Vietnam Journal of Marine Science and Technology; Vol. 19, No. 3; 2019: 395–404
DOI: https://doi.org/10.15625/1859-3097/19/3/14059
https://www.vjs.ac.vn/index.php/jmst

Building a set of criteria for selection of natural clam beds (*Lutraria rhynchaena*, Jonas 1844) as broodstock source for seed production and gene conservation in Cat Ba - Ha Long bay

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Received: 10 May 2019; Accepted: 7 August 2019

Abstract

Building a set of criteria used as a scientific basis for conservation zoning of natural marine resources is essential. In this study, we propose a set of criteria to select the suitable natural clam beds of *Lutraria rhynchaena* for protection priority from 16 sites in Cat Ba - Ha Long bay. Research results have built a set of 13 different criteria, including area, density, benthic substrate, food organisms, turbidity, water flow, water depth, predators, salinity, benthic organisms, pollution, area location and zoning conflicts. The criteria are divided into 5 groups, in which the area and density have the highest coefficient of 5, followed by the zoning conflict with the coefficient of 4, the pollution with the coefficient of 3, the efficient of benthic substrate and water flow is 2, the remaining criteria have a same coefficient of 1. The highest points are evaluated at Tung Sau bed (station 12) with a score of 273 points, followed by Van Boi beach (station 7) with a score of 253 points, the bed in Trinh Nu cave (station 13) with a score of 249 points, which are proposed to be prioritized sites for conservation.

Keywords: Natural clam beds, *Lutraria rhynchaena*, Cat Ba - Ha Long bay, set of criteria, seed production, gene conservation.

Citation: Do Manh Hao, Dao Thi Anh Tuyet, Nguyen Tien Dat, Le Minh Hiep, Nguyen Van Quan, Tran Dinh Lan, Hoang Phu Hiep, 2019. Building a set of criteria for selection of natural clam beds (*Lutraria rhynchaena*, Jonas 1844) as broodstock source for seed production and gene conservation in Cat Ba - Ha Long bay. *Vietnam Journal of Marine Science and Technology*, *19*(3), 395–404.

INTRODUCTION

The snout otter clam *Lutraria rhynchaena* is a bivalve animal with high economic and nutritional values. In the world, the clam is distributed in warm waters of the Philippines, Thailand, China, the US and Australia. In Vietnam, the clam is only confined to a very narrow range at the tidal flats along the limestone islands of Cat Ba, Ha Long, Bai Tu Long and Co To [1, 2]. However, this resource has been seriously reduced in both density and biomass. In 1979 the density and production of the clams in this area were about 1.07 tons/ha and 64.0 tons respectively [3], then in 2008 these figures are 0.01 tons/ha and 0.66 tons respectively [4]. This decline in resources not only affects the sustainable exploitation of natural resources but also greatly affects the supply of parent broodstocks to produce artificial seeds. Therefore, in order to restore cultivation of commercial clams as well as contribute to the conservation of genetic resources, it is necessary to identify and delineate the natural clam beds as a source of broodstock to produce disease-free, high adaptable seeds with the indigenous environment in the context of natural clam resources in great decline.

The development of a set of criteria as a scientific basis for selecting natural clam beds used as parent sources for seed production as well as restoration of natural resources is difficult and new. In order to select the most appropriate natural beds, we must determine the criteria and quantify them by scoring and then select the natural beds with the highest score.

MATERIAL AND METHODS

Study scope and times

In this study, the investigation area is littoral and sublittoral zones in Cat Ba - Ha Long bay from 20°43’25.56”N to 20°53’22.54”N and from 107°3’30.30” to 107°14’10.16”E. There are 16 surveyed sites in wet season and dry season during 2017–2018 (fig. 1).

