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Identification, Aquaculture Trials and Ecological Associations of Hexacorallian Zoanthids Collected from Selected Inter-Tidal and Underwater Rocky Sites of Pakistan

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Abstract

One intertidal (Buleji) and two diving sites (Mubarak village and Churna Island (2 stations)) were visited for the collection of zoanthids (with *morphotypes*) (*Zoanthus sansibaricus (6)*, *Z. vietnamensis (2)*, *Palythoa tuberculosa* and *P. mutuki (2)* from Buleji), while *Z. sansibaricus (morph-4)* and *P. tuberculosa* from both diving sites. Moreover, 29 intertidal (26 Molluscans and 1 Annelida, Arthropoda and Echinodermata) and 1 underwater Porite (*Tubipora musica*) associates were identified from Buleji and diving station-I of Churna Island, respectively, in order to study their ecology. All zoanthids (including morphotypes) and porite were reared in aquaria to determine their growth potentials in artificial settings. During culture, the recorded parameters ranged between 35-36.5‰ salinity, 7.3-7.7 pH, 25.4-31.0˚C temperature, 0.4-0.3ppm NO_2^-, 0.12-0.08ppm NO_2-N, 500-545mg/l Ca, 0.23-0.25mg/l NH_3 and 7-10mg/l DO_2. The *Z. sansibaricus* (morph-1c) and *Z. vietnamensis* (morph-1), *P. tuberculosa*(c), and *P. mutuki* (morph-1c & 2a) lived up to 21 weeks, showing 41, 51, 18, and 32 & 66 average growth rate or average polyp growth percentage (APG%) respectively, while *Z. sansibaricus* (morph-4a), *P. mutuki* (morph-2b) and *T. musica* were sustained up to 19, 6 and 4 weeks with APG% of 0, -92 and -93, respectively.

Keywords: Hexacorallia, Zoanthids, Buleji, Mubarak Village, Churna Island, Aquaculture, Identification, Ecology

1. Introduction

Hexacoralian zoanthids are delicate, radially symmetrical, diploblastic, and vibrant marine invertebrates attached to intertidal/deep-sea rocks (Gul, 2013; Trivedi and Vachhrajani, 2014) with basal discs (Budarf *et al.,* 2009). Mouth is surrounded by tentacles and six (paired) mesenteries, although 5th pair is complete in suborder Macrocnemina while incomplete in Brachycnemina (Khushali and Pradeep, 2013; Krishna and Gophane, 2013). Van der Land and Hartog (2001) proposed their taxonomy, although molecular grade identification is required in addition to morphological parameters (Reimer *et al*., 2007). They are famous epizoic animals (Nasir *et al*., 2018), associated with Annelida, Arthropoda, Cnidaria, Crustacea, Echinodermata, Mollusca, Porifera (Khushali, 2015), and photosynthetic algae, *Zooxanthellae* (Rabelo *et al*., 2015a). Recent anthropogenic stressors negatively affect wild zoanthids (Lin *et al.,* 2018), while their aquaculture seems to be difficult due to toxic secretion abilities (Hamade *et al.,* 2015). However, rearing experiments might conserve these species.

Pakistan possesses 990km coastline (Mangroves for the future, 2016). *Zoanthus sansibaricus* is found from Manora, Karachi (Gul, 2013; Morandini *et al.*, 2015; Nasir *et al.,* 2018), and a natural product zoanthaminone was isolated from *Zoanthus* sp (Atta-ur-Rahman, 1989). However, no published work was related to their aquaculture except Nasir *et al.*, (2018), who identified and reared *Zoanthus sansibaricus*, *Z. vietnamensis*, *Epizoanthus scotinus,* and *Palythoa tuberculosa.* Thus, the aim and objectives of this study are to provide awareness for their replenishment via aquaculture and identify not only the zoanthid species in and around Karachi coasts but also the factors affecting their normal growth rates and their ecological associations to highlight positive and negative interactions.

2. Materials and Methods

From October 2018 to April 2019, zoanthids were collected from intertidal rocks of Buleji (24^o 50’ 355” N 066^o 50’ 367” E), ~8m depth from Mubarak Village (24^o 51’ 599” N 066^o 38’ 804” E), and two stations of Churna Island (Station I: 24^o 54’ 890” N 066^o 36’ 840” E and Station II: 24^o 53’ 926” N 066^o 35’ 508” E), using SCUBA. A one-square-foot quadrate was placed randomly to collect the samples by hammer and chisel. Live samples were transferred to laboratory for rearing. The details are given below:

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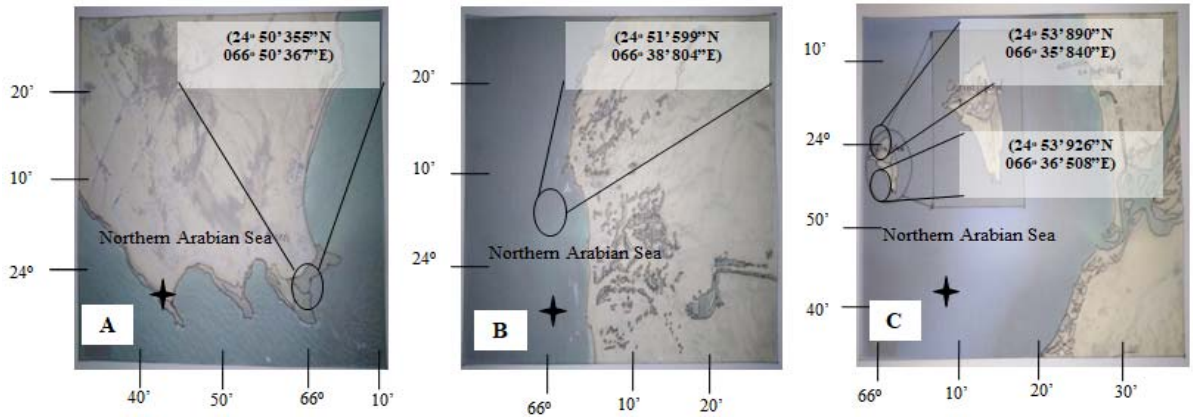


Figure 1: Maps of Collection Sites: (A) Buleji Coast, (B) Mubarak Village, (C) Churna Island Station I & II

2.1. Study Area (Fig 1)

2.1.1. Buleji:

Triangular, highly diversified, south-west to the Arabian Sea, almost 8km rocky parts, having tides-pools on west while sand/mud on east side (Nasir *et al.*, 2018).

