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Title: Happy as a clam in greater depths:

an investigation on the burrowing behavior of *Donax cuneatus*




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1. Abstract

Donax cuneatus is a species of marine molluscs within Class Bivalvia, commonly referred to as 'clams'. They reside inside a calcareous shell and live a sedentary lifestyle, with extension of siphons and burrowing being major observable activities.

Burrowing is a mechanism by which bivalves escape predators and immediate threats, and thus increasing their evolutionary fitness. *Donax cuneatus* is a species of infaunal bivalves, and are found buried in soft substrates in intertidal zones, where sediment generally remains damp. When buried in sediment, clams are sheltered from abiotic abnormalities as well as predators.

In this investigation, our objectives are to study the effects of gyroscopic motion, salinity and sound intensity on the digging behavior of *Donax cuneatus*.
Clams were collected from Shui Hau Wan mudflat, South Lantau. Experiments are carried out with the collected clams in the school laboratory over three weeks, and their behaviors are recorded.

From our investigation, *Donax cuneatus* responded to the three environmental factors that we manipulated. They tend to burrow deeper when larger gyroscopic motions are detected. *Donax cuneatus* clams take longer time to extend siphons under higher sound intensities. Lastly, *Donax cuneatus* clams are able to endure salinities that deviate slightly (~1%) from their original environment (~4%), as indicated by the extension of siphons in water with various salinities. They can cope with water of different salinities manipulated by sea salt, while water with salinities manipulated by sodium chloride noticeably affect their behavior.

2. Introduction

<u>2.1 Introduction of clams</u>

The shell of a clam, usually divided into two valves of similar sizes, is used to cover and protect its body. The two valves are held together by an elastic ligament and a hinge joint, which provide tension to bring the valves apart. The shell opens for foot extension when the clam is relaxed, while the two adductor muscles close the shell and keep the shell shut.

The mantle forms the outer wall of the bivalve body, encloses its visceral mass (ie. internal organs), and secretes calcium carbonate to form the shell.

Clams possess a pair of siphons, which are tube-like structures for water flow. Clams extend siphons to sediment surface for respiration, feeding, excretion and reproduction. The members of the pair of siphons are called the inhalant siphon and the exhalant siphon respectively. Water usually enters the mantle cavity, which is the space between the mantle and the internal organs, through the inhalant siphon, and leaves through the exhalant siphon. If the siphons get attacked by a predator, they break off. They can later be regenerated.

The foot is a muscular organ which clams use for burrowing. It is usually flat, blade-like and ciliated.

The sensory organs of clams are not well developed. Clams have mechanoreceptors and chemoreceptors located along the margins of their posterior mantle. Mechanoreceptor cells respond to mechanical pressure and are sensitive to touch. Siphons are equipped with chemoreceptor cells which can detect water quality for feeding.

Clams usually extend their siphons to substrate surfaces for feeding and respiration during high tide, but burrow to deeper locations or keep their shells tightly shut during low tides.

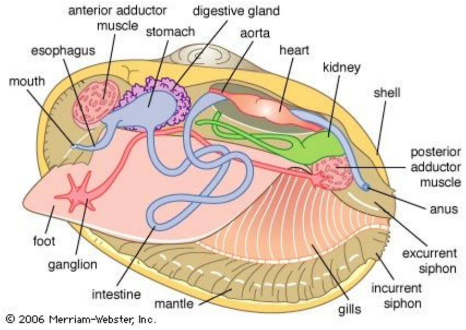

Fig.1 Structure of clams

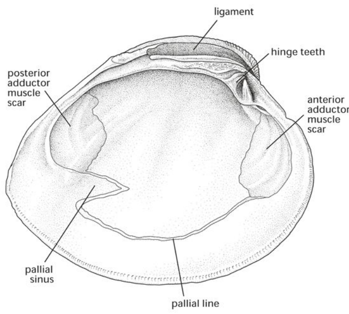

Fig.2

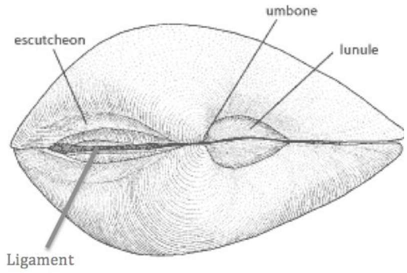

Fig.3

<u>2.2 Significance of investigation</u>

Lantau Island supports a rich diversity of plant and animal species due to its geographical location, geological features and low degree of urban development. Many ecologically important sites are found in Lantau, including montane forest, natural woodland, uncontaminated streams, and coastal waters. Chinese White Dolphins at Tai O, butterflies at Nam Shan, fish and amphibians at Tung Chung Au, precious mudflats at Shui Hau and Pui O are all vulnerable species and habitat to be conserved.

However, in the recently released Policy Address, the government has proposed to further develop various parts of Lantau Island to facilitate *"development for commercial, tourism and recreational purposes"*. Reclamation and construction of artificial islands, as drafted in the development blueprint, may pose irreversible damages to the natural habitats and hinder conservation of indigenous species. In particular, we have chosen to look into the impacts of human activities on the habitat and behaviour of *Donax cuneatus*, a species of bivalves that is commonly found at the South Lantau Shui Hau Wan mudflat.

Donax cuneatus is a type of wedge clams that feed on suspended particulates in water. These clams are considered as 'ecosystem engineers' because they are responsible for making the water clear by filter feeding, and have huge ecological importance and impact on other organisms sharing the mudflat.