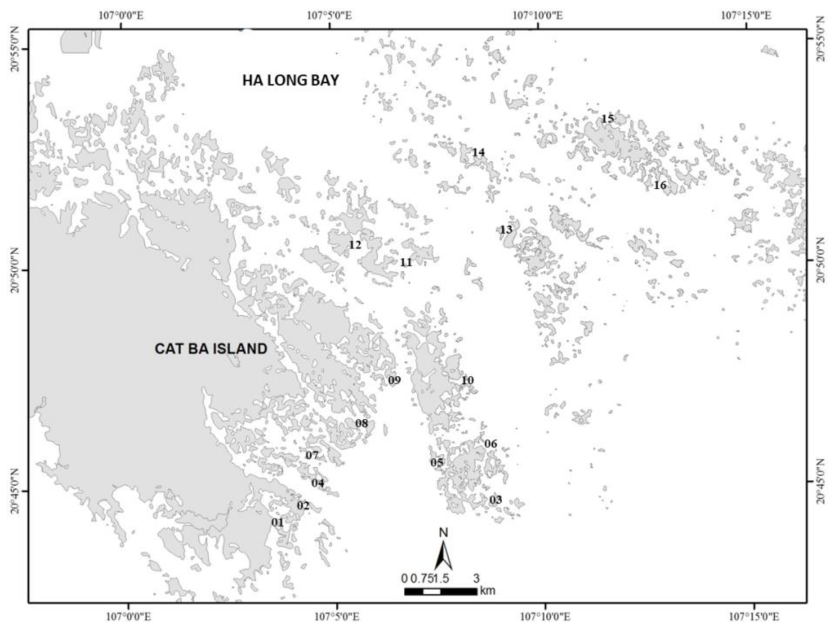

Fig. 1. Surveyed site diagram during 2017–2018

Methods

Investigation and sampling in the field

Clam samples are collected by using scuba equipment to dive directly to the bottom and look for holes (tricks), when we find the tricks, we will use spades to dig and capture the clams. The density of the clams at each site is estimated in 3 quantitative frames (5 m × 5 m).

Seawater samples on the surface are collected by bathometer, then the seawater from bathometer is distributed into glass bottles and glassic bottles (15–50 ml). The samples are stored in ice box and transferred to laboratory for analysis.

The salinity (S‰) is measured by a hand refractometer with an accuracy of 1‰. The pH is measured by a pH meter, reaching exactly 0.01 units. Dissolved oxygen (DO) is measured by an oxygen meter or a Winkler titration with an accuracy of 0.01 mg/l.

The area of natutal clam beds is determined by estimation in the field combined with measuring on the map.

Analysis in the laboratory

Chemical parameters

Biological oxygen demand (BOD_5) is determined by direct method without dilution, incubating in 20^{o}C, accuracy of 0.01 mg/l.

Chemical oxygen demand (COD) is determined by the oxidization of potassium permanganate ($KMnO_4$) in an alkaline medium, accuracy to 0.01 mg/l.

The concentrations of inorganic nitrogen nutrients: Nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3^+, NH_4^+) are determined by optical absorption density measurement method on DR/2000 HACH spectrometer, USA. Errors of ammonia, NO_2^- measurements are 0.1 µg/l, that of NO_3^- is 0.5 µg/l.

Selection method of natural clam beds for protection

Selection of natural clam beds to prioritize protection is conducted by quantifying the set of criteria affecting the growth and development of clam; in particular, quantifying the role of indicators by coefficients and quantifying natural harmonic sites for each criterion by the method of scoring by 10-point scale. Any bed with the highest total score will be given priority to select protection zone.

RESULT AND DISCUSSION

Scientific basis for selecting and quantifying criteria

The criteria for selecting a natural clam bed to prioritize protection as a broodstock source for seed production and resource conservation are environmental, biological and ecological factors that directly affect growth and development of the clams. When considering the priority of protection, we need to take into account the socio-economic factors. These are factors that do not directly affect the growth and development of the clams but are related to the feasibility of implementing resource protection, other relevant socio-economic plans,...

Area

The area is one of the important criteria to select natural clam beds. The larger area of clam bed results in the higher reserve. The larger quantity of the clam in natural beds presents a greater potential in genetic variability, makes the fertilization more efficient which results in higher ability to select new individuals and less effect by the risk of the environmental events.