2.1.2. Churna Island:

Arabia sea on its west, 9km away from Hub river, highly diversified rocky island (1.2x0.5km), less turbid, famous for snorkeling and SCUBA diving (Shahid, 2015; Khan, 2015).

2.1.3. Mubarak Village:

Variety of sediments from Boulders to pebbles, rocks with tidepools, caves and overhanging structures (Ali *et al.*, 2014).

2.2. Identification of Specimens

Zoanthids are identified morphologically based on the types of polyp (immersae/ liberae/ intermediae) (Reimer *et al.*, 2006), colors of polyps, tentacles and oral slits, number of tentacles, size of colonies and oral discs, and sediment encrustation inside tissue or not. The collected zoanthid and associated species were identified morphologically with the aid of online available literatures (Esper 1805; Haddon and Shackleton, 1891; Carlgren, 1900; Pax and Muller, 1957; Reimer *et al.*, 2011; Gul, 2013; Khushali, 2014; Koupaei *et al.*, 2014; Reimer *et al.*, 2014; Nasir *et al.*, 2018, and Linnaeus, 1758; Rudy *et al.*, 1983; Bosch *et al.*, 1995; Budd, 2007; Apte *et al.*, 2010; Bano *et al.*, 2011; Afsar *et al.*, 2012; Carmona *et al.*, 2014a, 2014b, Agustiadi and Luthfi, 2017; Ghani *et al.*, 2017; Ghani *et al.*, 2018a, 2018b; Kazmi *et al.*, 2018; Ghani *et al.*, 2019).

2.3. Laboratory Rearing Data Collection

The collected species were reared for 21 weeks in two aquaria containing 30L seawater, strong bio-filter aerators and 25 watts white LED bulbs. The parameters (pH, temperature, and salinity) were monitored weekly using hand-held digital pH-, thermo-, and refracto-meters, respectively, while nutrient concentrations like dissolved oxygen, calcium, nitrite-nitrogen, and ammonia were checked fortnightly with their respective kits: AQUA NITE Thailand, Merck KGaA, 84271 Darmstack, Germany, Aquacare 2000.10, PARA Test, Aquacare 2000.4, PARA Test. Lugol's iodine solution (few drops) were poured in aquaria weekly for growth nourishment and pathogens removal (Nasir *et al.*, 2018). The number of polyps counted before placing in aquaria (Polyps placed (PP)). The number of polyps grown (PG) were counted once every week (up to 21 weeks that is PG_1 to PG_{21}), to judge the increase or decrease in polyps quantity, then their sum was taken as: $(\Sigma_{PG})=PG_1+\ldots.+PG_{21}$. The average polyps grown over 21 weeks was calculated by: $APG/21wk=(\Sigma_{PG})/21$ to obtain the mean production of polyps grown throughout the production period i.e. 21 weeks. Then, Standard deviation (SD) is taken for each specimen from their (PG) of 21 weeks to analyze fluctuations in polyp number within overall observation period. The average growth rates of reared species were recorded as: Average Polyp Growth Percentage APG%=((APG/21wk-PP)/PP)*100 (Table 3).

3. Results

The findings of the research are categorized into three (3) parts: Part (I) identification of Hexacorallian zoanthids and their associated fauna, Part (II) growth patterns along collection sites, while Part (III) aquaculture trials. The details are mentioned below:

3.1. Part (I) Identification

Four species of zoanthids including their (morphotypes) were identified as *Zoanthus sansibaricus* (6), *Zoanthus vietnamensis* (2), *Palythoa tuberculosa* and *Palythoa mutuki* (2) from the intertidal rocks of the Buleji coast. Only two zoanthid species, *Zoanthus sansibaricus* (morphotype-4) and *Palythoa tuberculosa*, were collected from 8m depth of Mubarak Village and both Churna Island's stations (Table 1; Plate 1). The details are as follows:

Table 1: Morphological appearance and number of tentacles of observed in collected Zoanthids and a porite coral species

S.No	Species Names	Morph	Polyp color/ outside	Inside	Tentacles
1	*Zoanthus sansibaricus*[1]	1	Purple	Green	48
		2	Purple/Pink	Light green and few Orange	48
		3	Green	Brown	48
		4	Green	Green	48
		5	Green	Blue	40
		6	Brown	Brown and few Green	48
2	*Zoanthus vietnamensis*[2]	1	Brown	Pink	48
		2	Green	Pink ring and Brown or Grey	48
3	*Palythoa tuberculosa*[3]	Nil	Yellow/Cream	White	32 (Brown)
4	*Palythoa mutuki*[4]	1	Brown	Green	47
		2	Brown	Brown	40
1	*Tubipora musica*[5]	Nil	Bright Red	Nil	8 (Gray)

Note: [1](Carlgren, 1900; Gul, 2013; Reimer *et al.*, 2014; Koupaei *et al.*, 2014; Nasir *et al.*, 2018)

[2](Pax and Muller, 1957; Reimer *et al.*, 2011; Khushali, 2014; Nasir *et al.*, 2018)

[3](Esper, 1805; Reimer *et al.*, 2011; Koupaei *et al.*, 2014; Reimer *et al.*, 2014; Nasir *et al.*, 2018)

[4](Haddon and Shackleton, 1891; Reimer *et al.*, 2011; Koupaei *et al.*, 2014)

[5](Linnaeus, 1758; Agustiadi and Luthfi, 2017)

