**: 125–139. https://www.oieau.org/eaudoc/system/files/documents/7/36325/36325_doc.pdf

Buchmann, K., Bresciani, J. and **Jappe, C.** (2004) Effects of formalin treatment on epithelial structure and mucous cell densities in rainbow trout, *Oncorhynchus mykiss* (Walbaum), skin. *J. Fish Dis*., **27**: 99–104. https://doi.org/10.1111/j.1365-2761.2003.00519.x

Chen, J.C. and **Lin, C.H.** (2001) Toxicity of copper sulfate for survival, growth, molting and feeding of juveniles of the tiger shrimp, *Penaeus monodon*. *Aquaculture*, **192**:55-65. https://doi.org/10.1016/S0044-8486(00)00442-7

Choo, P.S. (2008) Population status, fisheries and trade of sea cucumbers in Asia. In V. Toral-Granda, A. Lovatelli and M. Vasconcellos (eds). Sea cucumbers. A global review of fisheries and trade. FAO Fish. and Aquac. Tech. Pap., 516: 81-118. https://hdl.handle.net/20.500.12348/1511

Claude, E.B. and Laurence Massaut (1999) Risks associated with the use of chemicals in pond aquaculture. *Aquac. Eng.*, **20**: 113–132. https://doi.org/10.1016/S0144-8609(99)00010-2

Deng, H., He, C., Zhou, Z., Liu, C., Tan, K., Wang, N., Jiang, B., Gao, X. and **Liu, W.** (2009) Isolation and pathogenicity of pathogens from skin ulceration disease and viscera ejection syndrome of the sea cucumber *Apostichopus japonicus*. *Aquaculture* **287**: 18-27. https://doi.org/10.1016/j.aquaculture.2008.10.015

Deng, H., Zhou, Z.C. and **Han, J.B.** (2008) An observation on the pathogeny of stomach atrophy in sea cucumber *Apostichopus japonicus* (Selenka) larva by electron microscope. *J. Fish. China*, **2**: 023. https://en.cnki.com.cn/Article_en/CJFDTotal-SCKX200802023.htm

Dolmatov, I.Y., Khang, N.A. and **Kamenev, Y.O.** (2012) Asexual reproduction, evisceration, and regeneration in holothurians (Holothuroidea) from Nha Trang Bay of the South China Sea. *Russ. J. Mar. Biol*., **38**: 243-252. https://doi.org/10.1134/S1063074012030042

Duncan, T.O. (1978) The Use of Potassium Permanganate (KMnO4) in Fisheries: A literature review. National Technical Information Service, PB275397, 56. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB275397.xhtml

Emson, R.H. and **Wilkie, I.C.** (1980) Fission and autotomy in echinoderms. Oceanogr. *Mar. Biol. Ann. Rev*., **18**: 155-250. http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=PASCALZOOLINEINRA8110075348

FDA (1995*) Environmental Impact Assessment for the Use of Formalin in the Control of External Parasites on Fish. Environmental Assessments*, Food and Drug Administration, Washington, D.C.

Flores-Nava, A. and **Vizcarra-Quiroz, J.J.** (1988) Acute toxicity of trichlorfon (Dipterex) to fry of *Cichlasoma urophthalmus* Günther. *Aquacult. Fish. Manage*., **19**:341-345. https://doi.org/10.1111/j.1365-2109.1988.tb00583.x

Forrest, B.M., Hopkins, G.A., Dodgshun, T.J. and **Gardner, J.P.A.** (2007) *Efficacy of acetic acid treatments in the management of marine biofouling. Aquaculture*, 262:319-332. doi:10.1016/j.aquaculture.2006.11.006

Francis-Floyd, S.R. (1996) Use of formalin to control fish parasites. University of Florida Cooperative Extension Service, *Institute of Food and Agriculture Sciences, EDIS*, **5**: 77. https://agrilifecdn.tamu.edu/fisheries2/files/2013/09/Use-of-Formalin-to-Control-Fish-Parasites.pdf

Harms, C.A. (1996) Treatments for parasitic diseases of aquarium and ornamental fish. In Seminars. *J. Exot. Pet Med.,* **2**: 54-63. https://10.1016/S1055-937X(96)80018-1

Jangoux, M. (1987) *Diseases of Echinodermata* (I). Dis. Aquat. Org., 2:147-162. http://www.int-res.com/articles/dao/2/d002p147.pdf