2.3 Objectives

- To study the effect of gyroscopic motion on the digging behaviour of *Donax cuneatus*
- To study the effect of salinity on the digging behaviour of *Donax cuneatus*
- To study the effect of sound intensity on the digging behaviour of *Donax cuneatus*

2.4 Background information of *Donax cuneatus*:

Classification:

Kingdom Animalia
Phylum Mollusca
Class Bivalvia
Order Veneroida
Family Donacidae
Genus Donax
Species cuneatus

Fig.4

Donax cuneatus clam, like other bivalves in Genus Donax, has a triangular shell shape. The anterior side of the shell is rounder than the posterior side. The external ligament is relatively short. *Donax cuneatus* has a whitish grey coloured shell with purplish brown radial stripes. It has a shell length of approximately 4 cm.

Donax cuneatus has internal structures fitting general characteristics of clams. However, its pallial line, which is a mark on the interior valve that shows where the interior tissues are attached, is curved inward to a larger extent when compared to other species.

Donax cuneatus clams are found in sandflats and mudflats. They are distributed in the Indo-Pacific region which includes the red sea, Arabian Gulf, Indian Ocean and the Pacific Ocean.

3. Materials and Methods:

3.1 Field Studies

Field studies were conducted at South Lantau Shui Hau Wan mudflat on 20/02/2016 and 27/02/2016. In the first field study, the natural habitat of *Donax cuneatus* was analysed and the burrowing behaviour of this species was observed. In the second field study, 35 clams of the species *Donax cuneatus*, soil samples and seawater were collected.

The site was divided into three zones: the supratidal, intertidal and subtidal zones. There were also salt marshes with grass growth. *Donax cuneatus* clams were mostly found in the intertidal zone, near the surface during low tide where the sediment was moist.
The sediment in the zone was of wavy bedding. Trails of gastropods were also present on the sediment.

Fig.6 Features of mudflat

Fig.5 Map of Shui Hau Wan

Fig.7 Trails of gastropods

Site Information

	Field trip 1	Field trip 2
Location	South Lantau Shui Hau Wan mudflat	South Lantau Shui Hau Wan mudflat
Date	20 Feb 2016	27 Feb 2016
Time	12:00-14:00	16:00-18:30
Air temperature	17.0°C	18.0°C
Substrate temperature	17.7°C	19.0°C
Salinity	39 ppt	40 ppt
pH	5	5
Current tide	low tide (1.08m ~ 1.21m)	low tide (0.82m ~ 1.14m)
Highest tide	1.44m (10:06)/ 2.34m (20:05)	1.75m (23:37)
Lowest tide	0.22m (02:40)/ 1.08m (13:45)	0.82m (18:11)
Wind direction	WNW	NE

Equipment for measuring abiotic factors

Abiotic factor	**Equipment**
Temperature	digital thermometer
Salinity	refractometer
Wind direction	compass
pH value	pH paper and pH colour chart

Other equipments

Equipment	**Quantity**
Shovel	2
Gloves	4
Forceps	2

<u>3.2 Behaviour of *Donax cuneatus*</u>

The *Donax cuneatus* clam is a shallow burrower, and burrows into the sediment for protection against predators, wave action, desiccation and overheating. From our observation, *Donax cuneatus* first extends its siphons out of its valves, then its muscular foot for burrowing. When the foot extends far enough and reaches the sediment, *Donax cuneatus* jerks and sediment nearby is displaced. After several jerks (generally ranges from 4-5 times), it reaches a vertical position in which the ligament is clearly observed from above. *Donax cuneatus* then burrows vertically into the sediment. Its siphons can still be observed on the water surface after its shell is completely covered by the sediment.

A current is occasionally observed at the water surface around the siphon. This is due to the water flow directed into and out of the siphons. Water is occasionally seen to be ejected out of the siphon, creating air bubbles in the water.

Fig. 8 Stages of burrowing

Original position Extension of siphons First jerk Vertical position

<u>3.3 Laboratory work</u>

After the soil and the seawater were collected from the field site, they were used to simulate the living environment of *Donax cuneatus.* 35 clams of the species *Donax cuneatus* were kept in two boxes. Water was changed regularly and nutrients (including *spirulina* and *kelp*) for filter feeders were provided. They were returned to the field site after experiments were conducted at the laboratory.

3.4 Experiments

3.4.1 Control:

Design of experiment:

Before abiotic factors are altered to investigate their effect on the digging behaviour of *Donax cuneatus*, 8 trials of the control set-up were conducted. The abiotic factors in the control set-ups were simulations of the original living environment of the species: the salinity was kept at 39 ppt, and sound intensity at 52 dB (room conditions). The data obtained from the control set-ups were used for comparison with later experiments.

Equipment per set-up:

1 x *Donax cuneatus*
1 x 750 cm^3 soil
1 x 250 cm^3 sea water
1 x transparent plastic container
1 x stopwatch

Procedures:

1. A transparent container with 750 cm^3 of mud was prepared.
2. 250 cm^3 of water was added into the container.
3. A clam was placed on the substrate with one of its valves facing upward.
4. The time for the clam to extend its siphon, its foot, carry out its first jerk and achieve a vertical position was recorded respectively.
5. After the vertical position was achieved, 15 minutes were allowed for burrowing.
6. The time when the clam's shell become totally covered by substrate was recorded.
7. After 15 minutes were allowed for burrowing (mentioned in step 5), the depth of the clam was measured. (length measured from the lowest point of the shell to the soil surface).
8. The experiment was stopped if only siphons were extended/ no action was observed after 25 minutes (timed from the beginning of the experiment).
9. The curved shell length of the clam was measured.
10. Steps 1-9 were repeated 8 times.