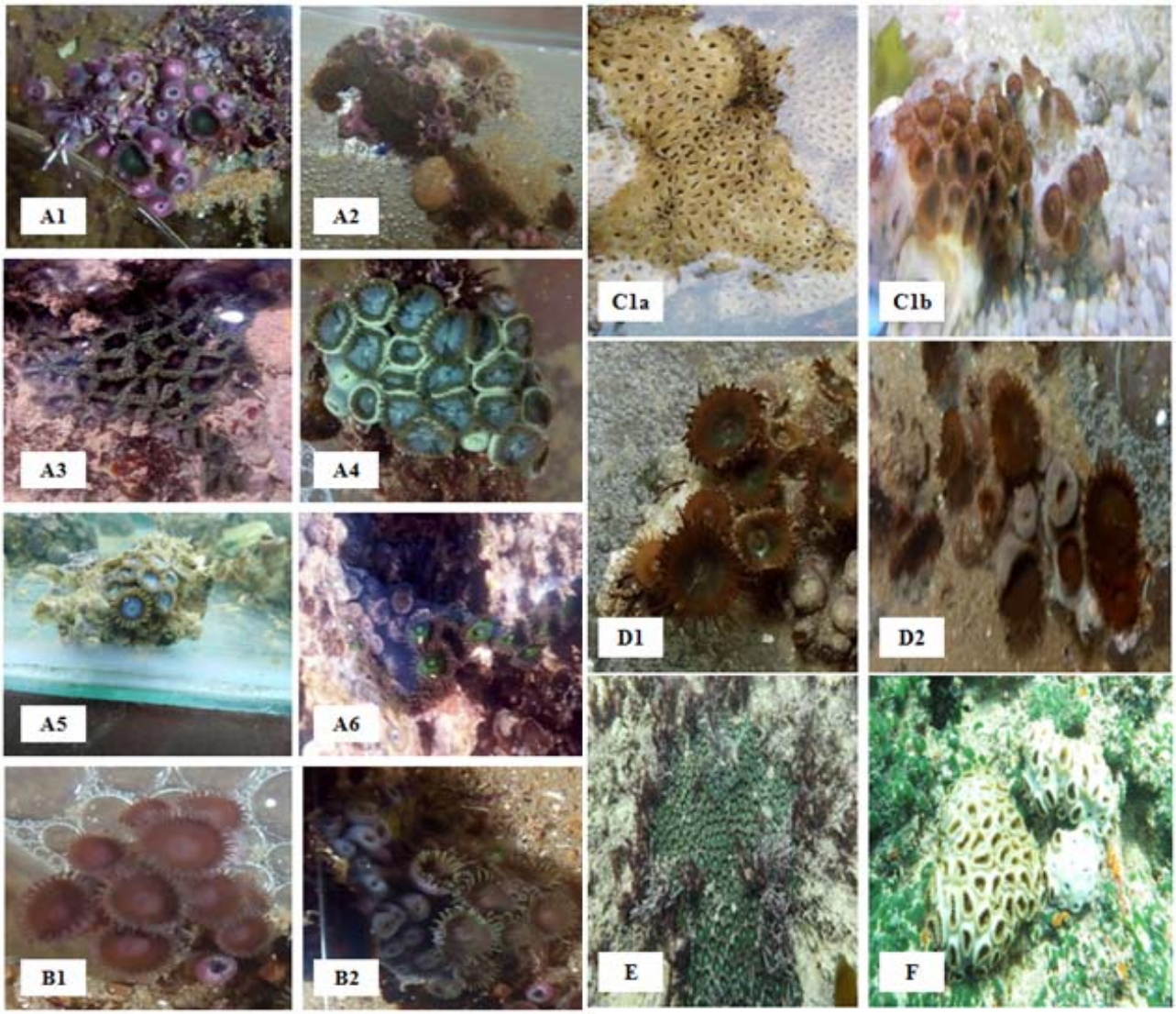


Plate 1: Intertidal Zoanthids species: *Zoanthus sansibaricus*: (A1) Morph 1, (A2) Morph 2, (A3) Morph 3, (A4) Morph 4, (A5) Morph 5 and (A6) Morph 6, *Zoanthus vietnamensis*: (B1) Morph 1 and (B2) Morph 2, *Palythoa tuberculosa*: (C1a) Closed Polyps and (C1b) Open Polyps, *Palythoa mutuki*: (D1) Morph 1 and (D2) Morph 2, Underwater Zoanthids species: (E) *Z. sansibaricus* and (F) *P. tuberculosa*

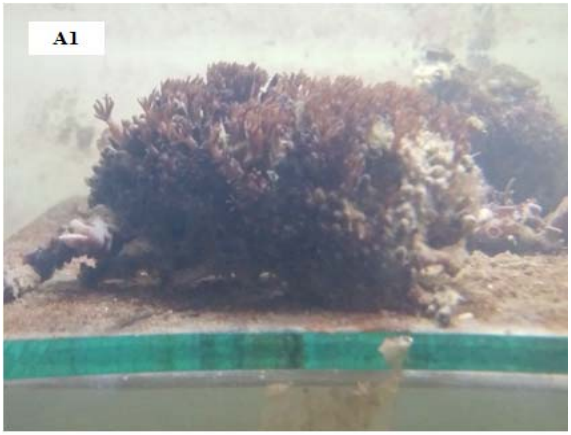
Moreover, only single species was associated with deep-water zoanthids of Churna Island (Table 1; Plate 2). However, 29 species belonging to various phyla such as Mollusca, Annelida, Echinodermata and Arthropoda, were found in association with intertidal zoanthids at Buleji coast (Table 2). It was observed that zoanthids have no negative impact with all mentioned species in (Table 2), except nudibranchs (*Baeolidia palythoae* and *Berghia* spp), which were found to be grazing on zoanthids. Additionally, an unidentified bacterial disease was observed (Plate 3). The details are mentioned below:

3.1.1. Zoanthids

Zoanthus sp. have delicate, erect and liberae polyps, do not accumulate sediments inside their tissues, gray/green/purple externally, while bright/green-brown internally. *Z. sansibaricus* are most stiff species, classified into morphotypes based on oral discs coloration like brown, green, purple, orange, yellow, red, white or fluorescent, containing about 60 tentacles (30 each row). *Z. vietnamensis,* have brown polyps with green/white/light pink tentacles, white oral slits with pink loop in their oral discs. The species of the genus *Palythoa* encrust sediments in tissues. The *P. tuberculosa* have immerse and round polyps, fused at base then extend individually, 30-34 tentacles (1/2 oral disc size). They have green-brown oral discs with creamy brown colors externally. Another species, *P. mutuki,* has 30mm long intermediae polyps (brownish apex) and 45-67 tentacles with green/brown oral discs (>1/2 tentacles' size) (Reimer, 2010; Khushali and Pradeep, 2013).