Density distribution

The natural beds with a high density of clam are related to favorable environmental conditions. The high density of clam makes the fertilization more efficient which results in a higher ability to select new individuals. Therefore, density distribution plays a very important role to select the natural clam beds. However, many clam beds have favorable environmental conditions but still present a low density of clam due to the strong exploitation of fishermen. Therefore, it is necessary to evaluate based on many other criteria, in which the history of the clam distribution in the past should be taken into account in selecting the natural clam beds.

Bottom substrate

Substrate composition, topography, and stability of the bottom are important criteria to select the natural clam beds. The clam lives buried in the bottom to avoid their predators and drifting by the water current. The substrate also helps the clam go to the bottom successfully in the D-shaped larval stage [5, 6]. Mud sand, gravel and sell debris are appropriate substrate environments for clam living. The thickness of the substrate also plays an important role, as the greater thickness and porosity, the more suitable for growth and development.

Food organisms

Main food sources of *Lutraria rhynchaena* are phytoplankton and organic debris. High primary productivity areas lead to high productivity and biomass of the clam. On the contrary, the areas presenting a lower density of phytoplankton lead to reduced growth and development [4]. However, the clams are filter feeder, so the ability to use food depends on a number of other factors such as flow and turbidity of the water source. The clam filters more food in a high flow rate of water movement, while the ability to filter food is significantly reduced if the flow is low. Filtering activity of the clam also decreases in the turbid water.

Turbidity

Turbidity directly affects the filtration ability of the clams, the more turbid the environment, the less likely it is to filter. High turbidity also affects the development of phytoplankton, thereby affecting the growth and development of the clam.

Water movement

The area with low water movement usually results in decrease of the filtering ability of the clam. The flow is also related to the level of risk of predators, a strong water movement can cause more difficulties to attach the food in particular for young clam. However, a very strong movement of the water flow will lead to negative effect on the stability of the substrate, the ability of egg fertilization and the ability of sedimentation of the D-shaped larvae.

Water depth

The depth of the clam beds is not usually a limiting factor for the clams, however the long exposure to the light during the extreme low tide will affect the filtering ability of the clams. When the bed is long exposed, many environmental factors also fluctuate like temperature and light intensity. There is also a certain risk of sunlight exposure time for egg and sperm fertilization and survival ability of larval stage of the clam. Conversely, the possibility of settling the D-shaped larvae will be limited in a high depth clam bed. As the depth increases, phytoplankton also decreases, leading to reduce in filtering opportunities. Moreover, the deeper the clam bed, the greater the energy consumption for their living activities.

Predators

The main predators of the clams are the crabs and fishes because the clams have incompletely closed cover, so the possibility of being attacked by predators is greater than other bivalve species [3]. The level of risk of predation by predators depends on the density of the predators and on some of the factors such as flow, substrate. In areas with a strong current, ability to catch prey of the predator is significantly reduced. The thick and porous bottom substrate area will make the clams avoid much better than the thin and smooth substrate.

Salinity

Salinity has an effect on the growth and development of the clam, they depend on the absolute value and stability of salt concentration. Although salinity is not a limiting factor for mature clams, it is a limiting factor to the early stages of development of the clams [6]. Some studies showed that the salinity around 28–30‰ is very suitable for growth and development of the clams but when the salinity is lower or higher than this range, it will inhibit the growth of larvae and offspring.

Benthic animals

Other organisms, particularly benthic animals play an important role in the growth and development of *Lutraria rhynchaena*. Crabs and some species of fishes that live on the bottom are predators of *Lutraria rhynchaena*. Other bivalve species can compete for living places and food sources with *Lutraria rhynchaena*, but they also have a useful role such as reducing the pressure of predators, particularly during the reproductive period. However, the quantitative assessment of the role of the benthic ecosystem for growth and development of the clam is still limited.