Results: Table showing the burrowing behaviour of *Donax cuneatus* under normal conditions

Control	Outer shell length of clam (mm)	Extension of siphon out of the valves (s)		Extension of foot (s)		First jerk (s)		Vertical position (s)		Shell totally covered by substrate (s)		Depth after 15 min of vertical digging (mm)	
			Avg		Avg		Avg		Avg		Avg		Avg
1	37	460	460	/	/	/	/	/	824.5	/	1370	/	/
2	33	68		/		/		329		810		33	
3	44	816		/		/		1320		1930		29	

4	34	364[1] 503 1218		/		/		/		/		/	
5	38	191 313 410		/		/		/		/		/	
6	39	1080		/		/		/		/		/	
7	37	332 1064		/		/		/		/		/	
8	40	368		/		/		/		/		/	

Analysis:
In a majority (75%) of set-ups, *Donax cuneatus* only extended their siphons in the control set-up, and there was no specific time range for the extension. It was also common to see extensions and retractions of siphons within the time allowed for burrowing. Clams were at a peaceful environment with no external stimuli. It did not detect any danger and thus burrowing was not required for protection.

<u>3.4.2 Gyroscopic motion:</u>
Design of experiment:
A transparent container was placed onto a gyroscopic stimulator (gyro rocker) to simulate swirling movements caused by human activities such as construction, running and walking movement of crowds and tourists, or passage of ships. This serves to investigate the effect of gyroscopic motion on the digging behaviour of *Donax cuneatus.*

IV: revolutions per minute
DV: depth of burrow after 15 minutes
CV: depth and volume of substrate, temperature, salinity of water, water depth

Equipment per set-up:
1 x *Donax cuneatus*
1 x 750 cm^3 soil
1 x 250 cm^3 sea water
1 x transparent plastic container
1 x gyro rocker
1 x stopwatch

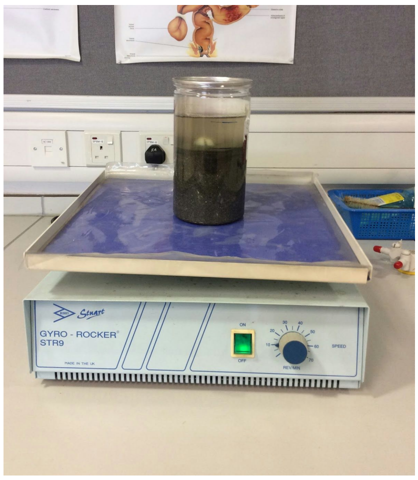

Fig. 9

Procedures:
1. A transparent container with 750 cm^3 of mud was prepared.
2. 250 cm^3 of water was added into the container.

[1] Siphons extended for 3 times after retraction.

3. The container was placed onto the centre of a gyroscopic stimulator (gyro rocker).
4. The gyroscopic stimulator (gyro rocker) was set at 10 revolutions/ minute.
5. A clam was placed on the substrate with one of its valves facing upward.
6. The time for the clam to extend its siphon, its foot, carry out its first jerk and achieve a vertical position was recorded respectively.
7. After the vertical position was achieved, 15 minutes were allowed for burrowing.
8. The time when the clam's shell become totally covered by substrate was recorded.
9. After 15 minutes were allowed for burrowing (mentioned in step 5), the depth of the clam was measured. (length measured from the lowest point of the shell to the soil surface).
10. The experiment was stopped if only siphons were extended/no action was observed after 25 minutes (timed from the beginning of the experiment).
11. The curved shell length of the clam was measured.
12. Steps 1-11 were repeated with 2 more trials, with a total of 3 trials.
13. Steps 1-12 were repeated with frequencies of the gyroscopic stimulator at 20 rev/min, 30 rev/min, 40 rev/min.

Results: Table showing the effect of gyroscopic motion on the burrowing behaviour of *Donax cuneatus*

Swirling motion (rev/min)		Outer shell length (mm)		Extension of siphon out of the valves (s)		Extension of foot (s)		First jerk (s)		Vertical position (s)		Shell totally covered by substrate (s)		Depth (mm)	
			Avg		Avg		Avg		Avg		Avg		Avg		Avg
10	Trial 1	38	36.7	292	546.3	320	655.3	386	992	434	1076	921	1652.7	21	21.3
	Trial 2	34		1252		1266		1346		1500		1938		21	
	Trial 3	38		95		380		1244		1294		2099		22	
20	Trial 1	38	37.3	102	256.3	717	737.7	854	966.3	920	1138.7	1244	1539.7	35	35.3
	Trial 2	41		604		646		920		956		1356		46	
	Trial 3	33		63		850		1125		1540		2019		25	
30	Trial 1	36	37.3	134	113.3	175	210	261	351.3	287	441.3	634	812	25	24.0
	Trial 2	39		79		163		253		293		601		20	
	Trial 3	37		127		292 469		540		744		1201		27	
40	Trial 1	39	35.7	158	265.7	714	545.5	989	591.7	1035	710.7	1560	1106.7	33	27.3

