PROTOCOLS FOR COLLECTING DATA TO SUPPORT AQUACULTURE OF BIVALVES IN DUBROVNIK-NERETVA COUNTY

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1. Introduction

This document presents the abbreviated results of both a top-down and bottom-up approach to determine the protocols and data relevant for bivalve aquaculture production in Mali Ston bay located in the Dubrovnik-Neretva county, Croatia.

The top-down approach identified the need for estimating the ecological carrying capacity of Mali Ston bay for the purpose of bivalve farming. A list of data to be collected locally, as well as the sampling and monitoring programs that would be required for this purpose have been covered in the scope of this document.

The bottom-up approach to collecting relevant data for bivalve farming was decided through a meeting with representatives of the Association of bivalve farmers in Ston. They expressed the need for a central dispatch and depuration centre for farmed bivalves located within the facilities of the University of Dubrovnik's Mariculture laboratory in Bistrina bay. In the scope of this document we present the study that was performed to determine its capacity and operational procedures.

2. Determining carrying capacity for bivalve aquaculture in Mali Ston bay

Current state

Mali Ston bay is divided into three aquaculture production Zones (Figure 1):

- Zone I - Located in the very interior of the bay. Bivalve farming is the only permitted aquaculture activity. Bivalve farm concessions occupy 4.72% of the surface of the zone.
- Zone II - Occupies the middle area of the bay. Bivalve farms occupy as much as 12.62 % of the total surface. Only a single, albeit larger, fish farm is present here.
- Zone III - only 0.95 % of the surface of Zone III is used for bivalve farming. Fish farming is not limited in this zone.

Zones I and II are the most sensitive to anthropogenic influences due to lower depth and smaller volume of seawater, as well as less water exchange with the open sea, but are under the influence of submerged freshwater springs. Thus, the impacts of bivalve aquaculture and especially fish farming are the most pronounced there.

Figure 1. Division of Mali Ston bay into three production zones.

According to a study done in 2004, the maximum available space for aquaculture was limited to 10-15 % of the total area of the bay, which is generally considered a conservative value in literature used for production sites in initial developmental stages of cultivation where a lot of unknowns still remain on how any level of farming would affect the environment. It was estimated that the concessions at that time occupied only 849,39 m2, which is 12.9 % of the recommended available area for aquaculture (6,592,000 m2) i.e. 10 % of the total estimated area of the bay of 65.92 km2. However, since then production and the surface allotted to aquaculture has increased and now equals to 1,445,460 m2, which is 21.93 % of the designated area for aquaculture (6,592,000 m2). Although this figure has almost doubled since the previous assessment in 2004, it can still be said that there is room for additional growth if considering very approximate estimations. However, it is highly recommended to carry out dedicated analyses and modelling of carrying capacity using case-specific parameters and not general approximations from literature for any further increases in production capacity. The need for such dedicated monitoring and analyses is further exacerbated by the fact that aquaculture activity is not equally distributed throughout the bay, but rather concentrated in certain hotspots where farming intensity exceeds previously recommended values. Even on the scale of whole production zones there is an obvious heterogeneity in the usage of space for aquaculture:

- Bivalve farming in production Zone I uses 47.21 % of the total designated surface for aquaculture activities
- The total surface for farming in Zone II currently exceeds the recommended one and covers 126.17 % of the surface designated for aquaculture
- In Zone III only 9.52 % of the designated surface for aquaculture is used.

According to these estimates, there are is still some space in Zones I and III for the expansion of bivalve farming to reach the lower estimated limit for conservative production capacities (10% of the total area), while in Zone II the recommended 10% has been exceeded and is approaching the upper limits of estimated capacities - 15% of total surface.

Unfortunately, due to the lack of available data, it is currently only possible to use such approximations, but for a more knowledge-based and realistic assessment of carrying capacity a significant amount of new data and modelling would be required. Given the nature of the bay and the importance of the natural environment to the condition and quality of farmed bivalves, an ecological approach to aquaculture regulation and management is suggested,

i.e. a comprehensive integrated management of activities based on the best available scientific knowledge about the ecosystem and its dynamics, with the aim of recognizing negative impacts that pose or may pose a threat to ecosystem health and taking measures to mitigate or remove them, thus achieving a sustainable use of the goods and services that the ecosystem offers and maintaining its integrity.
In order to achieve such a sustainable future, it is necessary to assess the ecological carrying capacity of the bay for bivalve aquaculture, from the perspective of the physical, chemical and biological characteristics of the ecosystem and the requirements and impacts of aquaculture production. The following parameters have been chosen for this purpose, as well as the minimum and preferred spatiotemporal distribution of required samplings and an estimation of the required budget for such activities:

