COMMON SET OF PROTOCOLS AND GUIDELINES FOR SUSTAINABLE PRODUCTION AND CB ADDED VALUE IN REARING OF COMMON INTEREST SHELLFISH SPECIES

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1. Introduction

Each aquaculture production site for bivalves along the Croatian coast is a geographically separate location and is often characterized by different ecological, technological and socioeconomic parameters. Two species of bivalves are cultured in all sites to varying degrees: Mediterranean mussel (*Mytilus galloprovincialis*) and European flat oyster (*Ostrea edulis*). These cultured bivalve species have been known to be sporadically transferred between locations, mainly to kickstart or renew production of a certain species and/or when local production was not able to meet market demand. This continues to be a dangerous practice as it has proven to be a vector for spreading bivalve pathogens and biofouling organisms, including non-native species of which some have proven to be invasive. These kind of practices can have devastating consequences for the ecosystems and the industries that depend on them, such as bivalve farming.

On the other hand, in the face of direct and indirect anthropogenic effects that are steadily increasing in scope and intensity and pose a threat both to cultured and natural populations of bivalves, translocation of certain species can have numerous benefits. It is an essential tool for restoring devastated production sites and/or natural populations of certain species and habitats. However, given the dangers associated with these activities, it is imperative that translocation procedures are properly managed and follow strict biosecurity guidelines.

In the scope of this document, we will cover the main issues with translocation of bivalves, describe the main Croatian production sites and suggest realistic biosecurity guidelines that should be put in place to protect and conserve these production sites and the ecosystems theyare located in. These biosecurity guidelines are heavily based on the Native oyster restoration alliance (NORA) publication "European Guidelines on Biosecurity in Native Oyster Restoration".

2. The issues with translocating bivalves

Translocation is the human-mediated movement of living organisms from one area to another. There are a number of risks associated with the translocation of bivalves and any accompanying biofouling or other organisms and in some cases substrate, mainly related to disease transfer and the introduction of invasive non-native species, but also the disruption of population genetics.

Bivalve translocation often involves the movement of people, equipment, live shellfish and sometimes substrate materials between sites and as such provides numerous opportunities for the unintended transfer of accompanying species as well. Whilst such movements are by no means the only vectors for non-native species or diseases, efforts to transfer bivalve populations must adopt rigorous biosecurity protocols in order to reduce the risk that an action with an intended positive commercial and/or ecological benefit results in a negative impact.

In the Adriatic, bivalves are mainly cultivated on floating long lines and as such are suspended in open water, which means that there aren't any obstacles to prevent harmful species from coming into contact with them. For this reason, cultured bivalves can potentially be in touch or infected by any disease or non-native species present in their environment. They are always accompanied by numerous biofouling organisms present on or within their shell, but are also filter feeders which means they indiscriminately inhale the surrounding water which contains numerous organisms and floating particles before selecting those fit for ingestion within the mantle cavity. This increases the chance that they contain harmful species within the mantle cavity as well.

European flat oysters are a particular issue, due to their susceptibility to and potential for introduction of diseases caused by *Bonamia ostreae* and *Marteilia refringens* that can decimate entire oyster populations. There is also a higher possibility of introduction of non-native species when translocating oysters in comparison to mussels due to their thick, hard and gibbous shell, which houses many organisms on both the outside and inside of the shell. Thus, it is extremely hard or even impossible to mechanically clear or remove all accompanying species from the shell.

The media that bivalves are cultured in, in this case seawater, is often transferred with the animals as well, even if this only refers to the seawater captured within the mantle cavity oncethe shell is closed. This seawater can contain pathogens and/or non-native species that could be transferred to the sites to which the bivalves are being introduced.

Equipment such as boots, containers, clothes and similar can also be in touch with the seawater from a donor area and, if not properly dried or cleaned, can be a way of introducing harmful species to the receiving area. Thus, non-native species and pathogens can be moved betweensites whenever people and equipment are moved as well as the bivalves themselves and any accompanying substrates that they are attached to. As such, it is important that all people participating in bivalve translocation, including farmers, scientists, technical personnel and others, comply with both standard 'Check, Clean, Disinfect, Dry' protocols.

2.1. Invasive non-native species (INNS) and their biosecurity management

There have been few successful eradication attempts for marine non-native species or diseases in open waters. Therefore, the most effective method of control is to prevent their introduction.
While a number of high-risk INNS are recognised in the Adriatic, the absence of a disease or species from the certifiable or high-risk list does not mean it does not pose a risk. While many non-native species have little or no impact on the receiving water bodies, it is difficult to predict which species will become problematic in an introduced range. Certain attributes related to both the life history of the species and the condition of the receiving site which can indicate the likelihood of species becoming problematic, and invasion history from other locations can also be a useful indicator. Assessments of whether a species is likely to become invasive in a new location requires expertise. Several databases exist for European and Mediterranean records of non-nativespecies (which are not necessarily invasive), but those on a national level, such as „Introduced marine species in Croatian waters (Eastern Adriatic Sea)" by Pećarević et al. (2013) should be updated further and preferably narrowed to organisms associated to bivalve farms to facilitate easier access to information for the purpose of bivalve translocation. While existing lists can be used to generally identify which species are of particular concern when considering bivalve translocation and deciding on where to source bivalves from, the information contained therein isnot set in stone, requires constant updating and should be discussed with experts.