	Trial 2	33		187		[2] /		243		451		715		[3] 23	
	Trial 3	35		452		377		543		646		1045		26	

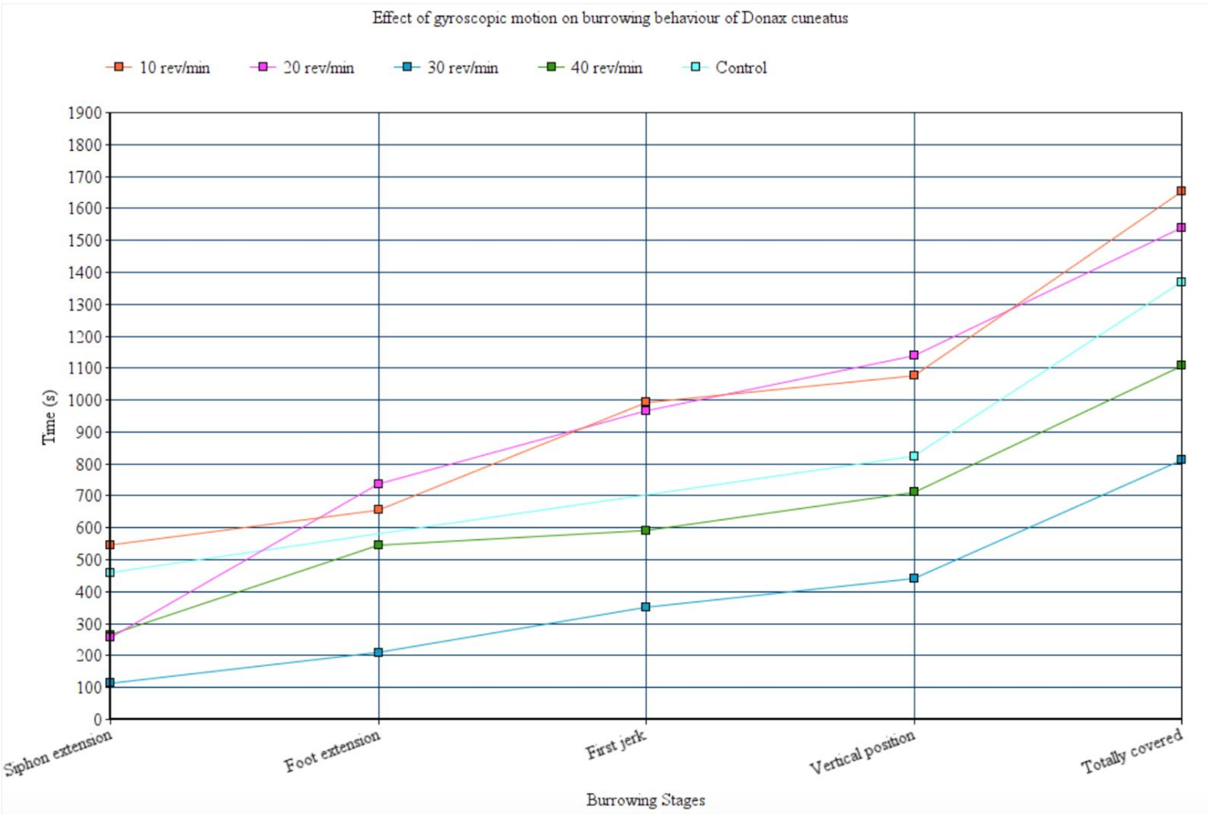

Fig.10

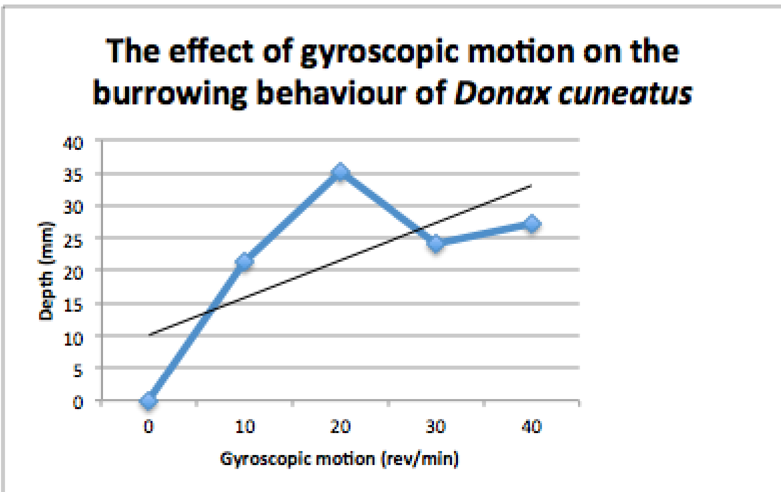

Fig.11

Analysis:

The average burrowing depth of *Donax cuneatus* increased from 21.3mm to 35.5mm when gyroscopic motion increased from 10 rev/min to 20 rev/min. When gyroscopic motion further

[2] Extension of foot cannot be seen.