3.1.2. Associated Species

Tubipora musica (scleractinian) associated with deepwater zoanthids were collected from 1st diving station of the Churna Island (Table 1; Plate 2). Furthermore, 29 species were associated with intertidal zoanthids at Buleji, including 26 species of the phylum Mollusca (three classes: Gastropods (23 species), Bivalvia (2), and Polyplacophora (1) and one species of each phyla: Annelida, Echinodermata, and Arthropoda. The abundantly found species with zoanthids in descending order were: *Turbo bruneaus* > *Tenguella granulate* > *Trochus fultoni*. No associated species harmed zoanthids but only two nudibranchs, namely *Baeolidia palythoae* and *Berghia* spp, fed on zoanthids. On the other hand, a harmful bacterial growth over zoanthids was observed (Plate 3).



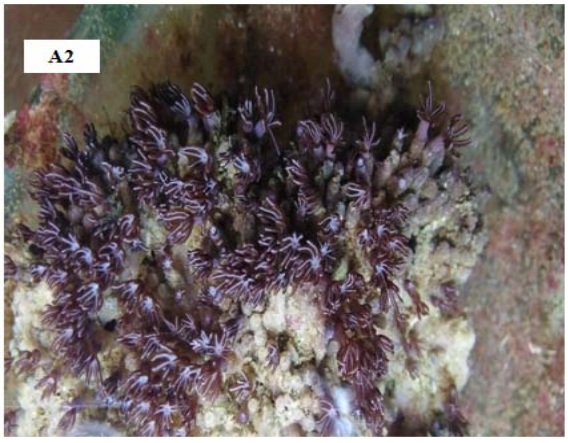


Plate 2: Underwater Zoanthids' Associates: (A1) *Tubipora musica* (Side view) and (A2) *Tubipora musica* (Top view)

Table 2: Checklist of associated fauna observed in association with zoanthids of Buleji coast, Karachi

Phylum	Class	Order	Family	Genus	Species
Mollusca	Gastropoda	Trochida	Trochidae	*Trochus*	*Trochus fultoni* (Melvill, 1898)
		Archeogastropoda	Turbinidae	*Turbo*	*Turbo bruneus* (Roding, 1798)
		Cycloneritida	Neritidae	*Nerita*	*Nerita albicilla* (Linnaeus, 1758)
		Caenogastropoda	Cerithiidae	*Cerithium*	*Cerithium caeruleum* (G. B. Sowerby II, 1855)
					Cerithium scabridum (Philippi, 1848)
				Clypeomorus	*Clypeomorus bifasciata* (G. B. Sowerby II, 1855)
		Littorinimorpha	Cypraeidae	*Naria*	*Naria turdus* (Lamarck, 1810)
			Cymatiidae	*Gyrineum*	*Gyrineum natator* (Roding, 1798)
		Neogastropoda	Muricidae	*Tenguella*	*Tenguella granulata* (Duclos, 1832)
				Purpura	*Purpura panama* (Roding, 1798)
				Drupella	*Drupella rugosa* (Born, 1778)
				Tylothais	*Tylothais savignyi* (Deshayes, 1844)
				Semiricinula	*Semiricinula tissoti* (Petit de la Saussaye, 1852)
					Semiricinula konkanensis (Melvill, 1893)
			Nassariidae	*Nassarius*	*Nassarius deshayesianus* (Issel, 1866)
			Pisaniidae	*Pollia*	*Pollia undosus* (Linnaeus, 1758)
			Olividae	*Oliva*	*Oliva bulbosa* (Roding, 1798)
			Conidae	*Conus*	*Conus namocanus* (Hwass in Bruguiere, 1792)
		Siphonariida	Siphonariidae	*Siphonaria*	*Siphonaria belcheri* (Hanley, 1858)
					Siphonaria savignyi (Krauss, 1848)
		Nudibranchia	Aeolidiidae	*Baeolida*	*Baeolidia palythoae* (Gosliner, 1985)
				Berghia	*Berghia spp* (Trinchese, 1877)
		Systellommatophora	Onchidiidae	*Peronia*	*Peronia verruculata* (Cuvier, 1830)
	Polyplacophora	Chitonida	Chitonidae	*Chiton*	*Chiton peregrines* (Thiele, 1909)
	Bivalvia	Orcida	Arcidae	*Barbatia*	*Barbatia obliquata* (Wood, 1828)
		Mytilida	Mytilidae	*Leiosolenus*	*Leiosolenus tripartitus* (Jousseaume, 1894)
Arthropoda	Malacostraca	Isopoda	Cirolanidae	*Eurydice*	*Eurydice pulchra* (Leach, 1815)
Echinodermata	Ophiuroidea	Amphilepidida	Ophiactidae	*Ophiactis*	*Ophiactis savignyi* (Muller and Troschel, 1842)
Annelida	Polychaeta	Phyllodocida	Nereididae	*Nereis*	*Nereis vexillosa* (Grube, 1851)
4	6	15	20	26	29