Regulation 1143/2014 on the prevention and management of the introduction and spread of invasive alien species, which entered into force on 1 January 2015, systematically deals with the problem of INNSs at the level of the European Union. This Regulation is binding in its entirety and directly applies to all Member States, including Croatia. It sets out rules to prevent, minimize, and mitigate the harmful effects of invasive alien species on biodiversity in the European Union. The framework for its implementation in Croatian legislation is set by the Act on the Prevention and Management of the Introduction and Spread of Alien and Invasive Alien Species and its subordinate legislation.

Although this requires significantly more resources for detailed samplings, analyses and monitoring programs, the current document will attempt to identify the main INNS relevant to bivalve translocation in the Adriatic in the moment of its publishing, as well as their presence and abundance in Croatia bivalve aquaculture sites, but would like to highlight that future translocation activities should check several platforms and ensure that the information they draw on is up to date.

Every non-native species introduced to a new area has the potential to become invasive. Therefore, while biosecurity protocols should prioritize the prevention of key identified problem species, translocation attempts should always strive to adhere to the precautionary principle and clean all materials and equipment moved, even if no INNS are believed to be present.

2.2. Diseases and their biosecurity management

Of the two main farmed species of bivalves in Croatia, European flat oysters in particular are susceptible to numerous diseases. Although incidences of these diseases has not been recorded in Croatian culture sites, they are still expanding their range in Europe and there is a very high risk of their introduction and transfer to Croatian culture sites. It is critical that relevant stakeholders familiarize themselves with the potential diseases and the disease status of any locations where work is carried out. When working in shellfish growing waters, consideration should also be given to the possible transmission between bivalve species. Some pathogens, such as *Marteilia refringens* (including the recently proposed species *M. parafringens* sp. nov.) can be transmitted between European flat oysters and blue mussels (*Mytilus edulis*), and there are indications that OsHV-1 µvar can be transmitted between Pacific oysters (*Magalana gigas*) and European flat oysters.

There are several diseases which are of particular note in the context of European flat oysters. These include the listed diseases (and the agents) of bivalves to the World Organisation for Animal Health (OIE) and/or to the European Commission (EC) (The Council Directive 2006/88/EC):

- Bonamiosis – *Bonamia ostreae* (OIE/EC – present in Europe),
- Bonamiosis – *Bonamia exitiosa* (OIE/EC – present in Europe),
- Marteiliasis – *Marteilia refringens* (OIE/EC – present in Europe),
- Herpes-like infection – Herpes virus OsHV-1-µvar (present in Europe) (notifiable in few zones in Ireland and UK only).

In the context of the Mediterranean mussel, the most common disease is:

- Marteiliasis – *Marteilia refringens* (OIE/EC – present in Europe).

In Croatia, a National surveillance program was put into place in 2000 to monitor and control shellfish diseases in farmed species. The last available report was published in 2011, and notes a prevalence of *Martelia* sp. in mussels of 0-10%, depending on the culture site and was not associated with culture density. Although both mussels and oysters are cultivated in close proximty, oysters remained free of both parasites *B. ostrea* and *M. refringens*. In 2016, first findings of the parasite *Bonamia exitosa* occurred in the north and south of the Croatian Adriatic coast and repeated findings have been reported up to 2020, with the addition of two more sites on the north coast. The infection prevalence ranged from 3,3% to 20% in the different sites, although culture sites around Šibenik and Zadar were not tested. However, no mortalities were reported from the infected sites, and it seemed that infection of flat oysters with *B. exitiosa* did not affect their health.

In the case the presence of a disease is suspected, the competent authority, in this case the Croatian Veterinary Institute or the Administration for Veterinary Medicine and Food Safety of theMinistry of Agronomy should immediately be notified, upon which they should investigate the occurrence and decide what measures are to be taken. Measures may include an initial survey, the inclusion of a site-based risk assessment and biosecurity plan with contingency measures, as well as follow-up monitoring of the site as part of the licensing conditions of the plan or project and movement restrictions. In the case of the suspicion of the presence of a disease or non-nativespecies, the practitioner must follow these steps:

1. Report immediately to the competent authority
2. Adopt a precautionary approach – do not carry on operations that might contribute to further dispersal
3. Carry out risk assessments

4. Seek and follow advice from the relevant authorities. This may include not moving any material

It is important for stakeholders to be aware not only of the listed diseases and the requirements to follow the rules on translocations that apply locally and internationally, but also to be mindful that there are a range of other parasites, pathogens and epibionts to which bivalves are susceptible, or may be a vector of. The following is a non-exhaustive list of known pathogens and parasites affecting commonly farmed bivalve species:

- *Boccardia* (genus of)
- *Cliona celata*
- *Cliona viridis*
- *Gyrodinium aureolum*
- *Haplosporidium armoricanum*
- *Herrmannella duggani*
- *Hexamita inflata*
- *Mytilicola intestinalis*
- *Nocardia crassostreae*
- *Ostracoblabe implexa*
- Papovaviridae (family of)
- *Perkinsus mediterraneus*
- *Polydora* (genus of)
- *Pseudoklossia* (genus of)
- Vibrio spp. (e.g. V. alginolyticus, V. anguillarum, V. coralliilyticus, V. neptunius, V. ostreicida, V. tubiashi)

Haemic neoplasia may also affect oysters. In this case, no disease agent is observed, but the neoplastic cells may be infectious and cause significant mortalities.