Biomass of cultured bivalves (by species)

Minimum:

- The **total mass of bivalves** located on aquaculture farms within the bay, expressed in kilograms
- Individual data for each species (mussels and oysters)
- Estimated once per year
- Annual sales of each species (kg)

Preferred:

- Spatial distribution of farmed species within the bay, at least amounts and percentages of species in each production zone (using current or new zonation)
- Quantities by size/age category of each species

Comments:

- Currently reported quantities are not realistic and an alternative, more direct, method of data collection is required, which would incur significant expenses
- Potential on-site data collection from representative farms from several size categories that could be used to extrapolate and estimate data for the whole bay; estimated budget for such an activity is *5.000,00 €*

Biomass of wild bivalves and other sessile filtratory organisms (by species/other taxa)

Minimum:

- Quantitative and qualitative data from **visual census** benthic surveys of **50 m transects** for coastal areas, expressed in numbers of individuals of individual species
- Survey performed once every five years at 10 sampling points
- Budget: 25*,000.00 €*

- Quantitative and qualitative data from **visual census** benthic surveys of **25 m^2 squares** for infralitorral areas away from shore, expressed in numbers of individuals of individual species
- Survey performed once every five years at 15 sampling points
- Budget: *25,000.00 €*

Preferred:

- More sampling points and/or larger transects/areas
- Quantities by size/age category of each species

Comments:

- Extrapolating data from transects and areas to the whole bay may yield significant errors which can be reduced by increasing the number and size of sampling points
- Potentially express quantitative and qualitative values for wild sessile filtratory organism for individual habitats and perform a more detailed mapping the surface covered by each habitat, or for individual production zones (using current or new zonation)

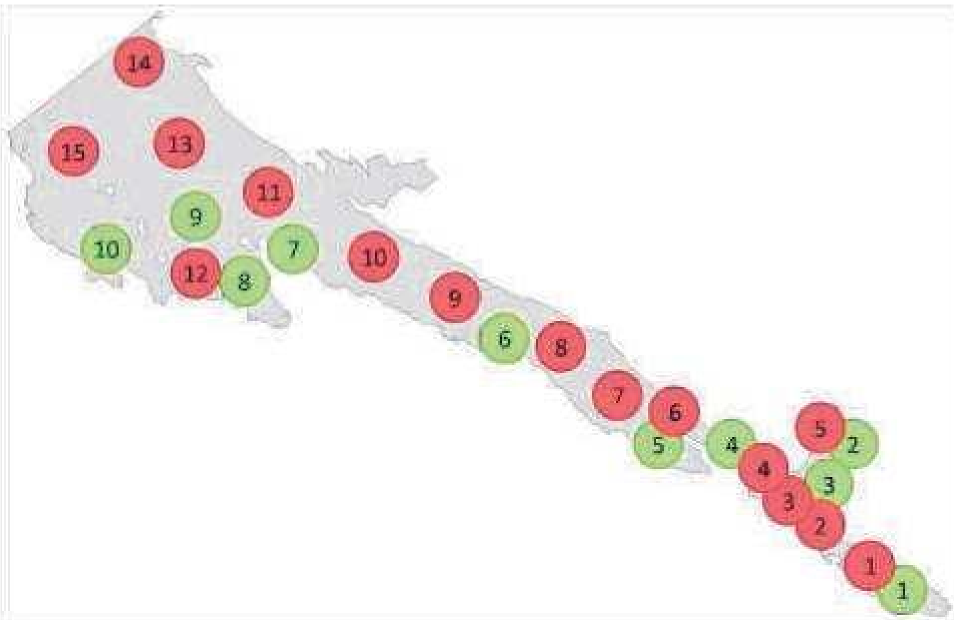

Figure 2. Suggested sampling points for benthic surveys in Mali Ston bay; 10 sampling points for 50 m transects originating from coast (green) and 15 sampling points for 25 m^2 squares away from coast (red).