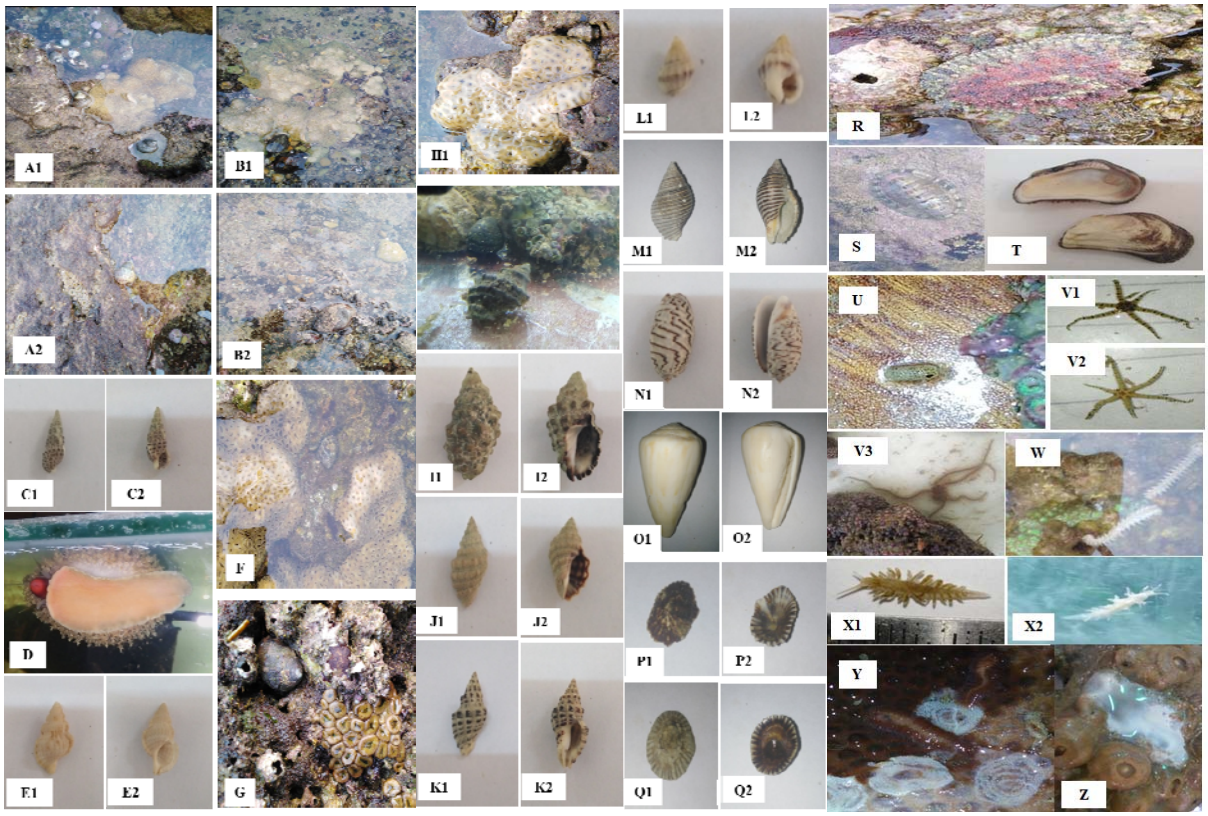


Plate 3: Intertidal Zoanthids' Associates: (A1) Trochus fultoni and Turbo bruneus with Palythoa tuberculosa, (A2) Trochus fultoni and Turbo bruneus with Zoanthus sansibaricus, (B1) Nerita albicilla, Cerithium caeruleum and Clypeomorus bifasciata with Palythoa tuberculosa, (B2) Nerita albicilla, Cerithium caeruleum, Clypeomorus bifasciata with Zoanthus sansibaricus, (C1) Cerithium scabridum (Dorsal view), (C2) Cerithium scabridum (Ventral view), (D) Naria turdus (Ventral view), (E1) Gyrineum natator (Dorsal view), (E2) Gyrineum natator (Ventral view), (F) Tenguella granulata with Palythoa tuberculosa, (G) Purpura panama with Zoanthus sansibaricus, (H1) Drupella rugosa with Palythoa tuberculosa, (H2) Drupella rugosa with Zoanthus sansibaricus, (I1) Tylothais savignyi (Dorsal view), (I2) Tylothais savignyi (Ventral view), (J1) Semiricinula tissoti (Dorsal view), (J2) Semiricinula tissoti (Ventral view), (K1) Semiricinula konkanensis (Dorsal view), (K2) Semiricinula konkanensis (Ventral view), (L1) Nassarius deshayesianus (Dorsal view), (L2) Nassarius deshayesianus (Ventral view), (M1) Pollia undosus (Dorsal view), (M2) Pollia undosus (Ventral view), (N1) Oliva bulbosa (Dorsal view), (N2) Oliva bulbosa (Ventral view), (O1) Conus namocanus (Dorsal view), (O2) Conus namocanus (Ventral view), (P1) Siphonaria belcheri (Dorsal view), (P2) Siphonaria belcheri (Ventral view), (Q1) Siphonaria savignyi (Dorsal view), (Q2) Siphonaria savignyi (Ventral view), (R) Peronia verruculata, (S) Chiton peregrines, (T) Barbatia obliquata, (U) Eurydice pulchra, (V1) Ophiactis savignyi (Dorsal view), (V2) Ophiactis savignyi (Ventral view), (V3) Ophiactis savignyi (In Aquarium), (W) Nereis vexillosa, (X1) Berghia spp (In aquarium) (X2) Berghia spp (Outside the aquarium), (Y) Baeolidia palythoae with eggs (grazing) and (Z) Bacteria (disease)

3.2. Part (II) Growth Patterns

All identified zoanthid species including *Zoanthus and Palythoa* were either totally absent or diminutive on high tide zones of Buleji. However, *Zoanthus* were more abundant at low tide, while *Palythoa* at mid tides. Only two zoanthid species, *Z. sansibaricus* (morphotype-4) and *P. tuberculosa*, were abundantly found across all diving sites at water depth of around 8m.

3.3. Part (III) Aquaculture

During aquarium trials, the average recorded parameters in aquaria varied between 35-36.5‰ salinity, 7.3-7.7 pH, and 25.4-31.0˚C temperature (Fig 2a), whereas the aggregate nutrients' concentrations were 0.3-0.4ppm NO^{2-}, 0.08-0.12ppm NO_2–N, 500-545mg/l Ca, 0.23-0.25mg/l NH_3 and 7-10mg/l DO_2 (Fig 2b). It was observed that most species lived less than one week then died completely, thus showing 0 APG/21wk and -100 APG%, namely *Z. sansibaricus* (morph-1(a, b), 2, 3, 4(b, c, d)), *P. tuberculosa*(a, b, d, e, f) and *P. mutuki* (morph-1a, b) (Table 3). A few survived about a week like *Z. sansibaricus* (morph-1d, 4e, 5, 6), *Z. vietnamensis* (morph-2), *P. mutuki* (morph-1d), hence shown -95 APG% while 5, 10, 1, 2, 13, and 5 APG/21wk respectively (Table 3, Fig 3a). *Tubipora musica* and *P. mutuki* (morph-2b) remained alive for 4 and 6 weeks, having -93 and -92 APG% with 24 and 27 APG/21wk respectively. *Z. sansibaricus* (morph-4a) lived for 19 weeks with 25 APG/21wk and 0 APG%. However, some species were successfully grown and sustained throughout the culture period of 21 weeks, for instance, *Z. sansibaricus* (morph-1c), *Z. vietnamensis* (morph-1), *P. tuberculosa*(c), *P. mutuki* (morph-1c and 2a) with 41, 51, 18, 32, 66 APG% and 189, 15, 59, 57, 28 APG/21wk respectively (Table 3, Fig 3a-c). The possible reasons of death or loss of polyps might be due to the nudibranchs grazing, bacterial disease outbreak, parameters fluctuation (including increase in salinity via evaporation), and provision of low light causing less photosynthesis. Small aquarium space available for aquaculture experiment can also be a factor for deterioration, although use of lugol's solution, removal of nudibranchs and diseased or dead polyps by hand picking, provision of freshwater bath treatment for 5 minutes, reducing salinity by adding freshwater seemed to boost the growth rates of some species. The date and site of