It is the responsibility of farmers and other stakeholders involved in translocation to implement appropriate national disease prevention and management requirements and to report any unusual or unexplained mortalities, as well as any suspicion of occurrence of a listed or emerging pathogen, to the relevant authority for investigation.

Screening for diseases is usually carried out by national reference laboratories or other national institutions, depending upon the jurisdiction. OIE reference laboratories can be found on the World Organisation for Animal Health website.

As with all introduced species, it is not possible to know before a disease is introduced, whether it will seriously impact in its introduced range. A disease may be subclinical in a population that has co-evolved with it, and therefore not apparent. Once transferred to a naive population it may cause high mortalities and disruption. For example, *Bonamia ostreae* was not known as a disease agent in its Californian source range but caused widespread mortalities in excess of 90% in its introduced range in Europe.

3. Biosecurity guidelines for translocation of bivalves

Given that all translocations carry with them a risk of accidental introduction, it is important that avoiding the risk by avoiding translocations be considered. If stakeholders do decide to proceed with translocations, despite the inherent risks, comprehensive protocols and actions to mitigate and reduce the risks presented should be developed on a case by case basis. It would be critical that the relevant authorities are informed of all planned activities and that translocators seek advice from, and work in partnership with, the relevant authorities. In fact, this should be a highly regulated activity similar to how farming of non-indigenous species is carried out. In the scope of this section we suggest some guidelines to follow for such activities.

3.1. Before translocation

It is critical that when considering translocating bivalves the following questions are addressed:

- Is translocation necessary? Consider why translocation is the best option. If possible, do not translocate bivalves.
- Is it possible to source the bivalves more locally? If not, try to obtain hatchery-reared spat.

If translocating, use the following general hierarchy in selecting donor material to minimize risk:

- Do not consider donor sites with high-risk invasive species or diseases that are not present at the receiving site. The ecological and socioeconomic risk of introducing either adisease or high-risk INNS into an area is unacceptable, given the possible impacts such an action could result in.
- Minimize the physical distance between the donor and receiving site. To reduce the risk of unknown diseases or INNS being introduced to an area, it is best to reduce the physical distance between the donor and receiving site. This will also allow for maintaining local or regional genetic structure in bivalve populations.
- Avoid movements across latitudinal gradients. Bivalves can be infected by a large number of pathogens. Within their co-evolved range and the local temperature regime, pathogens may have limited impact on their host. There is, however, a risk that pathogens may

become more virulent when moved to a different environment. As it is not possible to know which diseases may have an impact in the novel environment, and it is in any case challenging to screen for all known diseases, movement of bivalves to a largely different environment is not recommended.

- Never consider donor sites outside of the natural range of the species. Reintroducing species from outside their native range should be avoided at all costs in order to avoid the potential introduction of non-native species and diseases associated with the translocated bivalves. As an illustration of the risk, the European presence of more than sixty species, native to the Pacific Northwest USA, can be attributed to movements of the Pacific oyster since the 1960's alone.

3.2. Translocating live bivalves

If translocation is indeed deemed necessary and potentially appropriate donor material has been identified, the next step is to undertake thorough biosecurity measures, under advice from the relevant authorities, to reduce the risk of accidental transfer of hitchhiking species. Initial risk assessments should be undertaken in order to understand the risk and map out the appropriate action. Assessment of risk should include consideration of ongoing activities in both the donor and receiving site.

It is critical that adequate time for completing comprehensive biosecurity measures is planned into the activity. If sufficient data is not available for donor sites, their suitability should be evaluated ideally during summer months, when species are most abundant and therefore likely to be encountered and identified. Additionally, the time required to physically clean and screen bivalves should be accounted for, as this can be a rate or scale limiting step. Substantial epifaunal growth can mean that it takes one person one hour to clean around 100 oysters, while less time can be planned for mussels. Translocators should not seek to move a greater number of bivalves than they have time to clean and check thoroughly. Translocating large numbers of bivalves is an arduous and time-consuming process, and translocating more individuals increases the risk of unintended introductions. These realities should be considered when properly planning translocation activities.

3.2.1. Undertaking a risk assessment

The first steps in any risk assessment is risk identification or mapping and analysis. The identified risks should then be analyzed regarding likelihood and consequence. Moreover, to rank risks, they must first be comparable. In the current document, the characteristics of each production site along the Adriatic coast will be covered in detail in order to have a better understanding of the risks and benefits involved with translocation between individual sites. Even though many factors can be taken into account, the main ones should be presence of bivalve diseases (primarily bonamiosis and marteiliosis) and potential INNS.

3.2.2. Survey the donor site

Once a potential donor site has been identified, the current disease status of the site should be confirmed through further testing. Although a profile of individual production areas in Croatia will be provided, disease testing and especially biodiversity surveys take place at insufficient frequency to ensure that the current disease/INNS status of a site is accurately reflected in available information. If possible, dedicated field surveys and testing to ensure that the risk assessments are undertaken with the most current and relevant information should be performedfor each individual translocation activity.