Biomass of biofouling organisms (by taxa)

- Collection of biofouling organisms on commercial farms from different culture equipment (ropes, buoys, mussel stockings, curtain collectors for oysters, coupelle collectors for oysters, oyster lantern nets, oyster bags, cemented oysters)
- Subsequent qualitative and quantitative analysis of collected subsamples
- Once every five years, 4 times per year at 7 sampling points
- Budget: *5,000.00 €*

Condition index of farmed bivalves

- Sample collection and analysis (shell dimensions, shell mass, meat mass) of farmed adult oysters and mussels for calculating different types of **condition indices** of bivalves
- Each year, 4 times per year at 7 sampling points at 2-3 depths, depending on site depth; n=30 oysters and n=30 mussels for each depth
- Budget: *5,000.00 €*

Primary production (^{14}C-PP)

- Sampling at different depths for periods of 6 hours in order to measure the **incorporation of ^{14}C** into phytoplankton cells. Results in gr ^{14}C m^{-2} year^{-1} values
- In order to account for seasonal variations, samplings should be performed at least 4 times per year at 7 sampling points (every 5 m depth at each sampling point)
- Budget: *9,000.00 €*

Phytoplankton

- Seasonal changes **phytoplankton biomass** expressed in chlorophyl a concentration (µg Chla L^{-1})
- Sampling performed each year on a monthly basis, with measurements at least every two weeks during spring blooms at 7 sampling points
- Budget: *4,000.00 €*

- Qualitative **phytoplankton composition** and **size fractions** analysed using microscopy and microscopy and/or flow citometry, respectively
- Sampling performed annually, 4 times per year at 7 sampling points (28 samples)
- Budget: *6,000.00 €*

Zooplankton

- Qualitative and quantitative analysis of zooplankton (biomass and taxa)
- Sampling performed once in two years, 4 times per year at 7 sampling points
- Budget: *6,000.00 €*

Comments:

- Price may increase depending on the detail of taxonomic identification

Abiotic water parameters

- Sampling and subsequent analysis of **nutrient levels**: nitrites (NO2-), nitrates (NO3-), ammonia (NH4+), phosphates (PO4-) and silicates (SiO4)
- Sampling performed each year, 4 times per year at 7 sampling points
- Budget: *4,000.00 €*

- **Salinity**, **temperature** and **dissolved oxygen** measured using probes or loggers at different depths, continuously each year, at least on a monthly basis at 7 sampling points
- Measurement using probes requires more field work, but incurs less equipment costs, while loggers are the opposite. However, loggers can provide more precise and continuous data.
- Budget: *2,000.00 €*

* **Turbidity** and **particulate matter** measured using analog methods, probes or loggers and filtration, respectively
* Measured annually, 4 times per year at 7 sampling points
* Budget: *2,000.00 €*

Figure 3. Seven suggested sampling points for productio as well as biotic and abiotic ecosystem parameters in mali Ston bay. Sampling points are in: 1 – Kuta, 2 – Bistrina, 3 – Soca, 4 – Usko, 5 – Bjejevica, 6 – Brijesta, 7 – Sutvid.

Bay geography

- **Surface**, **bathymetry** and **water volume** is currently available

Comments:

- Current bathimetry (and subsequent volume calculation) data has been estimated from online navigational charts and not dedicated bathymetry surveys
- A dedicated bathymetry survey by boat would be advised but will likely require a significant budget

Bay hydrodynamics

- Perform hydrodynamic surveys, data anaylsis and modelling of the bay in order to obtain: freshwater input, spatio-temporal stratification of the water column, currents, water retention within the bay, ratio of local production of phytoplankton and introduced through currents

Minimum:

- Sampling performed a single year, 3 times per year for a duration of 2 months each
- Budget: *35,000.00 €*

Preferred:

- Sampling performed during 2-3 years, 4 times per year for a duration of 2 months each

Biological model for carrying capacity

- Simple model where biotic and abiotic parameters are separate from hydrodynamics
- The Dynamic ecosystem model (DEMO) is suggested
- Budget: *25,000.00 €*

Hydrodynamic model for carrying capacity

- Expands on biological model by including hydrodynamics and spatiotemporal distribution of data. We suggest use of this model due to the heterogenicity of the production site
- Deltares modelling software is suggested
- Budget: *40,000.00 €*

Table 1. Cost in Euro (€) for individual samplings and analyses required for estimating the ecological carrying capacity of Mali ston bay for bivalve farming. Values are grouped by the required frequency of the surveys.