collections, numbers of polyps counted per square foot and collected for cultivation, APG/21wk, APG % and SD are enlisted in (Table 3; Fig 3a-c). However, the average growth rates (APG%) of successfully reared species (i.e. species who had shown growth activities from 4 to 21 weeks, either increased or decreased from or reached to their initial quantities placed in aquarium) in ascending order are: *T. musica* (-93) < *P. mutuki* Morph-2b (-92) < *Z. sansibaricus* Morph-4a (0) < *P. tuberculosa*(c) (18) < *P. mutuki* Morph-1c (32) < *Z. sansibaricus* Morph-1c (41) < *Z. vietnamensis* Morph-1 (51) < *P. mutuki* Morph-2a (66). The details are mentioned below:

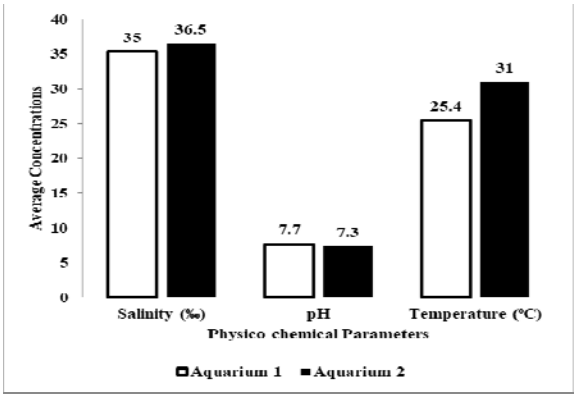


Figure 2a: Average physico-chemical parameters during culture trials

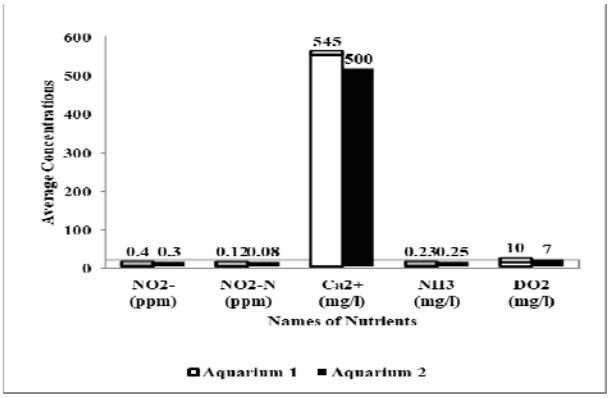


Figure 2b: Average nutrient's concentrations during aquarium trials

Table 3. Species, date and site of collection, Polyps observed per Square foot, number of polyps placed and grown over 21 weeks (all Morphotype of Zoanthids and Associated Coral species)

S.No	Species Names	Morph	Site	Date	Polyps/sq.ft	Aq #	Polyps Placed (PP)	Σ_{PG}	APG/21wk	APG%	SD (±)
1	*Z. sansibaricus*	1a	B	9/10/18	1000	1	26	0	0	-100	0
		b	B	7/11/18	1000	1	74	0	0	-100	0
		c	B	3/1/19	6432	1	134	3961	189	41	66
		d	B	18/4/19	7488	2	108	108	5	-95	24
		2	B	9/10/18	2000	1	130	0	0	-100	0
		3	B	9/10/18	1000	1	330	0	0	-100	0
		4a	B	3/1/19	3600	1	25	524	25	0	10
		b	CI I	9/3/19	3168	2	44	0	0	-100	0
		c	CI II	13/3/19	936	2	13	0	0	-100	0
		d	MV	15/4/19	1944	2	27	0	0	-100	0
		e	B	18/4/19	3600	2	206	206	10	-95	45
		5	B	18/4/19	1008	2	14	14	1	-95	3
		6	B	18/4/19	22176	2	47	47	2	-95	10
2	*Z. vietnamensis*	1	B	3/1/19	1440	1	10	318	15	51	5
		2	B	18/4/19	5760	2	273	273	13	-95	60
3	*P. tuberculosa*	0a	B	9/10/18	3000	1	132	0	0	-100	0
		b	B	7/11/18	3000	1	500	0	0	-100	0
		c	B	3/1/19	7200	1	50	1243	59	18	9
		d	CI I	9/3/19	2160	2	30	0	0	-100	0
		e	CI II	13/3/19	4536	2	63	0	0	-100	0
		f	MV	15/4/19	1440	2	20	0	0	-100	0
4	*P. mutuki*	1a	B	9/10/18	1000	1	302	0	0	-100	0
		b	B	7/11/18	10000	1	680	0	0	-100	0
		c	B	3/1/19	9456	1	43	1191	57	32	17
		d	B	18/4/19	6048	2	98	98	5	-95	21
		2a	B	3/1/19	1224	1	17	593	28	66	7
		b	B	18/4/19	1440	2	333	566	27	-92	73
5	*T. musica*	0	CI I	9/3/19	23328	1	324	500	24	-93	73

Note: B:Buleji, CI I:Churna Island Station I, CI II:Churna Island Station II, MV:Mubarak Village, Aq #:Aquarium No, Σ_{PG}=Sum of polyps grown, APG/21wk=Average Polyp Grown over 21 weeks, APG%=Average Polyp Growth Percentage, SD=Standard Deviation

APG%: -100=died completely within 1wk, -95=died after 1wk, -93=died after 4wk, -92=died after 6wk, 0=reached to initially placed quantity

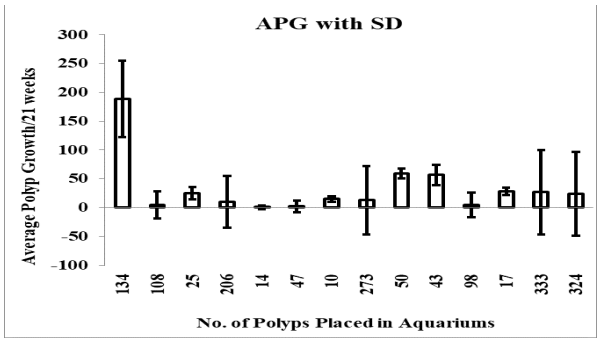


Figure 3a: Average polyp growth over 21 weeks (APG/21wk) and Standard Deviation (±SD) of Zoanthids and associated Porites reared in aquaria. **Note:** *Z. sansibaricus:* [Morph-1: 134(c) & 108(d), Morph-4: 25(a) & 206(e), Morph-5: 14, Morph-6: 47)]; *Z. vietnamensis:* [Morph-1: 10, Morph-2: 273]; *P. tuberculosa*: 50(c); *P. mutuki:* [Morph-1: 43(c) & 98(d), Morph-2: 17(a) & 333(b)] and *T. musica:* 324.