3.2.3. Screening for diseases

Pathogen screens should be done using recommended methods as specified in the OIE aquatic manual and as recommended by the EU legislation (Commission Implementing Decision (EU) 2015/1554 of 11 September 2015 laying down rules for the application of Directive 2006/88/EC as regards requirements for surveillance and diagnostic methods (notified under document C(2015) 6188).

This should at least include the diseases:

- Bonamiosis (*B. ostreae* and *B. exitiosa*)
- Marteiliosis (*M. refringens*)

Sample sizes should follow or exceed those recommended in the OIE aquatic manual and EU legislation. In the aforementioned decision there are specific recommendations about the surveillance and diagnostic of *B. ostreae* and *M. refringens*.

In addition to screening listed pathogens, general screening based on histology and bacteriology should be implemented. Consideration should also be given to diseases which are not listed. Attention should therefore be paid to the general health of the bivalves and the recent history of mortality at the donor site.

3.2.4. Surveying for INNS

When undertaking a biodiversity survey to inform the translocation risk assessment, particular care should be paid to potential and high-risk INNS. As INNS include a full range of species with differing life histories, no one sampling protocol will be best suited to all potential species of interest. Stakeholders should therefore consider using a range of methods that cover: species thatare likely to have low densities and are dispersed and species that are likely to have higher densities and/or be less patchily distributed. These should at least cover biofouling organisms and planktonic organisms present at the donor site.

3.3. How to respond to unexpected mortality events

A critical aspect of biosecurity relating to disease management is monitoring of increased and unexplained mortality. During monitoring, stakeholders may notice changes in bivalve growth, absence of larval settlement or increased or unexplained mortality. These may not have an immediate or obvious explanation and therefore require investigation.

Disease is not the only cause of unexpected mortality. Pulse events, such as heavy rainfall can cause fluctuations in temperature, salinity, and turbidity, and may contribute to adult and spat mortalities, loss of planktonic larvae and cessation of reproductive activity. Storms can also increase pollution, horizontal advection and abrasion, which can negatively impact bivalve condition and possibly influence the prevalence of diseases such as infection with *Bonamia ostreae* where it is present. Because of the risk posed by disease, translocators should always seekadvice from the relevant authority regarding actions required in the event of an increased and unexpected mortality event.

As a guideline in instances where there are sudden, increased and unexplained high mortalities or recruitment failures, stakeholders should report any abnormal mortality event to the authority and investigate the possible involvement of an infectious agent. For that purpose, bivalves, including moribund ones (but not dead ones) should be sampled and processed according to recommendations of the European Union Reference Laboratory (EURL) for Mollusc Diseases in order to carry out histology, bacteriology and PCR for the detection of specific pathogens. These diagnostic analyses must be carried out by a recognised or agreed laboratory

Currently, there is no 100% accurate method of disease-screening for all translocated organisms in a consignment.

1. Any biosecurity for the translocation of live bivalves runs the risk that not all individual INNS will be eliminated because, inevitably, the system must allow for the survival of the oysters.
2. Third-party activities in the area may have introduced a disease or INNS at or around the time of the translocation event.
3. The disease or INNS may have already been present and undetected in other biological reservoirs.

3.4. Conclusion

Once site surveys have been undertaken, the initial risk assessment should be revisited with the updated information in mind. If an aggressive INNS or a notifiable shellfish disease is recorded at the donor site, then bivalves should not be translocated from that site. Should other non-native species be identified from previous data or surveys of the donor site, then a marine biosecurity plan should be written to identify measures that can reduce the risk of those non-native species being introduced. This may be required by regulating bodies before consent is given for the translocation.

It is critical that the following ground rules be followed for all translocations:

- Animals must only be moved to recipient sites from donor sites with equal or higher health and INNS status. For example, moving oysters from an OsHV-1 µvar positive to negative site, or moving animals from an area with close proximity to a bonamiosis positive zone to a *Bonamia*-free zone, should not take place irrelevant of disease andINNS status, it is not recommended to move bivalves from smaller production sites to larger production sites as the potential negative scenarios are far more devastating in receiving locations with a booming aquaculture industry.

- Given the fact that potential stakeholders interested in performing translocation activities may find it difficult to obtain all the relevant data listed in this document, it is suggested that the bivalve centers established in the scope of the ARGOS project offer consultation services to farmers in this and other topics. Moreover, depuration centers established in the scope of such centers could act as quarantine systems for the purpose of translocation (see following chapter for translocation protocols).

4. Treatment protocols for translocating bivalves

4.1. Physical cleaning of adult bivalves

If the origin and donor sites have been deemed suitable by the preceding steps, the oysters obtained for translocation should first be inspected and then physically cleaned to ensure no visible epibiota persists. This process should be completed at the donor site pre-transport to ensure no epibiota is transferred elsewhere. It may be necessary to require farmers to cost forthis activity, which may be different to their normal aquaculture practices when preparing bivalves for market placement. Wastewater and biofouling organisms from the cleaning can be disposed of at the origin site rather than being transferred elsewhere. It is also recommended that treatment and transport of oysters takes place in the late autumn to late winter to minimise epibiotic growth.