Environmental quality

Activities from humans have been emitting toxic pollutants and potentially pathogenic microorganisms into the environment. *Lutraria rhynchaena* as well as many other bivalves are known to accumulate pollutants in tissue and may lead to negative effect on the health of the clam [5]. In the early stages, *Lutraria rhynchaena* is much more sensitive to pollutants than in the adult clam. Many pollutants have indirect effects on the clam, such as eutrophication, which can lead to blooms of phytoplankton or the development of potentially pathogenic microorganisms.

Protection ability

The feasibility of protection of natural ecology is also seen as one of the important criteria for selecting a natural clam bed [5]. Clam bed with easy protection will get higher point than those with difficult protection.

Planning conflict

If there are potential conservation areas, but they are planned for other purposes, such as marine protected area planning, aquaculture, diving, etc., they also need to be taken into account. It is feasible and effective if the natural site is considered for the MPA plan. On the other hand, it will be difficult to zone the protection in case of planning in the area of aquaculture, tourist areas,…

Other environmental factors

Some environmental factors such as temperature, pH, DO, biodiversity are ecological factors that affect the growth and development of clam. Because these factors do not present a large fluctuation between sites, they are not considered as criteria for selection in this paper.

Quantitative evaluation of criteria

A set of criteria is composed of 13 elements, including area, density, benthic substrate, food organisms, turbidity, water flow, water depth, predators, salinity, benthic organisms, pollution, area location and zoning conflicts. The criteria are divided into 5 groups, in which the area and density have the highest coefficient of 5, followed by the zoning conflict with the coefficient of 4, the pollution with the coefficient of 3, the benthic substrate and the water flow with the coefficient of 2, the remaining criteria have the same coefficient of 1. The highest total score calculated on this scale is 280 points (table 1).

Table 1. Quantify the criteria to choose the natural clam beach in Cat Ba - Ha Long bay

No.	Criteria	Coefficient	Point scale	Total score
1	Area	5	10	50
2	Density	5	10	50
3	Benthic substrate	2	10	30
4	Food organisms	1	10	10
5	Turbidity	1	10	10
6	Water flow	2	10	20
7	Water depth	1	10	10
8	Predators	1	10	10
9	Salinity	2	10	20
10	Benthic organisms	1	10	10
11	Environment	3	10	30
12	Area location	1	10	10
13	Zoning conflicts	4	10	40
	Total	29	10	290

Characteristics of natural clam beds in Cat Ba - Ha Long bay

Area and distribution density of the clam

In the waters of Cat Ba - Ha Long, the clam is distributed into the beds scattered around the islands, sandbank and in the coral reefs, in which the areas of the beds fluctuate significantly, from several hundred m^2 to several hectares. In this study, we focused on investigating 16 natural clam beds with the area ranging from 1.0 ha to 14.0 ha. Tung Sau is the largest area of 14.0 ha, followed by Van Boi with an area of 8.0 ha and Trinh Nu cave with area of 5.9 ha. The remaining sites are less than 4.5 ha (table 2).

Table 2. Area and density of distribution of natural clam beds in Cat Ba - Ha Long Bay

No.	Station name	Area (ha)	Average density (individuals/25 m^2)
1	Cai Beo	2.0	1.7
2	Hon Quai Xanh	4.3	6.7
3	Dau Be -1	4.5	5.7
4	Tung Cap Quan	4.0	7.7
5	Hang Moc	3.5	6.3
6	Dau Be - 2	1.5	5.7
7	Van Boi bed	8.0	14.7
8	Tai Keo bed	1.5	2.7
9	Cong Ngoai	1.0	2.3
10	Trai cave	3.0	4.3
11	Trinh Nu cave	5.9	6.3
12	Tung Sau	14.0	23.7
13	Vung Ha 1	4.4	8.3
14	Vung Ha 2	1.5	2.0
15	Cong Do 1	2.0	7.0
16	Cong Do 2	4.0	7.7
	Total	65.07	7.0

The density of the clam fluctuates in the wide range, from 1.7 individuals/25 m^2 to 23.7 individuals/25 m^2 with an average of 7.0 individuals/25 m^2. In particular, Tung Sau has the largest density of 23.7 individuals/25 m^2, followed by Van Boi with a density of 14.7 individuals/25 m^2. The remaining sites have a lower density of 8.3 individuals/25 m^2 (table 2).