Jangoux, M. and **Kinne, O.** (1990) Diseases of Echinodermata. Dis. Aquat. Org., 3:439-567. https://difusion.ulb.ac.be/vufind/Record/ULB-DIPOT:oai:dipot.ulb.ac.be:2013/89815/Details

Jo, Sadler and **Andrew, Goodwin** (2007) *Disease Prevention on Fish Farms.* SRAC, No. 4703. https://freshwater-aquaculture.extension.org/wp-content/uploads/2019/08/Disease_prevention_on_fish_farms.pdf

Leal, J.F., Neves, M.G.P., Santos, E.B. and **Esteves, V.I.** (2018) Use of formalin in intensive aquaculture: properties, application and effects on fish and water quality. *Rev. Aquac*., **10**: 81-295. https://doi.org/10.1111/raq.12160

Jung, S.H. and **Kim, J.W.** (1998) The lethal concentration (LC50) and lethal time (LT50) of formalin on olive flounder fingerlings, *Paralichthy olivaceus*. *NFRDI*, 54:125–129.

Lamela, R.L., Quintana, Y.C., Silveira, C.R., Martínez, M. and **Herrate, N.G.** (2008) Effects of formalin on total haemocytes count and histopathological changes in the shrimp *Litopenaeus schmitti* (Pérez-Farfante & Kensley 1997). *Aquac. Res.*, **39**: 1316-1321. https://doi.org/10.1111/j.1365-2109.2008.01997.x

Langdon, J.S. (1992) Major protozoan and metazoan parasitic diseases of Australian finfish. In: *Fin Fish Workshop. Proceedings 182*, Post Graduate Committee in Veterinary Science, University of Sydney, Sydney, Australia, 1– 26.

Li, L., Tian, X., Yu, X. and **Dong, S.** (2016) Effects of acute and chronic heavy metal (Cu, Cd, and Zn) exposure on sea cucumbers, *Apostichopus japonicus*. *BioMed Res. Int*. https://doi.org/10.1155/2016/4532697

Liu, X., Gu, B.X. and **Zhang, X.L.** (2002) Several problems in seed-rearing of *Apostichopus japonicus* (Selenka) and corresponding activities. *Mod. Fish. Inf.*, **17**: 26-27.

Locke, A., Doe, K.G., Fairchild, W.L., Jackman, P.M. and **Reese, E.J.** (2009) Preliminary evaluation of effects of invasive tunicate management with acetic acid and calcium hydroxide on non-target marine organisms in Prince Edward Island, *Canada*. *Aquat. Invasions*, **4**: 221-236. https://doi: 10.3391/ai.2009.4.1.23

Lu, M., Zhang, P., Li, C., Zhang, W., Jin, C. and **Han, Q.** (2015) MiR-31 modulates coelomocytes ROS production via targeting p105 in *Vibrio splendidus* challenged sea cucumber *Apostichopus japonicus* in vitro and in vivo. *Fish Shellfish Immunol*., **45**: 293-299. https://doi.org/10.1016/j.fsi.2015.04.024

Ma, Y., Xu, G., Chang, Y., Zhang, E.P., Zhou, W. and **Song, L.S.** (2006) Bacterial pathogens of skin ulceration disease in cultured sea cucumber *Apostichopus japonicus* (Selenka) juveniles. *J. Dalian Fish Univ*., **21**:13-18.

Meira-Filho, M.R.C., Ramirez, J.R.B., Vianna, R.T. and **Júnior, J.P.** (2017) Efficacy of glacial acetic acid in the control of *Trichodina* sp. and *Apiosoma* sp. associated with Mugil liza. *Aquaculture*, **479**: 7-12. https://doi.org/10.1016/j.aquaculture.2017.05.014

Meyer, F.P. (1966) A new control for the anchor worm *Lernea cyprinacea*. *Progressive Fish-Culturist*, **28**:33-9. https://doi.org/10.1577/1548-8640(1966)28[33:ANCFTA]2.0.CO;2

Michael, J.S. and **Kelly S.B.** (2015) Diseases in marine invertebrates associated with mariculture and commercial fisheries. *J. Sea Res*., 16–32. https://doi.org/10.1016/j.seares.2016.06.001

Mischke, C.C., Griffin, M.J., Wise, D.J. and **Greenway, T.E.** (2014) Potassium permanganate is not an effective pond disinfectant to control *Dero digitata*. **J**. *World Aquac. Soc.***, 45**: 350-353. https://doi.org/10.1111/jwas.12118

Noga, Edward (2010). *Fish disease: diagnosis and treatment*. John Wiley & Sons.