As part of the visual inspection, a experts should be engaged to oversee the cleaning process and prepare a qualitative and quantitative record of species present on bivalve shells pre- and post-treatment. This is not only helpful as an audit trail to demonstrate statutory compliance but could contribute to the evidence base for best practice of similar future activities. Note that oysters with associated heavy infestations of boring organisms will have holes which can be difficult to clean. Heavily undermined shells with many crevices should be discarded along with other oysters with physically compromised shells. These should be discarded responsibly at the donor site. If further fouling is found at a later stage, or if cleaning must occur remotely, material should be disposed of responsibly. Under circumstances of enhanced risk, disposal should be to a specified biological waste disposal route (possibly including incineration). During cleaning, care should be taken to ensure that there are no small bivalves hidden in the hinge-line of the bivalves (mainly relevant for oysters).

Physical cleaning can be done by hand (scrape/scrub off) and/or automated methods, such as cleaning drums or other shellfish cleaning machines used on farms. If using automated methods, bivalves can be cleaned in large batches and so it is a more time-effective procedure for a larger numbers of bivalves, but is not as precise as hand cleaning and may require repeated cleaning as some or parts of biofouling organisms, such as holdfasts, may persist. It is critical that, if automated treatment (as opposed to cleaning by hand) is undertaken, a large sample size of the treated oysters is examined by hand in order to determine that the epifauna have been effectivelyremoved. Hand cleaning should be performed if automated cleaning has limited efficiency in removing biofouling organisms.

Following physical cleaning, bivalves should be left to recover in running filtered seawater for a minimum of three days in the equivalent of a depuration system before undergoing chemical treatment. Subjecting them to immediate chemical treatment would put bivalves with chipped shells at risk of unnecessary exposure and may result in increased mortalities. During this time, bivalves also have the opportunity to release some of their internal microbiota. Disposal of water used in this phase should therefore be subject to biosecurity and chemical pollutant risk assessment and where necessary, treatment before disposal as is the case in registered depuration systems.

4.2. Chemical treatment of adult bivalves

The purpose of chemical treatment is to kill any shell epibiota that may have survived the physical cleaning of the bivalves and therefore reduce the risk of INNS transfer. Remaining epibiota might include scraps of colonial organisms such as sponges, sea squirts or certain types of seaweed, as well as hardy spores and resting/reproductive stages of other organisms. As well as the bivalves themselves, some biofouling organisms can clamp-shut to avoid ingress of fluids: they are therefore theoretically able to survive the chemical treatment just as well as the bivalve being cleaned. Care should therefore be taken in the physical cleaning stage to make sure that hard housings of tube worms, bivalves and other biofouling organisms are removed or broken open.

Various chemicals have been used for the surface sterilisation of bivalves and they range in their expense and availability, including hypochlorite, formaldehyde, and commercial fish-farm treatments such as Virkon. There is not a clear evaluation of the relative effectiveness of different treatments, but the obvious abiding principle is that it should be toxic to the epibiota in the concentration and exposure time used. Exposure-times can vary, and bulk dunking methods have been used, akin to the use of fryer baskets. Dunking methods may be preferable and efficient with mussels and younger oysters (e.g. 10 g) because the shells appear to seal-shut well. Sponging with the chemical treatment might be deemed more appropriate in larger adult oysters where the gape of the shell may be worn or damaged and therefore less likely to seal well if fully submerged in a chemical bath.

The method recommended for field tests is immersion in freshwater (salinity < 2ppt) for at least 24 hours.

4.3. Quarantine

Once bivalves have undergone both physical and chemical external cleaning, it is important that the efficacy of the biosecurity protocol is quantitatively assessed and that more time is given internal contaminants to be expelled. A quarantine period should therefore be imposed, during which the bivalves are given time to recover from the treatment under controlled conditions and given time to be completely depurated of internal microorganisms. During this period, they canbe monitored to assess the efficacy of treatments thus far.

Given that it is not yet known whether the bivalves have been successfully cleaned, water used in this period should be handled as potentially high-risk waste and should be disposed of accordingly. At this stage, bivalves may be kept in closed circulation or flow through systems. Filtered water from the receiving site may be used for this stage. Ideally, moderate numbers of bivalves should be kept in each tank, and tanks should not share water circulation. In circumstances of high risk, it may also be desirable to use artificial seawater and u/v recirculation systems to 'flush' in-shell water and decontaminate the oysters from waterborne organisms, butit is important to note that such "decontamination" will not "flush out" bivalve diseases. During this time, individual batches of bivalves can be screened and assessed independently. The bottom of the tanks should be checked daily for evidence of recently dead organisms that may have crawled out of crevices or from within the shells. Anecdotally, it seems that slightly reduced salinities may encourage this process. If any evidence of living associated biota is found, oysters should be subjected to further treatment. It should be noted that currently there is insufficient evidence regarding the effective duration of a quarantine period, but experience from restoration practices of oysters suggests that remnant live epibiota may take 3 days to emerge under these conditions, therefore a shorter period is not recommended.

4.4. Preparing spat for translocation

In cases where bivalve spat (2mm to 10mm) not originating from a biosecure hatchery is required for translocation, it should be considered that the sensitivity of young bivalves, especially oysters, may mean that many biosecurity treatments (both physical and chemical) are inappropriate. In these cases, locally sourced spat from sites with the same (or better) disease and INNS status may be the only appropriate option. The relevant authorities should be informed throughout the process as dictated by the precautionary principle, spat which have been in contact with open water prior to translocation, should only be translocated locally.