Parameter	One-time	Every 5 years	Every year
Biomass of farmed bivalves			5,000.00
Biomass of wild bivalves and other filtratory organisms		50,000.00	
Biomass of biofouling organisms		5,000.00	
Condition index of farmed bivalves			5,000.00
Primary production			9,000.00*
Phytoplankton - chlorophyl			4,000.00
Phytoplankton - composition and size fractions			6,000.00
Zooplankton			6,000.00
Bay geography	X**		
Bay hydrodynamics		35,000.00	
Abiotic water parameters			8,000.00
Biological model		25,000.00	
Hydrological model		40,000.00	
Total		155,000.00	43,000.00

*Preferably should be performed every year, but due to lack of laboratory infrastructure in Croatia to support assays using radioactive isotopes, these surveys may have to be spread to 5-year periods

**Preferred, but not essential surveys with a high, but undefined budget

Conclusion

A dedicated monitoring program should be established, or adapted from existing ones to better suit the needs for the initial estimation and continuous updating of an ecological carrying capacity model for Mali Ston bay.

One-time surveys, those that should be performed every 5 years and on an annual basis (accounting for numerous samplings in each year) have been determined (Table 1). Costs are listed for individual surveys as if they were standalone field trips, but these expenses could be reduced if the surveys were performed together.

3. Determining requirements for a central dispatch and depuration facility for farmed bivalves in Mali Ston bay

Feedback from the bottom-up approach went in the direction of determining the requirements and capacity of dispatch and depuration infrastructure for bivalve farming in Mali Ston bay. Production data was collected in the form of a questionnaire that allowed all farmers to express interest for use of such infrastructure (Annex I). The collected data was then cross-analyzed with the history of sanitary conditions of individual production zones in order to estimate the capacity of depuration systems that should be established within the bay.

Review of Questionnaire

The questionnaire distributed to all local shellfish producers gave an overview of the maximum quantities of bivalves individual farmers wanted to move through a central dispatch center on an annual and weekly basis, as well as the origin of their wares in terms of sanitary production zones. The bay is divided into 12 production zones for mussels (Figure 2) and 6 production zones for oysters (Figure 3), which are used for sanitary control of *Escherichia coli* levels in seawater and bivalve tissues. A total of 21 farms, including the majority of the largest producers, expressed interest in using the services of a central dispatch and depuration facility. No farmers from the Sutvid production zone (both for mussels and oysters) did not express interest in the services of a depuration faacility and thus have not been included in the calculations.

Figure 4. The 12 sanitary production zones for mussels in Mali Ston bay

Figure 5. The 6 sanitary production zones for oysters in Mali Ston bay

Mussels

Density distribution of mussel harvests (quantity of harvest in kg) indicated that the majority of weekly harvests are of low quantity (median of 300 kg per harvest) increasing to a maximum of 4000 kg (with estimated 15 occurrences per year).

Table 2. Summary of weekly mussel harvest by area. Sutvid not included due to lack of interest.

Zone	Predicted quantity (kg)	Max quantity (kg)	Operators
Brijesta II	4154	14170	8
Usko Kanal	1096	4800	5
Soca	663	3450	3
Brijesta I	625	2750	2
Banja	617	3000	4
Bistrina	337	1650	3
Bjejevica	308	2400	3
Mali Ston	173	500	3
Blazevo	58	30	1
Hodilje	38	200	1
Kuta	19	100	1

Table 2 summarises the following:

- Probable weekly harvest of mussels, based on size and frequency of harvests (operators do not harvest every week of the year).
- Scenario of all operators performing a harvest in the same week.
- Number of operators per defined area.