Environmental characteristics of the natural clam beds

Water quality

The pH at the surveyed stations ranged from 7.7 to 8.1. The lowest pH value was recorded at the station 1 and the station 2 in the rainy season, and the highest pH value was also recorded in the rainy season at stations 5, 6 and 12. pH in the rainy season fluctuated wildly and was often lower than that in the dry season (fig. 2a).

Salinity ranged from 22.0‰ to 30.0‰. Salinity fluctuated strongly in the rainy season and was stable in the dry season. The salinity ranged from 28.8‰ to 30.0‰ in the dry season and from 22.0‰ to 29.5‰ in the rainy season. Seasonal salinity fluctuated most strongly at station 1, followed by the stations 2, 4, 15 and 16, respectively. Station 6 presented the lowest seasonal variation, followed by stations 3, 5 and 10, respectively (fig. 2b).

Dissolved oxygen (DO) ranged from 5.8 mg/l to 8.5 mg/l, DO in the rainy season was often lower than that in the dry season. DO presented low concentration in the rainy season at stations 1, 2 and 4, with a range from 5.6 mg/l to 5.8 mg/l. At the remaining stations, DO presented more stable with a range from 7.6 mg/l to 8.5 mg/l (fig. 2c).

Biological oxygen demand (BOD_5) ranged from 1.2 mg/l to 3.4 mg/l. In particular, BOD_5 in the rainy season was usually higher than that in the dry season, with an average BOD_5 concentration of 1.5 mg/l in dry season and 1.8 mg/l rainy season. BOD_5 presented high concentration at the stations 1, 2 and 3, respectively, with a range from 1.9 mg/l to 3.4 mg/l, whereas the remaining stations presented the lower and more stable concentration of BOD_5 with a range from 1.2 mg/l to 1.9 mg/l (fig. 2d).

Chemical oxygen demand (COD) ranged from 2.0 mg/l to 4.9 mg/l. The stations 1, 2, 4 presented the highest COD with a range from 2.9 mg/l to 4.9 mg/l, followed by the stations 7, 15 and 16, respectively with a range from 2.5 mg/l to 2.9 mg/l. The remaining stations presented low and more stable COD concentration with a range from 2.0 mg/l to 2.5 mg/l (fig. 2e).

