### INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

**Research Article** 

# Maintenance, survival and behaviour of Green mussel *Mytilus viridis* – A Rare mollusk of Gujarat coast in laboratory conditions M. R. Patel<sup>1</sup>, M. B. Patel<sup>2</sup> and K. A. Pithawala<sup>2</sup>.\* <sup>1</sup>Department of Chemistry, Gujarat Arts and Science College, Ellis bridge, Ahmedabad, Gujarat, India - 380006.

Timedabad, Odjalad, mala 0000001

<sup>2</sup>Department of Biology, Gujarat Arts and Science College, Ellis bridge,

Ahmedabad, Gujarat, India - 380006.

#### ABSTRACT

Dwarka (22°25'N, 69°04'E) and Mocha (21°20'N, 69°53'E) situated on the coast of Gujarat are two spots where green mussel *Mytilus viridis* is located. This is a rare mollusk as only few splattered colonies are seen. The colony of Mytilus located at Dwarka is very old and consists of very adult animals more than 10-20 years. Also these are located upstream in the mouth of river Gomti. The bed is rocky as well as muddy and estuarine type, whereas the mocha spot is rocky, sandy and purely marine. The animals are located at distance of 10-50 meters from the lowest tide mark. The animals are of different age and size distribution ranging from spat of 1cm size to 10cm largest specimen. Since Dwarka and Mocha are different locations and ecology of both spots being different the animals belonging to same species do differ. But these animals can be maintained under laboratory conditions together as well as separately without showing much difference in their behavior. The animals could survive under laboratory condition for more than 5 months. These animals refused to consume whatever ration were supplied to them and hence they were fed fresh cultured Chetoceros algae as well as amino acid powder. Yeast extracts and other powdery nutrients were rejected vigorously by the animals. The animals under starvation showed severe emaciations and their guts were completely empty and gonad regressed however the byssus productionwas not much hampered as it is crucial for their survival in natural conditions.

Key words: Mytilus viridis, aquarium keeping, byssus

#### **INTRODUCTION**

There has been a record of *Mytilus (Perna) viridis* on the coast of Gujarat at Dwarka (Lat.  $22^{\circ}25^{\circ}$ N, Long.  $69^{\circ}04^{\circ}$ E) and Mocha (Lat.  $21^{\circ}20^{\circ}$ N, Long.  $69^{\circ}53^{\circ}$ E.).<sup>1,2,3</sup>It is an important marine food in India. They may be procured naturally or may be cultured. Mocha is a small coastal village near Porbander and has big alkali, fishing and chalk industries, a major source of pollution thereby. Dwarka is a pilgrimage town and is very ancient place whose waters are polluted only by domestic wastes, apart from a few fish-processing industries.

Numerous pollution studies especially heavy metal accumulation and bacterial contamination have been carried out.<sup>4</sup> Also work has been done to analyze nutritionally important constituents in mussel and it has been established that these mussels are fitfor human consumption.<sup>5</sup>

Mocha is a rocky as well as sandy shore of Arabian Sea located on the Saurastra coast of Gujarat. *Mytilus viridis* is located in patches in a 1.5 km long and 50 meter wide area of intertidal rocky substratum along with numerous other mollusks as Cowries, Onichidium, Thais, Haemastoma, Drupa sps., Chitonsetc., animals of other phyla include numerous species of crabs. Barnacles are numerous in this locality and are epifaunic on these partly sessile animals so much so that a few specimens were fully covered with them; however the animals were indifferent to them as seen from the others that had one or two barnacles attached on them. Among vertebrates numerous carnivorous eels haunt this place. The endemic flora of this area includes numerous seaweeds like *Gracillaria corticata*, *Gelidiella acerosa, Ulva lactuca* and *Enteromorpha prolifera*.

Density studies have been carried out by Tewari et al. in the year 1999. However in the year 2004 during our study very few colonies have been recorded and collection of animals when done in this patch only few samples were collected (Figure-1). Also it was observed that in the year 2006 in the month of August numerous colonies consisting of lacs of individuals of very small spat size 5-10 mm (Figure-2) were located. These were found more towards the shore and distributed in a patch of 500m x 20m where there was no much human interference. The adult population was located towards the seaside and beneath the rocks (Figure-3) whilst the juvenile were found near the shores and on the top of rocks, forming encrustations on the surface of flat rocks and the curvatures seaside of the rocks (Figure-4).