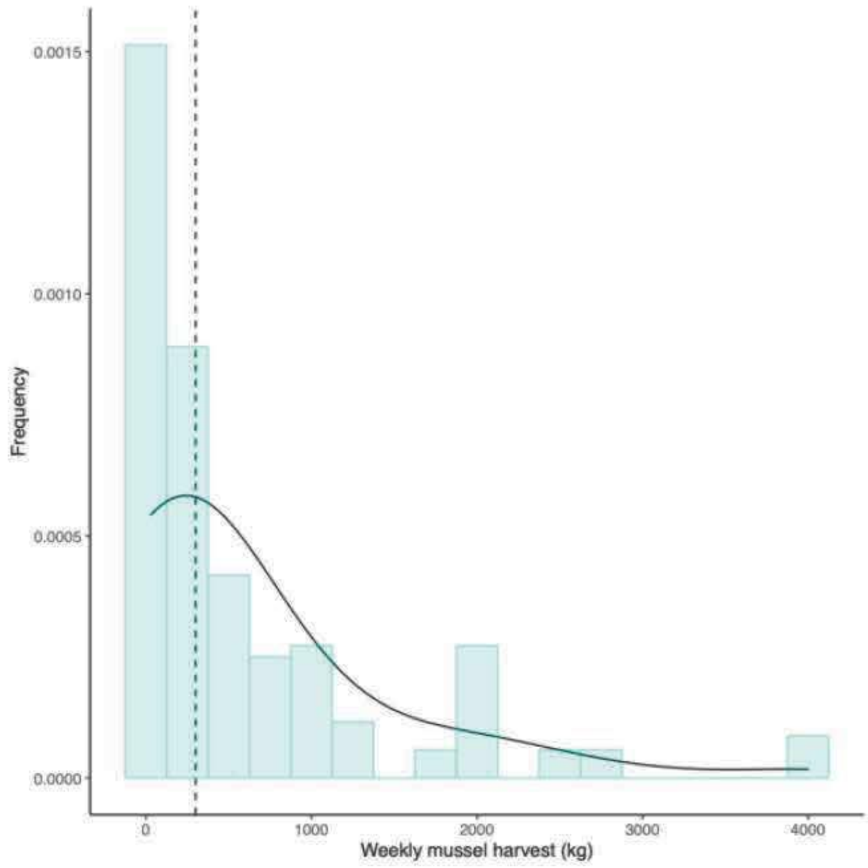

Figure 6. Density plot of weekly mussel harvest quantity in kilograms.

Oysters

Density distribution of oyster harvests (quantity of harvest in pieces) indicated that the majority of harvests are low quantities (median of 1250 pieces per harvest) increasing to a maximum of 10,000 pieces (with estimated 5 occurrences per year). The most prolific producer, 200,000 per year with an estimated 40 harvests of 5000 pieces per harvest.

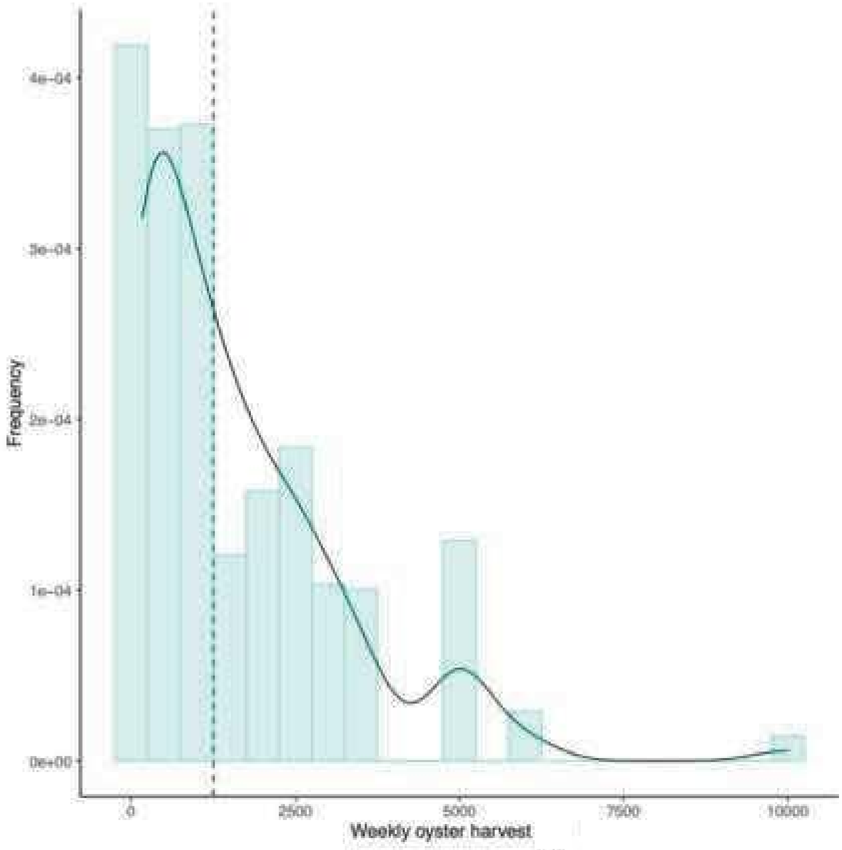

Figure 7. Density plot of weekly oyster harvest quantity in pieces.