Moss, J.L. (1978) Toxicity of selected chemicals to the fairy shrimp, *Streptocephalus seali*, under laboratory and field conditions. Progress. *Fish Cult*., **40**:158-160. https://doi.org/10.1577/1548-8659(1978)40[158:TOSCTT]2.0.CO;2

Park, I., Se-Won, B. and **Ki, H.M.** (2019) The Sterilization Effect of Methylene Blue, Formalin, and Iodine on Egg and Adult Stage of Marine Medaka, *Oryzias dancena*. *Dev. Reprod*., **23**:199-211. https://10.12717/DR.2019.23.3.199

Scott, P. (1993) Therapy in aquaculture. In: Brown L (ed.) *Aquaculture for Veterinarians: Fish Husb*. Med., pp. 447. https://agris.fao.org/agris-search/search.do?recordID=XF2016043746

Shao, Z.J. (2001) Aquaculture pharmaceuticals and biologicals: current perspectives and future possibilities. *Adv. Drug Deliv. Rev*., **50**: 229-243. https://doi.org/10.1016/S0169-409X(01)00159-4

Shepherd, J. and **Bromage, N.** (2001) *Intensive Fish Farming.* Blackwell Science Ltd, Oxford.

Smith, T. (1984) Ultrastructure and function of the proboscis in *Melanella alba* (Gastropoda: Eulimidae). J. Mar. Biolog. Assoc. U.K. *J Mar Biol. Assoc. Uk*., **64**: 503-512. https://doi.org/10.1017/S0025315400030198

Speare, D.J., Arsenault, G., MacNair, N. and **Powell. M.D.** (1997) Branchial lesions associated with intermittent formalin bath treatment of Atlantic salmon, *Salmo salar* L, and rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis., 20: 27–33. https://doi.org/10.1046/j.1365-2761.1997.d01-103.x

Stoskopf, M.K. (1993) *Fish Medicine*. W. B. Saunders Company, Harcourt Brace Javanovich, Inc., Philadelphia, Pennsylvania. http://www.worldcat.org/oclc/925348013

Tonguthai, K. (2000) The use of chemicals in aquaculture in Thailand. In: J. R. Arthur, C. R. Lavilla-Pitogo, & R. P. Subasinghe (Eds.) Use of Chemicals in Aquaculture in Asia: *Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia 20-22 May 1996, Tigbauan, Iloilo, Philippines* (pp. 207-220). Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center. http://hdl.handle.net/10862/597

Tresnati, J., Yasir, I., Aprianto, R., Yanti, A., Bestari, A.D. and **Tuwo, A.** (2019) Predators effects on mortality of sandfish *Holothuria scabra* cultured in multitrophic system. *J. Phys. Conf. Ser*., **1341**: 022-026. https://doi:10.1088/1742-6596/1341/2/022026

Tucker, C.C. and **Robinson, E.H.** (1990) *Channel catfish farming handbook*. Springer Sci. Busi. Media. https://www.springer.com/gp/book/9780412123313

Turnipseed, S.B., Roybal, J.E., Plakas, S.M., Pfenning, A.P., Hurlbut, J.A. and **Long, A.R.** (1997) Determination of Methylene Blue in Channel Catfish (*Ictalurus*

punctatus) Tissue by Liquid Chromatography with Visible Detection. *J. AOAC Int*., **80**: 31-36. https://doi.org/10.1093/jaoac/80.1.31

Wang, Y.G., Zhang, C.Y., Rong, X.J., Chen J.J., Shi, C.Y., Sun, H.L. and **Yan, J.P.** (2004) Diseases of cultured sea cucumber (*Apostichopus japonicus*) in China. Advances in sea cucumber aquaculture and management. FAO Fish. Tech. Rep., 463:297-310. http://www.fao.org/3/y5501e/y5501e10.htm

Wang, P., Chang, Y., Yu, J., Li, C. and **Xu, G.** (2007) Acute peristome edema disease in juvenile and adult sea cucumbers *Apostichopus japonicus* (Selenka) reared in North China. *J. Invertebr. Pathol*., **96**:11-17. https://doi.org/10.1016/j.jip.2007.03.001

Wang, Y.G., Rong, X.J., Zhang, C.Y. and **Sun, S.F.** (2005) Main diseases of cultured *Apostichopus japonicus*: prevention and treatment. *Mar. Sci*., **29**: 1-7 (in Chinese with English Abstract).