4.5. Screening of treated bivalves

Once all the steps have been taken to identify possible biosecurity risks and to address identified risks, it is critical that the effectiveness of the measures is assessed prior to bivalves being relayed into the receiving site. Only when the parties involved in translocation and relevant authorities are confident that the associated risk is acceptable, should the translocation be completed.

There is currently no method that, when applied, renders living bivalves completely biosecure for translocations. Although general protocols for cleaning aquatic organisms exist, protocols suitable for relaying of live bivalves between culture sites have yet to be tested and confirmed effective. There are knowledge gaps regarding the efficacy of possible treatments, in particular for large- scale translocation activities. It is therefore critical that each translocation attempt validates the efficacy of the biosecurity measures undertaken with a thorough screening of the treated bivalves.

The sample size should be large enough to ensure a high degree of confidence that the consignment of bivalves has met the desired biosecurity standard. Screening for epifauna should, as a minimum, involve visible examination of the shell and hinge. Screening for associated biota should as a minimum involve examination of the base of quarantine tanks for signs of recently emergent and dead organisms for several days. While disease screening is one of the first steps undertaken in determining whether the stock is suitable for translocation from an approved donor site, a further and final screening for diseases may be undertaken before the stock are released into the target environment. The rationale for this further final testing is that it ispossible that bivalves start to express the disease when under stress (e.g. having undergone treatment). Therefore, tests taken towards the end of the quarantine period may pick up disease presence overlooked in the initial stages.

5. Characteristics of bivalve production sites in Croatia

In the current document, the characteristics of each production site along the Adriatic coast will be covered in detail in order to have a better understanding of the risks and benefits involved with translocation between individual sites.

5.1. Mali Ston bay

Figure 1. Aerial view of Mali Ston bay interior (source: https://www.vecernji.hr/vijesti/akcija-ciscenja-malostonskog-zaljeva-okupila-je-50-ronilaca-1609406/galerija-522143?page=3).

Geography

The bay of Mali Ston is located in the Dubrovnik-Neretva county at the south of Croatia. It is a 28 km long and 6.1 km wide (at its widest point) bay enclosed between the main shoreline and the Pelješac peninsula at its south-east point, covering an area of 66 km2 (Figure 1.). It is connected to the Adriatic Sea at its northwest point, where it opens into the Neretva channel that is influenced by a large delta formed by the river Neretva. The total length of the bay's shoreline is around 100km and its largest depth is around 35 m. It is currently divided into 3 biogeographical zones which correspond to 6%, 7% and 87% of the bay's surface and have average depths of 7.82 ± 4.27 m, 11.04 ± 5.42 m and 24.61 ± 8.11 m, respectively. These zones have an individual water volume of 0.029 km^3, 0.052 km^3 and 1.414 km^3, respectively. The „interior“ of the bay (mainly coinciding with zones 1 and 2) is connected to the „exterior“ (roughly coinciding with zone 3) by the Usko strait which is around 330 m wide at its narrowest point and characterized by strong currents.

Apart from freshwater introduced into the bay's exterior by the Neretva river, the whole bay is influenced by submerged freshwater springs and surface runoff from precipitation, all of which contribute to bringing nutrients into the bay. Given this input of nutrients and abundance of phytoplankton populations, the bay is considered a mildly eutrophic system.

Three main production zones are currently in place based on their biogeochemical potential: Zone I, II and III. Zone I covers the very “interior” of the bay, has an average depth of 11.4 m, surface of 7.09 km^2 and volume of 0.078 km^3. Zone II covers an “interior” to “middle” area and has an average depth of 13.23 m, surface of 22.3 km^2 and volume of 0.301 km^3. Zone III is by far the largest, accounts for the bay’s “exterior” and has an average depth of 22.9 m, surface of 38.4 km^2 and volume of 0.879 km^3.

Anthropogenic activity

The coastline of Mali Ston bay is covered by several municipalities, including the municipalities of Ston (14,2 inhabitants/m_2) and Janjina (18,4 inhabitants/m_2) on the Pelješac peninsula and Dubrovačko primorje (11,0 inhabitants/m_2) and Slivno (29,2 inhabitants/m_2) on the mainland. There are a total of around 20 significant settlements surrounding the bay of Mali Ston, includinga part of the mainland belonging to the country of Bosnia and Herzegovina. This segment of accounts for around 20km of coastline in the form of a 6 km long and 1.2 km wide bay housingthe city of Neum (population of around 3000 inhabitants). The sewage system of Neum passes along to coast, thorough Croatia, crosses the bay of Mali Ston at the narrowest point of the Usko strait and continues over the Pelješac peninsula opening into the open Adriatic Sea on the other side. Many of the smaller Croatian settlements on both sides of the bay are also connected to this sewage system, but numerous households still rely on private cesspits that are not connected to any municipal sewage system. Irregular maintenance of the main Neum sewage line over the years has resulted in cracks and subsequent leakage on several occasions, which was likely a cause of reduced sanitary conditions of approximate aquaculture sites.

Touristic beaches are present in the bay, but generally located within or adjacent to inhabited areas and do not require specific mention outside the context of settlements.