[3] Lateral movement of clam is observed during experiment.

increased to 30 rev/min, the average burrowing depth decreased to 23.0mm, and rose to 27.3mm again when gyroscopic motion increased to 40 rev/min.
The average burrowing depth at 10 rev/min was the shallowest because 10 rev/min gyroscopic motion caused the least disturbance to clams. They may not feel as threatened and hence did not burrow as deep for self-protection. As gyroscopic motion further increased from 30 rev/min to 40 rev/min, the average burrowing depth also increased. There was a general increase in burrowing depth as the swirling movement increased. The conclusion that *Donax cuneatus* clams tend to burrow deeper when they feel a larger degree of external movement in the environment could be drawn.

<u>3.4.3 Sound intensity</u>
Design of experiment:
A speaker connected to a white noise stimulator was clamped vertically above a transparent container to generate different sound intensities at 170.00 Hz. This was to simulate noises produced by construction, passage of ships, tourists and crowds in the abiotic living environment of *Donax cuneatus*. This serves to investigate the effect of sound intensity on the digging behaviour of *Donax cuneatus.*

Equipment per set-up:
1 x *Donax cuneatus*
1 x 750 cm^3 soil
1 x 250 cm^3 sea water
1 x transparent plastic container
1 x clamp
1 x stand
1 x speaker
1 x stopwatch
1 x Sensor Meter

Fig.12

IV: sound intensity (dB)
DV: depth of burrow after 15 minutes
CV: depth and volume of substrate, temperature, water depth, salinity of water

Procedures
1. A transparent container with 750 cm^3 of mud was prepared.
2. 250 cm^3 of water was added into the container.
3. A speaker connected to a white noise stimulator was clamped vertically above the transparent container.
4. A sound intensity of 70 dB (measured at a vertical distance of 6.5 cm away from Sensor Meter) was produced by the white noise stimulator at 170.00Hz. (average of male and female voice range)
5. A clam was placed on the substrate with one of its valves facing upward.

6. The time for the clam to extend its siphon, its foot, carry out its first jerk and achieve a vertical position was recorded respectively.
7. After the vertical position was achieved, 15 minutes were allowed for burrowing.
8. The time when the clam's shell become totally covered by substrate was recorded.
9. After 15 minutes were allowed for burrowing, the depth of the clam was measured. (length measured from the lowest point of the shell to the soil surface).
10. The experiment was stopped if only siphons were extended/no action was observed after 25 minutes (timed from the beginning of the experiment).
11. The curved shell length of the clam was measured.
12. Steps 1-11 were repeated for 2 more trials, with a total of 3 trials.
13. Steps 1-12 were repeated at sound intensities of 80 dB, 90 dB and 100 dB.

Results: Table showing the effect of sound intensity on the burrowing behaviour of *Donax cuneatus*

Sound Intensity (dB)		Outer shell length of clam (mm)		Extension of siphon out of the valves (s)		Extension of foot (s)		First jerk (s)		Vertical position (s)		Shell totally covered by substrate (s)		Depth (mm)	
			Avg		Avg		Avg		Avg		Avg		Avg		Avg
70	Trial 1	39	39.7	155	156.7	178	370.5	201	837.5	347	957	1085	1549.5	30	28.0
	Trial 2	40		148		563		1474		1567		2014		26	
	Trial 3	40		167		/		/		/		/		/	
80	Trial 1	38	39.3	200	234.0	786	1081	957	957.0	1315	1315	/	/	37	37
	Trial 2	40		261		1376		/		/		/		/	
	Trial 3	40		241		/		/		/		/		/	
90	Trial 1	39	38.7	308	328.0	/	/	/		/		/		/	/
	Trial 2	37		395		/		/		/		/		/	
	Trial 3	40		281		/		/		/		/		/	
100	Trial 1	39	38.0	369	332.7	/	/	487	592	638	734.5	986	1096.5	28	30.5
	Trial 2	38		373		/		697		831		1207		33	
	Trial 3	37		256		/		/		/		/		/	