Wilkie, I.C. (2001) Autotomy as a prelude to regeneration in echinoderms. *J. Microsc. Res. Tech*., **55**: 369-396. https://doi.org/10.1002/jemt.1185

Willford, W.A. (1967) Toxicity of 22 therapeutic compounds to six fishes. Invest. Fish. Control, Bur. Sport Fish. *Wildlife*, 3-10. http://www.nativefishlab.net/library/textpdf/13281.pdf

Yin-Geng, W., Chun-Yun, Z., Xiao-Jun, R., Jie-Jun, C., Cheng-Yin, S., Hui-ling, S. and **Jing-Ping, Y.** (2005) Diseases of cultured sea cucumber, *Apostichopus japonicus*, in China. FAO Fish. *Tech. Pap*., 297-310. http://www.fao.org/3/y5501e/y5501e10.htm

Zhang J., Cao, Z., Li, Z., Wang, L., Li, H., Wu, F., Jin, L., Li, X., Li, S. and **Xu, Y.** (2015) Effect of bacteriophages on Vibrio alginolyticus infection in the sea cucumber, *Apostichopus japonicus* (Selenka). *J. World Aquac. Soc*., **46**: 149-58. https://doi.org/10.1111/jwas.12177

تأثير المطهرات على زريعة خيار البحر (*Holothuria scabra*) في ممارسات الاستزراع

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المستخلص. يشكل التطور السريع لصناعة خيار البحر المكثف ظهور تهديدات محتملة، مما يجعل من الضروري تطوير تركيزات آمنة للمطهرات الشائعة المستخدمة في الاستزراع المائي للسيطرة على مسببات الأمراض أو لو أصابه المفترسات. في هذه الدراسة، تمت دراسة تأثير الغمر والتعرض المطول للمطهرات على خيار البحر لتكوين تركيزات المعالجة المثلى. تم استخدام حمض الخليك، والفورمالين– FTM، وكبريتات النحاس، والميثيلين الأزرق، والترايكلورفون، وبرمنجنات البوتاسيوم، والمياه العذبة منزوعة الكلور كمطهرات. تم تحديد التأثيرات مثل نزع الأحشاء وآفات الجلد والوفيات أثناء التعرض وبعد التعرض. كان الغمر لمدة دقيقة لجميع المواد الكيميائية المستخدمة في الدراسة آمنًا للمعالجات المطهرة، ولم يُظهر أي تأثير سلبي على خيار البحر. اختلف تأثير التعرض المطول بناءً على السمية الكيميائية والتركيز الكيميائي ومدة التعرض. كان خيار البحر أقل حساسية لحمض الأسيتيك والترايكلورفون للمعالجة المطولة، ويتحمل مجموعة كبيرة من التركيزات الآمنة تصل إلى ١٠٠ مجم لتر$^{-1}$ لحمض الأسيتيك و٤ مجم لتر$^{-1}$ للترايكلورفون. وكان خيار البحر أكثر حساسية للتعرض المطول للفورمالين، وكبريتات النحاس، والميثيلين الأزرق، مما تسبب في تأثيرات شديدة. وكانت تركيزات المستوى الأعلى التي تعتبر آمنة ولم تظهر تأثيرًا معنويًا هي: ١ مجم لتر$^{-1}$، و٠,٠٥ مجم لتر$^{-1}$، و٤ مجم لتر$^{-1}$ للفورمالين وكبريتات النحاس والميثيلين الأزرق على التوالي. وكانت برمنجنات البوتاسيوم عند تركيز ٢ ملجم لتر$^{-1}$ شديدة السمية لخيار البحر. ويعتبر حمام المياه العذبة لمدة ١ و١٠ دقائق أحد العلاجات الآمنة لخيار البحر، ولم يلاحظ أي تأثير سلبي. وخلصت الدراسة إلى أن الغمر بالغطس القصير هو علاج محتمل لخيار البحر، ويعتبر آمنًا للسيطرة على مسببات الأمراض الخارجية وإصابات الحيوانات المفترسة. ويمكن أيضًا النظر بشكل إيجابي في المعالجة المطولة بالمياه العذبة، وحمض الأسيتيك، والترايكلورفون، لمعالجة خيار البحر بسبب الحساسية المنخفضة. وعلى الرغم من أن بعض المطهرات المستخدمة في هذه الدراسة كانت سامة، إلا أنه يمكن استخدامها بتركيزات أقل.

الكلمات المفتاحية: تربية الأحياء المائية، خيار البحر، شوكيات الجلد، مكافحة الأمراض، إدارة الأحواض.