There are no Naval routes within the bay, but the city of Ploče, located on the mainland immediately after the Neretva delta is a port for commercial shipping vessels and also connects Pelješac to the mainland via a regular ferry line from Trpanj. The Ploče port is a multipurpose port for the trans-shipment of almost all types of goods present in international marine traffic. The annual handling capacity of Ploče is estimated at 5 million tons of general and bulk cargo, while the total liquid cargo storage capacity is around 600 thousand tons.

History of bivalve farming and translocation of bivalves

Oysters from Mali Ston bay have been found in middens in archeological sites of surrounding Ilyrian settlements (Vid and Ošlje) dating back to the Roman empire. The first written records on oyster trading in the area are from the 16th century, while documents from 1667 mention oyster farming on farms made of wooden beams. The “First rational Dalmatian oyster and mussel farm” was established in 1889 and was in function until the First World War. In the 1930s, oyster farming was significantly modernized and oysters from Ston won the Grand Prix and gold medal awards for quality in the World Exhibition held in London in 1936. In the decade leading up to the Second World War, annual bivalve production combined was around 58 tons, 53 tons of which were from oyster production and 5 tons from mussels. After the Second World War, during which bivalve production was completely devastated, farms began to be rebuilt and production restarted. For this purpose, a national farm for the production of oysters and mussels called “Kamenica” was established in 1946. Farms made of reinforced concrete were constructed in Bistrina bay and production steadily increased, soon leading of the evolution of this company into a centre of oyster farming for all of Mali Ston bay called “Jedinstvo”. At the end of the 1960s, mussel farming began to gain popularity, leading to a significant increase in production quantities.Mussel farming was very profitable despite predation by gilthead sea bream (*Sparus aurata*), which was significant even then. In order to reduce mortalities caused by predation, farmers installed nets 1.5 m from the seabed which seemed to be successful in reducing predation due to the epibenthic habits of gilthead sea bream.

The average production during the 1970s was around 50 tons of oysters and 159 tons of mussels. A factory based in Ston called "Chromos" started production of plastic buoys to be used for establishing of floating long-line farms.

In the 1980s, 92.4% of all Yugoslav bivalve production was from Mali Ston bay. In 1984, the farmer's cooperative called "Dalmacijabilje" becomes the owner of the company and facilities in Bistrina bay and encompasses 129 registered farms. This results in a peak historical annual production of the cooperation equalling to 3000 tons of mussels and around 1.5 million pieces of oysters. In 1985, Dalmacijabilje starts with experimental production of European sea bass and gilthead sea bream in Bjejevica bay. The early 1990s was marked by war in Croatia, which led to the destruction or deterioration of most aquaculture farms after which recovery of the industry was slow, exacerbated by a reduction of wild oyster spat. From the start of the 21st century, almost all oyster production in Croatia was reduced to Mali Ston bay. However, production did recover and reports from 2007 estimate a production of 1.5 million pieces of oysters and 1.500 tons of mussels. By 2011 and 2012, production tripled, but in 2013 reverted back to previous levels, reducing even further on subsequent years. In 2020, the oysters from Mali Ston receive theEU Protected Designation of Origin brand, making the Mali Ston oyster the first marine food product with this brand in Croatia.

Although no records are readily available, there is ample anecdotal information from farmers and research bodies on the translocation of bivalves, especially oysters, from this site to other Croatian sites and vice-versa. On the other hand, mussel production started in the 1970's and translocations were more present during this early start-up period.

Production quantities

At the moment of writing this document (December 2022), there are 71 registered bivalve farms located in Mali Ston bay, which use a total of 200 marine concessions for cultivating oysters and/or mussels. The total surface of all concessions used for farming bivalves is a little over 1,5 million m2, which averages at 7,604 ± 10,803 m2 per concession. This equates to a maximum allowed production of 3751 tons of bivalves, which is an average of 19 ± 30 tons per concession. However, the total registered production in 2019 was only around 500 tons (73.6 tons of oysters and 425,4 tons of mussels; values for previous years are in Table 1). It is important to take into consideration that only roughly a 1/3 of all longlines are used for holding market sized bivalves, while the rest has bivalves of smaller size and earlier stages of development. Even when takingthis into account (dividing total allowed capacity by three), there is a discrepancy between total registered production and allowed production – only around 40% of the total production capacity is used. This is likely due to a high amount of unreported sales which seem to be a common practise on many farms.

Finfish aquaculture is present in Dubrovnik-neretva county and in the “outer” parts of Mali Ston bay, but to much a lesser degree than in other parts of the coast. Total production of gilthead sea bream and European sea bass (the two cultured species) in Dubrovnik-neretva county has varied between 200 and 300 tons in previous years, in comparison to total national production which reached 16500 tons in 2021. Furthermore, due to significant loses on bivalve farms caused by gilthead sea bream predation, fish farms in and around Mali Ston bay are urged to culture only European sea bass. Thus, this species accounts for more of the production than gilthead sea bream.

Table 1. Aquaculture production of bivalves in Mali Ston bay

	2014	2015	2016	2017	2018	2019
Oysters (pcs.)	502853	878891	1071415	1147895	1008125	1051198
Oysters (kg)	35200	61522	74999	80353	70569	73584
Mussels (kg)	379204	372093	346045	493258	441144	425446
Total (MT)	414	434	421	574	512	499

Ecosystem

In addition to the officially recorded habitats above, in the Mali Ston bay area, especially in its “outer” part, habitats typical of habitat type 1150 - coastal lagoons and habitats were also recorded, as well as 1120 - posidonia settlements and 1110 - sandy bottoms permanently coveredby the sea.