When these colonies were observed in the month of December in the same year, the number of colonies was reduced to 10%. This was located towards the shore and not in the seaside. The probable reason could be intensetidal actions or from predatory sea animals. Also possibility of predation from birds was nil. During this time the size of these individuals was not much larger indicating a very small growth rate.

As previously mentioned Dwarka is a pilgrimage place and the site where the animals are located is estuary formed by the river Gomti. The mouth of this river is subjected to intense tidal action and the animals are found at a distance 1.25 km from the sea and in the rocky and muddy beds, a sheet covering an area approximately  $25 \times 10$  sq. meters on smaller sized rocks less than half meter in diameter and beneath them.

The co-species include numerous colonies of edible oysters- of course! A filter feeder and a competitor in this aspect, also crabs inhabit these range, even few eels are located, a bit distance from them upstream. This area is frequently visited by the natives for fishing purpose and is to the notice of the fishermen there by. Also these colonies are thought to be the oldest ones and the life of the specimens range from more than 10 to 50 years as observed from the growth rings on the shells (Figure-5). The animals are covered occasionally by a barnacle or some tubicolous worms. The surface of the shells was covered by brownish colored algae giving a velvety feel to the surface and obstructing the bright green color of the animals. Also some animals were found to be covered with orange colored material and few crumbs of sponges not definitely identified (Figure-6). The byssus threads by which the animals remained attached, were dark brown and in bunch.

The present study was undertaken with the reasoning that the maintenance of these animals under laboratory condition was an essential requirement for the further use of this animal for other experimental work. Much less work has been reported on Mytilus viridis from coast of Gujarat. In nature the feeding behavior of Mytilus species other than M. viridis have been studied in details.<sup>6,7</sup>Also seasonal variations and other factors have been studied in other mytilus species.<sup>8,9,10</sup> Also byssus production under clear waters of aquarium gives neat threads for various biochemical studies.

#### MATERIALS AND METHOD

## *i.Setting up tank and Maintenance of animals under laboratory conditions:*

An all glass low basic style aquarium tank 122 x 38 x 47cm (150-liter capacity) was constructed so that surface area could be increased.<sup>11</sup>Subgravel filterwas of acrylic sheet holed at regular intervals on which was spread plastic mosquito net to hold the coral sand layer 10 centimeters thick. Coral gravel, crushed molluscan shells and dolomite (CaCO<sub>3</sub> and MgCO<sub>3</sub>) were also added. Two airlift pumps were attached at corners diagonally; moreover, two outside filters containing activated carbon plus cotton were used for constant water purification. These outside filters had inbuilt mechanism for sparging air hence no aerators were needed. Also an immersion heater 50 Watt rating was submerged to maintain constant temperature (Figure-7). Illumination was through fluorescent tube light and that too for limited time only as these animals preferred darkness because of their lurking habits underneath the rocks only exposed during lowest tides. Artificial sea salt (Dophin Co. China) was purchased to make seawater (1 kg. per 33 liters of distilled water). Aquarium was kept for a week without animals, after which for stabilization black molly (Poisella latipinna) was introduced and the whole setup was kept for another week, after that the animals were introduced.<sup>12</sup>

Also sea waters from both the places was collected in sterilized plastic bottles and were analyzed for various parameters as temperature, salinity, Biological Oxygen Demand, Chemical Oxygen Demand and presence of some heavy metal. The condition of the seawaters of both the places is realized from the data given in table-1. Similar parameters were studied for the aquarium water (table-2).

#### *ii. Procurement of animals:*

The animals were collected from Dwarka and Mocha. during low tide in early morning hours offshore about 1.25 km and 10-30 meters away from the shore respectively. The animals lie attached to huge rocks and are cryptic as they are covered by algae seaweeds and barnacles, other co-species include cowries, cockles etc. They were detached carefully by breaking rocks or by carefully cutting off thread sand kept in sea water constantly under aeration and were transported to laboratory. Animals are hardy and no mortality is seen even during transportation for more than 36 hours. When animals were brought to laboratory and transferred to tanks with all necessary precautions, no mortality occurred. Even a colony of very young animals (spat) sized 5-8 mm were collected along with substratum (hold) and were transported to the laboratory in the same condition as the adults. These were equally comfortable as the adults during transportation as no mortality was seen in these very juvenile ones.