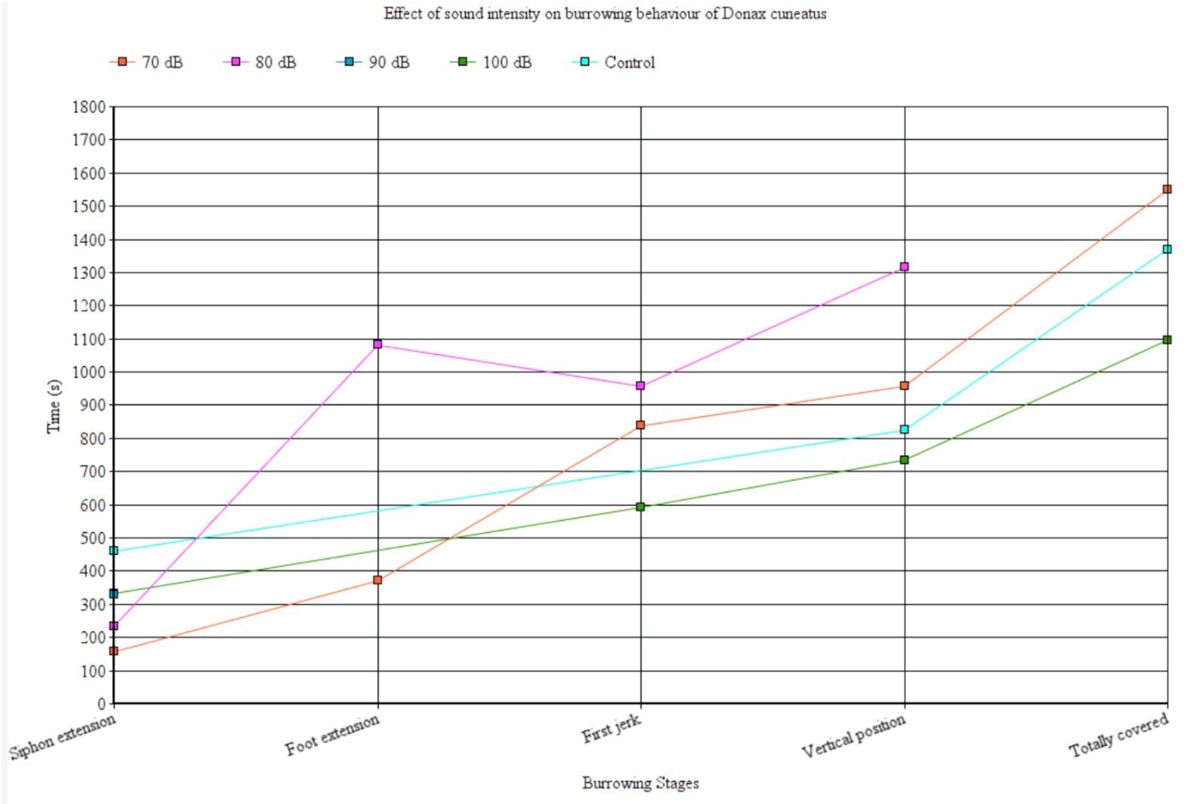

Fig.13

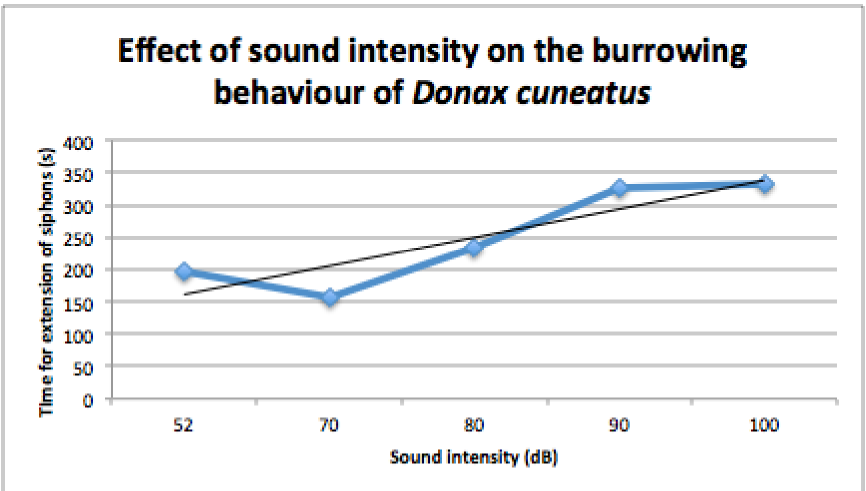

Fig.14

Analysis:

Donax cuneatus burrowed into substrate in some of the trials under sound intensities 70, 80 and 100 dB but did not burrow under sound intensity 90 dB. No general trend could not be seen from their burrowing depths. It could be concluded that sound intensity had no apparent effect on the digging behaviour of *Donax cuneatus*. However, the time needed for the extension of siphons increased from 156.7s to 332.7s from sound intensity 70 dB to 100 dB. This may be due to the presence of chemoreceptors at the siphons, which may have detected a larger degree of water vibrations caused by sound waves of higher amplitude. Thus it took a longer time for the clam to adapt to the environment and to feel safe in extending siphons for feeding and respiration.

3.4.4 Salinity

Design of experiment:

Salinity of water was altered within the range of 20-150 ppt to investigate the effect of salinity on the digging behaviour of *Donax cuneatus.* The manipulation of relative salt content in the water aims to simulate saline pollutants as a result of human activities, such as urban development, road construction, and climate change. People also pour table salt on the mud surfaces when digging clams, which may have adverse effects on clams.

Equipment per set-up:

1 x *Donax cuneatus*

1 x 750 cm^3 soil

1 x 250 cm^3 sea water

1 x transparent plastic container

1 x stopwatch

1 x spatula

20% sodium chloride solution/ Marinium Reef Sea Salt

IV: salinity (ppt)

DV: depth of burrow after 15 minutes

CV: depth and mass of substrate, temperature, water depth

Procedures:

Preparing set-ups with salinity <4%

1. A transparent container with 750cm^3 substrate was prepared.
2. 250 cm^3 of seawater diluted to 2% with distilled water was added into respective containers.
3. The substrate was mixed with the water with a spatula and the salinity of water was measured again to ensure a salinity of 2%.
4. The sediment was allowed to sink and not suspend in the water.
5. A clam was placed on the substrate with one of its valves facing upward.
6. The time for the clam to extend its siphon, its foot, carry out its first jerk and achieve a vertical position was recorded respectively.
7. After the vertical position was achieved, 15 minutes were allowed for burrowing.
8. The time when the clam's shell become totally covered by substrate was recorded.
9. After 15 minutes were allowed for burrowing, the depth of the clam was measured. (length measured from the lowest point of the shell to the soil surface).
10. The experiment was stopped if only siphons were extended/no action was observed after 25 minutes (timed from the beginning of the experiment).
11. The curved shell length of the clam was measured.
12. Steps 1-11 were repeated 3 more times.
13. Steps 1-12 were carried out with salinity of 3%.