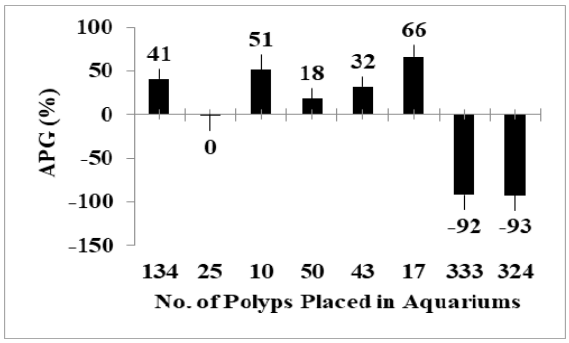


Figure 3b: Average polyp growth percentage (APG %) of Zoanthids and associated Porites reared in aquaria. **Note:** Polyp number: [**134**: *Z. sansibaricus* Morph-1(c)], [**25**: *Z. sansibaricus* Morph-4(a)], [**10**: *Z. vietnamensis* Morph-1], [**50**: *P. tuberculosa* (c)], [**43**: *P. mutuki* Morph-1(c)], [**17** & **333**: *P. mutuki* Morph-2 (a) & (b)] and [**324**: *T. musica*].

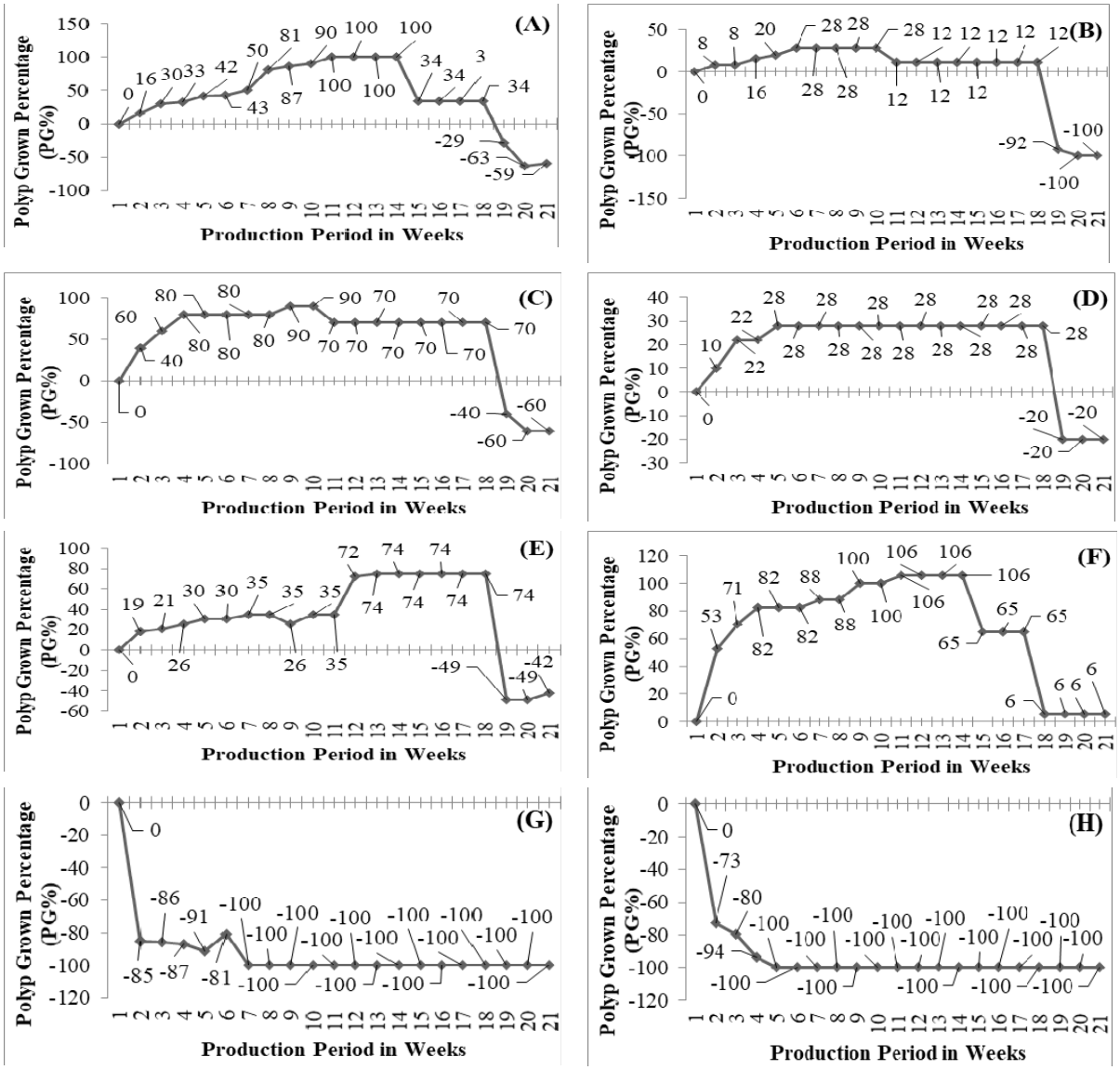


Figure 3c: Percentage of polyp grown/21 weeks (Zoanthids and Associated Coral): (A) 134 polyps of *Z. sansibaricus* (morph 1c), (B) 25 polyps of *Z. sansibaricus* (morph 4a), (C) 10 polyps of *Z. vietnamensis* (morph 1), (D) 50 polyps of *P. tuberculosa* (c), (E) 43 polyps of *P. mutuki* (morph 1c), (F) 17 polyps of *P. mutuki* (morph 2a), (G) 333 polyps of *P. mutuki* (morph 2b) and (H) 324 polyps of *T. musica.*
Note: 0 indicates quantity of polyps remains same as initially placed, the points above 0 indicates increase while below 0 indicates decline in growth rates. While -100 indicates that organisms completely died.