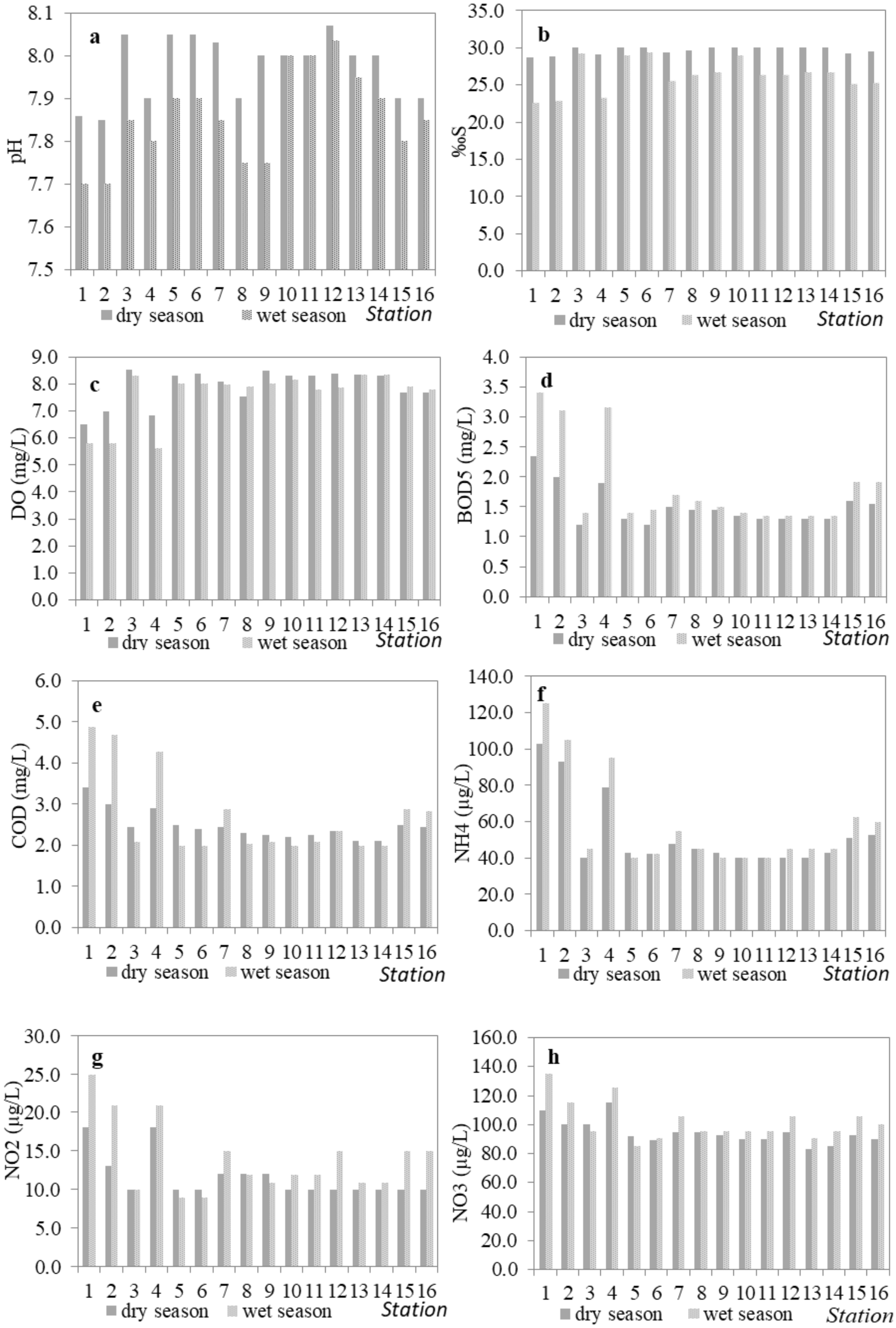

Fig. 2. Environmental quality of water in Cat Ba - Ha Long Bay
in rainy and dry seasons in 2017–2018

Ammonia (NH_4^+-N) ranged from 40.0 µg/l to 125.0 µg/l, with an average of 55.4 µg/l. Average ammonia concentration in the rainy season was 58.1 µg/l and higher than that in the dry season (52.7 µg/l). Ammonia was high at the stations 1, 2 and 4 with a range from 79.0 µg/l to 125.0 µg/l whereas presented a low value at remaining stations with a range from 40 µg/l to 62.5 µg/l (fig. 2f).

Nitrite (NO_2^--N) ranged from 9.0 µg/l to 25.0 µg/l, with an average of 12.8 µg/l. Average nitrite concentration in the rainy season was 14.0 µg/l and higher than that in the dry season (with an average of 11.6 µg/l). NO_2^--N was high at the stations 1, 2 and 4, with a range from 13.0 µg/l to 25.0 µg/l. NO_2^--N concentration in rainy season reached about 15.0 µg/l at the stations 7, 12, 15 and 16. The remaining stations presented low nitrite concentration, with a range from 9.0 µg/l to 12.0 µg/l (fig. 2g).

Nitrate (NO_3^--N) ranged from 83.0 µg/l to 135.0 µg/l, with an average of 98.1 µg/l. Average nitrate concentration in the rainy season was 101,6 µg/l and higher than that in the dry season (94.7 µg/l). The highest nitrate concentration was recorded at the station 1, followed by the stations 4, 2, 7, 12, respectively. The lowest nitrate concentration was recorded at station 13, followed by stations 14, 5, respectively (fig. 2h).