#### iii. Care taking:

Aquarium was maintained at 8.3 pH, 33 ppt. - salinity (sp.gr.-1.030), and temperature 27° to30°C. Aeration as well as filtration was continuous at every 5 minutes interval. Light was illuminated only when the animals were exposed to air by emptying the tanks periodically in accordance with periodicities of lowest tides, this was done to mimic the natural environment of seas. The idea about the condition of the aquarium can be had from the data given in table-1.2.

#### iv.Feeding:

The first day animals were kept without any food next day onwards animals were fed on fresh chetoceros algae and amino acid powder(used as cattle feed supplement). Although most of larger bivalves can and will eat small zooplankton of the size of rotifers or possibly even as large as newlyhatched baby brine shrimp. The vast majority of their diet is still typically composed of smaller particles in the size range of phytoplankton (approximately 2-20 µm). These mussels will do best with at least daily feedings of tiny planktonic foods. The most nutritious alternative for feeding is to maintain live cultures at home. These cultures include algae belonging to genera *Chaetoceros, Isochrysis, Monochrysis*, *Tetraselmis and Thalassosira*. Feeding them on a mixture of alga is better from the point of view of nutrition value.<sup>13</sup>In nature the feeding behavior of Mytilus species have been studied a lot in details

If one is to have any chance of keeping these animals alive in an aquarium, one has to feed some sort of tiny plankton supplement to the animals on a regular (at least daily, if not several times per day) basis or they are doomed to slowly starve to death in the aquarium.

Most aquarists believe that if a suspension-feeding marine invertebrate reacted to a given food, then they must be eating it. It is a fact known that even if an animal appears to be feeding. The active filtering of particles from the water by animals such as mussels or clams does not necessarily mean that they are eating to the food. In many filter-feeding marine invertebrates, the action of feeding is done by the gills, and therefore filtering and respiration (breathing) are intimately linked: an animal cannot really isolate one activity from the other. Furthermore, many suspension feeding marine invertebrates filter all particles of the appropriate size out of the water column, but then discard the particles if they do not find tasty, as "pseudofeces." Pseudofeces is a fancy word that means quite simply "fake poop" - the animals filter the particles and to the naked eye, they appear to eat it, but for some reason (typically the taste, surface texture or particle organic composition) they decide not to actually ingest the filtered particles. Instead, the animals ball up the filtered particles before they are eaten, wrap them in mucus and spit them out as fake poop. These fake poop particles are very hard to distinguish from the real thing, and unless one is able to observe the animals under a microscope, it would be very hard to tell whether the animals are really eating the food provided or not.

Similar experiments have not been done for these green mussels, so one cannot say for sure whether or not these animals will ingest any particle of the correct size or whether they are capable of selecting particles based on taste and rejecting distasteful particles. However, that experiments with the common mussels found along the coast of North America (*Mytilus species*) suggest that these animals are not very discriminating in terms of swallowing particles provided that they are of the size range (roughly 2-20  $\mu$ m) of phytoplankton.<sup>14,15,16</sup> In experimental feeding trials, *Mytilus* mussels appear to ingest unflavored beads or particles that are chemically defended at about the same rate as they eat the tiny phytoplankton that they filter from natural seawater, despite the fact that eating such particles can significantly decrease their growth rates.

It is important to realize that even if an animal is expanded and observed to be feeding, it can be expected to have about the same shor life span on food of in sufficient nutritional quality as it would have if it was being starved.

Large bivalve mollusks need to filter a surprising amount of food to keep them healthy and growing. It is estimated that mussels can filter as much as eight times their body weight each day from the water that passes over them.<sup>17</sup> Obviously, if one has a tank that is already prone to nutrient export problems, it is certain to increase nutrient loading and make those problems worse by adding the copious amounts of planktonic food necessary to keep a single mussel (let alone an entire cluster of them) healthy in the tank. If, on the other hand, a well-balanced reef tank with plenty of export is capable of handling the additional nutrient inputs of feeding a mussel such as these.

The juveniles when maintained under laboratory condition did not show growth in size or increase in weight. These tiny ones survived for more than ten months.