Table 3. Summary of weekly oyster harvest by area. Sutvid not included due to lack of interest.

Zone	Predicted quantity (pcs)	Max quantity (pcs)	Operators
Brijesta	8303	26775	8
Bistrina	5154	10500	5
Kanal	4663	18600	8
Ston	2361	7075	7
Luka	1485	3550	3

Table 3 summarises the following:

- Probable weekly harvest of oysters, based on size and frequency of harvests (operators do not harvest every week of the year).
- Scenario of all operators performing a harvest in the same week.
- Number of operators per defined area.

Review of history of zone closures

From 2015 to 2022, the 12 zones for mussels culture and the 6 overlapping zones for oyster culture were closed for at least one bivalve species, at least once. Some were closed more often than others, with Sutvid (P-13-MLZ-13 for mussels and P-13-MLZ-19 for oysters) being closed the most often, while interestingly enough, no farmers from this zone expressed an interest in the use of the centralized depuration facility. Thus, a solution such as establishing small-scale depuration facilities for individual/personal use in certain farms may be required in addition to a centralized depuration facility.

Apart from this, most incidences of high *Escherichia coli* levels were associated with the Brijesta and Blaževo zones, the Kuta and Mali Ston zones and the Hodilje, Banja and Soca zones. From a legislative standpoint, the closure of a mussel zone does not mean the closure of an oyster zone, even though they overlap. However, from an ecological standpoint, there is a high risk of contamination of both bivalve species in these instances. Thus these zones were analyzed in three groupings according to the oyster zones, each of which encompasses 2-3 mussel zones. Based on previous years, these had the highest chance of closure due to sanitary conditions in at least one of the zones from the grouping; 56 %, 44 % and 38 %, respectively (Figure 8). Thus, the associated predicted quantities of mussels and oysters produced in these zones obtained through the questionnaire were used as the basis for determining the capacity of a central depuration centre.

Figure 8. Spatial overview of calculated probabilities of closure of individual production zones (grouped to the dimensions of oyster production zones) due to inadequate sanitary conditions.

Projected production capacity

Two scenarios have been explored based on closure of three zones at any one time. Since batches from different zones and different species (mussels and oysters) should not be depurated within the same system, six independent systems are recommended as a minimum for operational flexibility. Scenarios explored include: scenario 1, average weekly harvest (based on annual production and harvest number), scenario 2, maximum potential weekly harvest (based on top 3 production zones by quantity per species).

Information given by national Veterinary institute indicates that species microbiological test results are independent – scenario 2 is thus considered the optimal strategy for maximum capacity in the event of shellfish zones being temporarily reclassified as class B waters. In the case of such a scenario, where 3 zones are closed off for both bivalve species, a total of six different systems would be required (Table 4).

Table 4. Capacity (processing) requirements per week for depuration systems

System	Species	Production volume (scenario 2)
System 1	Mussel	14170 kg
System 2	Mussel	4800 kg
System 3	Mussel	3450 kg
System 4	Oyster	26775 pcs
System 5	Oyster	10500 pcs
System 6	Oyster	18600 pcs

However, due to the large quantitites of bivalves that would originate from each of the zones and the fact that multiple farmers would likely depurate their stock simultaneously, more smaller systems instead of six larger systems are recommended. Furthermore, this would better use up the limited space of the target facility and enable a more energy efficient running of the systems during lower production out of season, by using only a small number of systems. The proposed system sizes and capacities have been listed in Table 5.

Table 5. Proposed system layout and production capacity (note: systems can accept either species, mixing species / zones not permitted within individual systems)

System	Tray capacity	Stock Capacity (kg)	Target Species
1a	40	600 / 4800 pcs	Oyster
2a	40	600 / 4800 pcs	Oyster
3a	60	900	Mussel
4a	60	900	Mussel
5a	60	900	Mussel
6a	60	900	Mussel
1b	40	600 / 4800 pcs	Oyster
2b	40	600 / 4800 pcs	Oyster
3b	60	900 / 7200 pcs	Oyster
4b	60	900	Mussel
5b	60	900	Mussel
6b	60	900	Mussel
7b	60	900	Mussel