Preparing set-ups with salinity >4%

1. A transparent container with 750cm^3 substrate was prepared.
2. Concentration of 250 cm^3 of seawater was increased to 5% with Marinium Reef Sea Salt, and was added into respective containers.
3. The substrate was mixed with the water with a spatula and the salinity of water was measured again to ensure a salinity of 5%.
4. The sediment was allowed to sink and not suspend in the water.
5. A clam was placed on the substrate with one of its valves facing upward.
6. The time for the clam to extend its siphon, its foot, carry out its first jerk and achieve a vertical position was recorded respectively.
7. After the vertical position was achieved, 15 minutes were allowed for burrowing.
8. The time when the clam's shell become totally covered by substrate was recorded.
9. After 15 minutes were allowed for burrowing, the depth of the clam was measured. (length measured from the lowest point of the shell to the soil surface).
10. The experiment was stopped if only siphons were extended/no action was observed after 25 minutes (timed from the beginning of the experiment).
11. The curved shell length of the clam was measured.
12. Steps 1-11 were repeated 3 more times
13. Steps 1-12 were repeated at salinity of 8%, 10% and 15% sea salt.
14. Steps 1-12 were repeated at 5%, 8%, 10% and 15% sodium chloride (ie. table salt) solution, each with 3 trials.

Results: Table showing the effect of salinity on the burrowing behaviour of *Donax cuneatus*

Salinity (%)		Outer shell length of clam (mm)		Extension of siphon out of the valves (s)		Extension of foot (s)		First jerk (s)		Vertical position (s)		Shell totally covered by substrate (s)		Depth (mm)	
			Avg		Avg		Avg		Avg		Avg		Avg		Avg
2 (sea water)	Trial 1	33	35.0	/	/	/	/	/	/	/	/	/	/	/	/
	Trial 2	37		/		/		/		/		/		/	
3 (sea salt)	Trial 1	38	37.0	/	/	/	/	/	/	/	/	/	/	/	/
	Trial 2	36		/		/		/		/		/		/	
5 (sea salt)	Trial 1	40	37.0	191.0	227.5	502.0	438.5	565.0	590.5	869.0	842.0	/	/	15.0	15.5
	Trial 2	34		264.0		375.0		616.0		815.0		/		16.0	
6 (NaCl)	Trial 1	35	35.5	/	21.0	/	/	/	/	/	/	/	/	/	/

	Trial 2	36		21.0 447.0		/		/		/		/		/	
8 (NaCl)	Trial 1 (Na Cl)	35	35.0	[4]181.0	181.0	/	/	/	/	/	/	/	/	/	/
8 (sea salt)	Trial 1	31	32.0	/	/	/	/	/	/	/	/	/	/	/	/
	Trial 2	33		/		/		/		/		/		/	
10 (NaCl)	Trial 1	36	36	[5]96.0	96.0	/	/	/	/	/	/	/	/	/	/
10 (sea salt)	Trial 1	37	35.5	/	/	/	/	/	/	/	/	/	/	/	/
	Trial 2	34		/		/		/		/		/		/	

*To minimize and prevent further harm done to clams, only one trial was carried out per NaCl concentration in seawater.

Analysis:

Donax cuneatus did not burrow for most seawater with salinities that deviate more than 1% from that of its original habitat (~4%), hence a trend cannot be observed for the effect of salinity on average burrowing depth of *Donax cuneatus.*

The effect of using sodium chloride and Marinium Reef Sea Salt to manipulate salinity of water were compared. Under the same salinity, water manipulated with sodium chloride did more physical harm to clams than did that with sea salt, as there were evident tissue damage on the clams when 8% and 10% sodium chloride solutions were used. At extreme salinities using sea salt, *Donax cuneatus* did not extend their siphons out of their shells, or retracted siphons immediately after assessing the environment with siphons.

4. Discussion

Burrowing depth has various implications[6]: *"deeper burial increases survival, but slows the feeding rate"*. When clams burrow deeper, the risk of being washed away by wave actions or eaten by predators, such as crabs, is reduced. However, since clams are filter feeders and obtain food mainly from the liquid medium, they become less exposed to water at a greater burrowing depth, and filter feeding can be hindered. An observation from all experiments indicated that once *Donax cuneatus* clams reached a vertical position, they burrowed with a similar rate regardless of difference in external factors.

[4] Siphons were broken off.
[5] Siphons were broken off.
[6] Stephanie D. Zaklan and Ron Ydenberg,1996. The body size-burial depth relationship in the infaunal clam *Mya arenaria*

4.1.1 Effects of gyroscopic motion on *Donax cuneatus*
There was a general increasing trend when the gyroscopic motion increased from 10 rev/min to 40 rev/min. Under larger swirling motion, temporary disturbance was caused to *Donax cuneatus*. *Donax cuneatus* may have sensed the possibility of being washed away by constant water movements and thus burrowed deeper for protection. If large ships pass by waters near the Shui Hau Wan Mudflat, such swirling movements can be produced and amplified, which may disturb the feeding ability of *Donax cuneatus*.