All grown species multiplied after 1st week of their placement in aquaria may be because they were adapting changes to new environment. *Z. sansibaricus* morph-(1c) shown peak growth rates from 11 to 14 weeks then, started diminishing and after 18th week declined sharply. However, survived up to 21 weeks (Fig 3c(A)). *Z. sansibaricus* morph-(4a) had shown growth acceleration in between 6-10 weeks, then started reducing with dominant death rates from 18th week and survived till 19th week then died completely (Fig 3c(B)). *Z. vietnamensis* morph- (1) had the shortest peak growth activities from 9 to 10 weeks, and started declining sharply after week 18 but survived to overall production period of 21 weeks (Fig 3c(C)). *P. tuberculosa* (c) multiplied with the longest growth peaks from 5-18 weeks but declined suddenly till 19th week after then nearly stable until 21 weeks (Fig 3c(D)). *P. mutuki* morph- (1c) shown peak growth rates in between 12-18 weeks, then sharp diminishing rates till 19th then slightly increased and remained alive until 21 (Fig 3c(E)), (2a) shown the highest growth rates than others from week 11 to 14, then declined twice sharply (i.e. after 14th and 17th weeks), but never died completely within 21 weeks of study period (Fig 3c(F)), and its morph (2b) had fast declining rates till 2nd week with a little multiplication at the end of the 5th week but started declining again while completely died on the 7th week thus sustained only 6 weeks (Fig 3c(G)). *T. musica* resulted almost similar to *P. mutuki* (morph-2b) but never multiplied, yet survived up to 4 weeks only, then died completely (Fig 3c(H)).

4. Discussion

In this research, the diversity of intertidal as well as deeper zoanthids in and around the Karachi coast were given. The identification of the collected zoanthids along with their intertidal and deep sea associated species was done with the help of online keys. The cultivation trials have been made to observe their growth potential in artificial media.

4.1. Identification of Zoanthid and Associated species

4.1.1. Zoanthids

An article published from Pakistan has revealed the occurrence of about four zoanthid species such as *Zoanthus sansibaricus, Zoanthus vietnamensis, Epizoanthus scotinus,* and *Playthoa tuberculosa* from Sunera, Paradise, Buleji and Manora intertidal rocks of Pakistan's Northern Arabian Sea (Nasir *et al*., 2018). Another article has evidenced the occurrence of varied morphotypes of *Z. sansibaricus*, *Z. vietnamensis*, *P. tuberculosa,* and *P. mutuki* from the Saurashtra coast of Gujarat, India. All of the aforementioned species are dominant along tropical to temperate intertidal rocky shores where corals are not abundantly present (Khushali and Pradeep, 2013). In the present study, the collected species and their morphotypes were found to be similar to that described by aforementioned literatures.

4.1.2. Associated species

Numerous studies have evidenced the symbiotic associations of zoanthids with other organisms that belong to different groups; for instance, Porifera (*Spongia officinalis*), Annelida (*Lanice conchelega*, *Nereis pelagica*, *Lepidonotus squamatus*), Polychaete larvae, Arthropoda (*Alpheus melabaricus*, *Alphaeus sp*, *Charybdis truncata*, *Menippe rumphii*, *Eurydice pulchra*, *Corophium* sps), Mollusca (*Cellana radiate*, *Cyprea tigris*, *Turbo bruneus*, *Cerithidea sps*, *Thais calvigera*, *Baeolida palythoae*), Echinodermata (*Asterias sps*, *Ophiotrix fragilis*, *Ophiocomina nigra*, *Stomopneutes variolaris* and *Tropiomera sp*) (Chakravarty *et al*., 2016). In this study, we observed almost the same associated fauna.

4.2. Growth Patterns

Zoanthids' population dynamics and their allocation patterns on inter-tidal rocks are elaborated in various studies (Trivedi and Vachhrajani, 2014). Despite this, fewer studies have been conducted in Pakistan on their growth rates and abundance (Ali *et al*., 2014). In the present study, the growth patterns of zoanthid species were found in a similar manner as described by Nasir *et al*., (2018) and Rabelo *et al*., (2015b); that is *Zoanthus* species are rich at low tides while *Playthoa* species are plentiful at mid tides. However, both are absent at high tidal levels.

5. Aquaculture

A single paper has been contributed from Pakistan on the cultivation trials of zoanthids. The article evidenced that zoanthids can be cultured successfully by just maintaining their required parameters, although the data was presented for 8 weeks only (Nasir *et al.,* 2018). Consequently, a comparative study is premeditated for 21 weeks, and freshly obtained erratic data related to the growth rates of identified zoanthids such as *Z. sansibaricus*, *Z. vietnamensis*, *P. tuberculosa,* and *P. mutuki,* including *T. musica* (deepwater zoanthid associates). The present study also revealed that zoanthids could be cultivated artificially by gratifying their requirements. However, mortalities occurred most likely due to small space, nudibranch grazing, bacterial disease outbreak. Lack of defense mechanism and internal immunity, and salinity changes due to evaporation of seawater from aquaria could also be considered. Nevertheless, few steps alleviated the growth rates of some species such as: lugol's iodine solution (few drops) controlled pathogens; nudibranchs, dead and diseased polyps were removed immediately, while freshwater bath treatment for at least 5 minutes using strong aerator (for water flushing flow) was given to remaining polyps in separate tanks, the hypersaline seawaters were mixed with freshwater in order to maintain salinity, and polyps were provided with enough space in aquarium to grow easily by placing racks and stones.

6. Conclusion

From the above research, it can be concluded that the rocky coasts of Pakistan are enriched with hexacorallian zoanthids. They form positive associations with other organisms such as molluscs, arthropods, echinoderms, annelids, scleractinians, etc., while negative with bacteria and nudibranchs. They can not only be sustained but also cultured in artificial environments if their required parameters are maintained successfully. The zoanthids species should be treated with freshwater bath including few drops of lugols iodine for ~5 min, and acclimatized before placing in aquarium, in order to get rid of

pathogens, boost immunity and eradicate hidden enemies that may come out after some time in aquarium, such as nudibranchs.

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