#### **RESULTS AND DISCUSSION**

#### i. Byssus production:

The animals upon introduction into the tanks start producing threads immediately (some produced even during transportation) and attached themselves to sand, corals, stones, shells, glass-walls or each otherwhichever support is nearby. Adults (>5cm) generally did not move more than 15 cm. distance whilst tiny young (2 cm) were seen to crawl on the walls of the glass tank and cover large distances 2-3 feet within a period of day or two, even adherence to glass walls was seen. Adults were known to move by voiding off the old byssi from the holdfast inside their foot and concomitantly forming new byssi at some distance as much long as their foot can be extended. This way the animals could move on horizontal walls of aquarium; of course- a difficult task indeed! (Figure-8). Some animals were intentionally made to adhere on to wooden piece which was initially submerged by putting extra weight. When anchorage was powerful (c.a. 16 threads within a day) wooden pieces were allowed to float with the animal in the aquarium. The animals were quite comfortable in such condition and were normal in behavior as others which were attached to bottom or side walls (Figure-9).

#### ii. Behavior of Mytilus during byssus production:

Mechanism of thread production was quite interesting, the length of the thread depended on the length of the foot protruded out of the animal's body. The animal would slowly monitor the surrounding by exploring out the foot, moment any support sways the way, the tip of the gland elaborates a secretion which sticks onto wet surface; be it smooth or rough, then groove also gets filled with the another secretion which forms the body of the thread. The animal then retracts the foot and solid thread remains (Figure-10). The threads' opposite end remains inside the foot cuff, enabling a firm anchorage. The thread when fresh is translucent but after 2-3 day attains brownish color. This way the animal makes 50-60 threads all attached to a common root, giving appearance of a broomstick (Figure-11). This appearance is unique and not seen in other *Mytilus* species.<sup>18</sup> The animal when needs to change the substratum it detaches itself from the byssi root moves some distance and again forms a new anchorage. When the animal is forcefully pulled out to detach from its substratum, the entire byssi from its root is pulled out, after that the animal will once again secrete new byssi. The animals sometime did not feed on artificial food and underwent starvation and in this condition survived for three months but till the end they were known to elaborate threads and upon autopsy it was revealed that their gonads atrophied and guts were empty but foot, muscles as well as byssus production were not affected.

#### CONCLUSION

*Mytilus viridis* is found only at Mocha and Dwarka in Gujarat. They can be successfully transported to the laboratory for more than 36 hours without any mortality. They can be successfully maintained under laboratory conditions for more than 6 months. Young animals (spat) <1cm size can thrive in the aquarium up to a period of one year. The growth rate of the young is very slow in the laboratory condition. Feeding them on a proper ration becomes difficult. A fresh chetoceros alga is the best food of this animal in the aquarium. Byssus production is not affected during the times of starvation.

#### ACKNOWLEDGEMENT

The author is grateful to UGC- New Delhi for financial support.

S. no.	Parameters	Unit	Data of	Data of
			Mocha water	Dwarka water
1	Salinity	‰	36.96	38.67
2	Temperature	°C	25 to 28	25 to 28
3	Dissolved Oxygen	mg/l	6.30	6.53
4	Biological OxygenDemand (3days 27°C)	mg/l	2.35	2.02
5	ChemicalOxygenDemand	mg/l	85.00	90.00
6	Pb (Heavy metal)	mg/l	0.01	0.01
7	Fe "	mg/l	0.047	0.049
8	Zn "	mg/l	0.003	0.005
9	Cr "	mg/l	0.008	0.004
10	Cu "	mg/l	0.002	0.003
11	Ni "	mg/l	0.009	0.007

 Table-1

 Data on various parameters of sea water from Mocha and Dwarka.

 Table-2

 Data on various parameters of sea water in the aquarium.\*

	Parameters	Unit	Data		
S. No.					
1	Salinity	‰	35.67		
2	Temperature	°C	28		
3	Dissolved Oxygen	mg/l	12.55		
4	Biological Oxygen Demand (3days 27°C)	mg/l	113		
5	ChemicalOxygenDemand	mg/l	186		
*Data collected from Chemical Testing Laboratory, Env. Div. ATIRA, Ahmedabad.					