Bottom substrate

Based on the grain size characteristics, bottom substrates were separated into 5 main types, consisting of (1) very coarse sand, (2) very coarse sand and coral reef, (3) coarse sand, (4) coarse sand and coral reef and (5) medium sand. In the areas with the bottom of the sand being very large sand or large sand and coral reefs, the clams live in sandy areas or in sandy rocks covered with little sand. The survey results show that the density of humankind in coral reefs is often lower than that in non-coral reefs (table 3).

Table 3. Characteristics of the bottom substrate of natural clam beds in Cat Ba - Ha Long Bay

Study sites	Name	Average size (mm)	Sediment types
1	Cai Beo	708.1	Coarse sand
2	Hon Quai Xanh	581.5	Coarse sand
3	Dau Be -1	1560.8	Very coarse sand and coral reef
4	Tung Cap Quan	399.4	Medium sand
5	Hang Moc	1560.9	Very coarse sand and coral reef
6	Dau Be - 2	1580.2	Very coarse sand and coral reef
7	Van Boi bed	757.8	Coarse sand
8	Tai Keo bed	665.6	Coarse sand and coral reef
9	Cong Ngoai	1570.0	Very coarse sand and coral reef
10	Trai cave	1560.0	Very coarse sand and coral reef
11	Trinh Nu cave	500.5	Medium sand
12	Tung Sau	491.5	Medium sand
13	Vung Ha 1	1176.7	Very coarse sand
14	Vung Ha 2	1170.2	Very coarse sand
15	Cong Do 1	1154.5	Very coarse sand and coral reef
16	Cong Do 2	1150.0	Very coarse sand and coral reef

Proposal of the protection priority of the clam beds

Based on the survey results and the assessment of current situation of environmental conditions and natural resources in 16 natural clam beds in Cat Ba - Ha Long bay, the highest point was evaluated at Tung Sau bed (station 12) with a score of 254 points, followed by Van Boi bed (station 7) and Trinh Nu bed (station 13) with a score of 235 points and 229 points, respectively. The lowest point was evaluated at Ben Beo bed (station 1) and Quai Xanh bed (station 2) with a score of 128 points and 167 points, respectively. The remaining stations presented the value from 178 points (station 4) to 222 points (station 11) (table 4). Based on the results of scoring, Tung Sau bed, Van Boi beach and Trinh Nu cave are proposed to be prioritized sites as a broodstock source for seed production and resource conservation.

Table 4. Assessing the quality of the clam beds according to the set of criteria

No.	Criteria	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Area	20	30	30	30	25	15	40	15	10	25	35	50	30	15	20	30
2	Density	4	20	16	22	20	16	32	8	8	12	20	40	24	4	22	22
3	Benthic substrate	30	30	15	30	15	15	30	15	15	15	30	30	30	30	15	15
4	Food	5	6	10	7	10	10	8	9	9	10	8	8	10	10	10	10
5	Turbidity	5	6	10	7	10	10	8	9	9	10	8	8	10	10	10	10
6	Water flow	10	12	20	14	20	20	16	18	18	20	16	16	20	20	20	20
7	Water depth	7	7	10	8	10	10	8	8	10	10	8	8	10	10	10	10
8	Predators	7	7	10	8	10	10	8	8	10	10	8	8	10	10	10	10
9	Salinity	10	12	20	12	20	20	16	16	18	20	18	18	20	18	18	18
10	Benthic organisms	5	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
11	Environment	15	18	30	21	30	30	27	30	30	30	30	27	30	30	27	27
12	Protection of the beds	5	5	5	5	5	5	10	10	8	8	10	10	5	5	5	5
13	Planning	20	20	40	20	40	40	40	40	40	40	40	40	40	40	40	40
	Total	143	183	226	194	225	211	253	196	195	220	241	273	249	212	217	227

Acknowledgment: The work was supported by Vietnam Academy of Science and Technology (grant number VAST06.04/17–18, VAST.DA47.12/16–19) and Ministry of Science and Technology (grant number KC.09.11/16–20). The authors would like to thank the Vietnam Academy of Science and Technology and the Ministry of Science and Technology for funding this research.

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