4.1.2 Effects of sound intensity on *Donax cuneatus*
Clams are not known to have any hearing structures to detect sounds. However, as sound waves from the air can be transmitted to the water medium, they generate water vibrations that can be detected by chemoreceptors on the siphons of *Donax cuneatus.*

There are no significant effects of sound intensity on the burrowing depth of *Donax cuneatus*, hence the time required for them to extend their siphons was compared instead. When sound intensities increased from 70 dB to 100 dB, the time taken for *Donax cuneatus* to extend its siphons lengthened from an average of 156.7s to 332.7s, which shows a general increasing trend. When sound intensities increased to a higher amplitude, the degree of water vibration increased. *Donax cuneatus*'s siphons were sensitive to such water movements caused by sound waves. The higher the degree of motion of surrounding waters detected by the clam's siphons, the less safe it feels, and hence the longer it takes to extend its siphons for feeding. As noticed from our experiments, *Donax cuneatus* clam is quite tolerant and adaptive to environmental changes. Once the clams have adapted to the continuous high sound intensities and the water motions generated, they behaved normally and there were no significant differences in burial depths or the burrowing stages following siphon extension. However, it was observed that *Donax cuneatus* react to sudden bursts of sounds, as the clams immediately retract their siphons when [7]sudden loudness was produced during experiments.

Due to development of South Lantau, construction work and the increase in number of tourists will cause more noises, such as sounds from construction machines and ships and crowds. There will also be many abrupt sounds, as the noises produced in the actual environment will not be continuous white noise, as they were in our experimental set-ups. This would make *Donax cuneatus* clams feel threatened and fail to adapt to their environment due to constant, unpredictable changes. They may not feel safe in extending their siphons, hence affecting their ability to filter-feed. This may indirectly affect the clarity of water at the site, due to the reduction in efficiency of these filter feeders.

4.1.3 Effects of salinity on *Donax cuneatus*
The salinity of seawater directly affects the water quality of *Donax cuneatus*'s feeding medium. At salinity 40 ppt, which is equal to that in its natural habitat, *Donax cuneatus* feel

[7] Sudden loudness were due to walking sounds of heels, and abrupt movements of other students and teachers in the laboratory.

unthreatened and thus no digging behaviour was observed. However, siphons were still extended out of the valves for filter feeding and respiration. At lower salinities, *Donax cuneatus* was able to cope with the environment, but siphons were not extended due to the unsuitable environment. At higher salinities close to 40 ppt, extension of siphons was still observed if sea salt was used to manipulate the salinity. This may be an adaptation in clams as a result of tidal movements (there are two tidal movements daily in South Lantau Shui Hau Wan mudflat). During low tides, the salinity of sediment increases as water evaporates, thus *Donax cuneatus* can cope with a slightly higher salinity.
When sodium chloride was used, a similar trend was observed. However, there were physical damage to *Donax cuneatus* clams' siphons at salinities 80 ppt and 100 ppt. After the top part of their siphons broke off, they immediately retract their siphons and closed their valves until the experiment ended.

The two *Donax cuneatus* with siphons broken were separated from other clams in a small tank for identification. The tank was of normal living conditions for the clams. It was observed that after their siphons were damaged, they still extended their siphons and feet, as preparatory actions to burrow under normal conditions (i.e. salinity 39 ppt, sound intensity 52 dB). However, after they extended their feet, they were held in position and the clams did not jerk or burrow, occasional lateral movements at the surface of the sediment were observed. It was suspected that the sodium chloride may have caused other physical damages that led to the abnormal behaviors.

Construction at the South Lantau may lead to leakage of chemicals into the groundwater or into the sea, subsequently affecting salinities. Ions in such chemicals may not be found in seawater under normal conditions, and can disrupt *Donax cuneatus* clams' burrowing behaviour or even cause tissue damage, making them vulnerable and unable to survive.

4.2 Limitations

- The distribution of clams and their relative abundance in sediment of varying characteristics cannot be obtained during fieldwork because they are buried in the mud. The ideal clam habitat is assumed to be the area clams were collected, and sand is collected from there.
- Sample size is not large enough and clam burrowing activities may be affected by the selected individual's health and physical fitness, as well as their sizes.
- Revolutions and white noise used in experiments cannot fully simulate vibrations and noises caused by human activities, thus the clam digging behavior in the experiments might deviate from actual scenarios under human impacts.
- It was assumed that there is only vertical movement during burrowing. However, lateral movement can be observed during the experiment, thus depth may not directly imply the distance burrowed by the clam. Lateral movement might not be observed clearly all the time especially when the clam is totally covered by the soil.

4.3 Error

- Human error is involved in measuring the depth of the clams which burrowed.
- Slight disturbances in the environment during experiments, such as abrupt noises, shadows casted onto the experimental bottles by group members, can cause clams to retract their siphons and cease movement.

4.4 Improvements

- More trials can be carried out to minimize human errors and provide more data for data analysis and trend reading.
- A larger range of intensities of different factors can be conducted for a more comprehensive view of the effect of abiotic factors on clams.
- Factors can be manipulated at smaller intervals to obtain more data and increase the accuracy of the results and trends obtained.

5. Conclusion

It was found that *Donax cuneatus* clams were sensitive to gyroscopic motion, sound intensities and salinities. Firstly, larger swirling movements led to a deeper depth of burial in general. Secondly, larger sound intensities caused a slower extension of siphons. Thirdly, *Donax cuneatus* were able to cope with salinities from 2-10% when sea salt was used, but did not extend siphons at very low (2%) and very high (8-10%) salinities; while salinities higher than 6% caused tissue damage when sodium chloride was used.

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