**Figure-1**Colony of *Mytilus* at Mocha showing few animals



**Figure-2**Colony of juvenile *Mytilus* (spat size 8mm) at Mocha



**Figure-3***Mytilus* seen lurking beneath the rocks (Mocha).





**Figure-5***Mytilus* shell showing growth rings indicating the age of animal.



**Figure-6***Mytilus* covered with orange colored material at Dwarka.



**Figure-7**Aquarium containing *Mytilus* in laboratory.

**Figure-8***Mytilus* crawling on the walls of glass aquarium by means of byssus (spider-man style).



**Figure-9***Mytilus* seen hanging on a floating wooden piece by means of byssus in an aquarium.



**Figure-11**Bunch of byssus showing broomstick appearance. 1- Stem, 2- Thread proper, 3- Plaque.

#### REFERENCES

- 1. Gosling EM, The Mussel Mytilus Ecology Physiology Genetics and Culture, Part-1, Elsevier Science.1992; pp1-100.
- Soot-Ryen T, Family Mytilidae Rafinesque 1815. In: R.C. Moore (Ed.), Treatis on Invertebrate Paleontology, Mollusca 6 Bivalvia. Geographical Society of American and University of Kansas Press, Lawrence. 1969; Part N. Vol-1: pp N271-N280.
- 3. Pai MV, Kuriakose PS. Mussel culture at Karwar, Karnataka state. Mar.Fish, Inv. Serv, 1981, 33, 13-16.
- 4. Trivedi CR, Bhaskaran M, Wadher DJ, Khhayya FD. Successful long distance road transport of green mussel from Calicut to Port Okha. Indian Counc. Agricult res. Mar. Fish. Inv. Serv. 1986; 70: 24-28.
- 5. Tewari A, Joshi HV, Raghunathan C, Shrankumar VG, Koliwar OS. New record



**Figure-10***Mytilus* with foot extended to produce byssus in laboratory aquarium.

of M. viridis (Linn) Its density, growth and accumulation of heavy metals on the Saurastra coast, Arabian sea. Current Sci. 2000; 78(1): 97-101.

- 6. Connor KM and Robles CD. Within-Site Variation of Growth Rates and Terminal Sizes in Mytilus californianus Along Wave Exposure and Tidal Gradients. Biol. Bull. 2015; 228(1): 39-51.
- 7. Fitzgerald-Dehoog L, Browning J and Allen BJ Food and heat stress in the California mussel: evidence for an energetic trade-off between survival and growth. Biol. Bull. 2012; 223(2): 205-216.
- 8. Díaz C, Figueroa Y and Sobenes C. Seasonal effects of the seeding on the growth of Chilean mussel (Mytilus edulis platensis, d'Orbigny 1846) cultivated in

central Chile. Aquaculture. 2014; 428: 215-222.

- Dowd WW, Felton CA, Heymann HM, Kost LE and Somero GN. Food availability, more than body temperature, drives correlated shifts in ATP-generating and antioxidant enzyme capacities in a population of intertidal mussels (Mytilus californianus). J. Exp. Mar. Biol. Ecol. 2013; 449: 171-185.
- Riisgard HU, Pleissner D, Lundgreen K and Larsen PS. Growth of mussels Mytilus edulis at algal (Rhodomonas salina) concentrations below and above saturation levels for reduced filtration rate. Mar. Biol. Res. 2013; 9(10): 1005-1017.
- 11. Hawkins AJS, Smith RFM, Tan SH, Yasin ZB. Suspension-feeding behaviour in tropical bivalve molluscs: Perna viridis, Crassostrea belcheri, Crassostrea iradelei, Saccostrea cucculata and Pinctada margarifera. Marine Ecology Progress Series. 1998; 166: 173-185.
- 12. Spotte S. In: Marine Aquarium Keeping. John Wiley and Sons. Inc.N.Y.1974; pp 9.
- Bayne BL. Physiological ecology of marine molluscan larvae. In: N. H. Verdonk, J. A. M. van den Biggelaar, and A. Tompa (Ed.),

The Mollusca, Vol. III, Development. Academic Press, NY. 1983; pp 299-343

- 14. Beninger PG, St-Jean SD. The role of mucus in particle processing by suspension-feeding marine bivalves: Unifying principles. Marine Biology. 1997; 129(2): 389-397.
- 15. Beninger PG, Veniot A. The oyster proves the rule: Mechanisms of pseudofeces transport and rejection on the mantle of Crassostrea virginica and C. gigas. Marine Ecology Progress Series. 1999; 190(3): 179-188.
- Duggins DO, Eckman JE. Is kelp detritus a good food for suspension feeders? Effectsof kelp species, age and secondary metabolites. Marine Biology. 1997; 128(3): 489-495.
- Tenore KR. Food chain pathways in detrital feeding benthic communities: A review, with new observations on sediment re suspension and detrital recycling In: B. C. Coull, (Ed). Ecology of Marine Benthos. University of South Carolina Press, Columbia, SC. 1977; pp 37-53.
- Eckroat LR, Stelle LM. American Malacological Bulletin, 1993; 10(1): 103-108.