The infralitoral, especially of the “interior” part, is dominated by anthropogenic habitats with two types of biocenoses according to the National classification of habitats (Nacionalna klasifikacija staništa, NKS): bivalve farms (G.3.8.4.2.) and infralitoral communitites under mariculture structures (G.3.8.4.).

The supralittoral rocks (F.4.2.) of the rocky coast (F.4) are inhabited by the biocenosis of supralitoral rocks (F.4.2.1.), while the mediolitoral solid bottom and rocks (G.2.4.) offer a habitatto the biocenoses of upper mediolittoral rocks (G.2.4.1.) and lower mediolittoral rocks (G.2.4.2.), and in the bay of Bistrina, an endemic association with the species *Fucus virsoides* (G.2.4.2.6.) has also been recorded.

Aside from the mentioned anthropogenic habitats in the infralittoral (G.3.), there are also several other habitats present, which, according to the NKS, are: infralittoral fine sands with more or less silt (G.3.2), *Posidonia* settlements (G.3.5), infralittoral coarse sands with more or less silt (G.3.3.), infralittoral solid bottoms and rocks (G.3.6.), which are the habitat of silty sand biocenoses of protected coasts (G.3.2.3., N2K 1660), associations with the species *Cymodocea nodosa* (G.3.2.3.4., N2K1660), biocenoses of *Posidonia oceanica* habitats (G.3.5.1., N2K 1120), biocenoses of coarse sands and fine gravels under the influence of bottom currents (G.3.3.2, N2K 1110), biocenosis of infralittoral algae (G.3.6.1., N2K 1170), degraded facies with encrusting algae and urchins (G.3.6.1.1., N2K 1170) and facies enclaves and associations of coralligenous biocenoses (G.3.6.1.20., N2K 1170).

Among target species that could be of interest Mali Ston bay, we can list:

- *Ostrea edulis* - economically (aquaculture) and ecologically important shellfish of Mali Ston Bay
- *Arca noae* – low populations along the eastern Adriatic, but plentiful in Mali Ston bay
- *Pinna nobilis* - populations are highly endangered and have disappeared from most of the Adriatic Sea
- *Cymodocea nodosa* - important ecological role of seagrass meadows
- *Fucus virsoides* – endemic and endangered species present in Mali Ston bay
- *Savalia savaglia* – a strictly protected species in Croatia, and it is also included in the list of strictly protected species of the Berne Convention and the Barcelona Convention and the SPA / BD Protocol.
- *Sparus aurata* - poses a potential threat for the existing state of the ecosystem and causes significant economic losses through predation of bivalve farms
- *Caulerpa cylindracea* – non-native, highly invasive species
- *Paraleucilla magna* – non-native species present as biofouling on farmed bivalves
- *Hydroides elegans* - non-native species present as biofouling on farmed bivalves
- Toxic phytoplankton species – require constant monitoring to prevent poisoning of consumers

Sea temperature values in the Mali Ston bay recorded in multi-year monitoring ranged from 12 to 29 °C, which is usual for that area. In addition, the formation of a thermocline was observed in the summer months (June, July, August), while the isotherm of the entire water column is typical for October to December. The salinity values in previous years have ranged from 32 to 38, while surface values were from 25 to 38, indicative of a greater inflow of fresh water. Furthermore, oxygen saturation greater than 85% can be recorded at all points in the bay. Nutrient concentrations vary over the years, with highest concentrations recorded in 2012, 2015 and 2016, and lower concentrations in 2011, 2014, 2017 and 2018. At the same time, it is important to pointout that in 2012 and 2015, the salinity also decreased, which points to an increased flow of nutrients by the Neretva River, underwater springs and/or precipitation runoff from the steep shores of the bay. River inputs as well as irregular discharges of urban waters can increase the concentration of nutrient salts, and thus increase the abundance of phytoplankton. Thus, a large amount of organic matter is produced, the decomposition of which consumes dissolved oxygen. However, it should be emphasized that even in years with increased values of nutrient salts, the water column was well aerated.

Based on the monitoring and analysis of the frequency distribution of population density and biomass of microphytoplankton, nanophytoplankton and total phytoplankton, Mali Ston bay can be classified as a naturally moderately eutrophic ecosystem. During the multi-year monitoring of biotic ecological parameters, a total of 195 taxa (species and lower taxonomic categories) of microphytoplankton (cells larger than 20 µm) were determined in the bay. Of these, two taxa belonged to silicoflagellates, seven to coccolithophorids, 101 to diatoms, 84 to dinoflagellates and one to euglenophytes. The structure of phytoplankton populations indicates stable conditions throughout the year. The presence of some toxic and/or potentially toxic species of phytoplankton have been recorded but a toxic effect on bivalves and the end consumer have not been recorded.

Bivalve pathogens

The World Organisation for Animal Health has been collecting data in Croatia since 2005. To date, the only incidences of infections that have been reported in Mali Ston bay were recorded in Mediterranean mussels and were caused by the following pathogens:

- *Marteilia refringens*:
    - 2 records in 2013 (10 positive samples)

    - 1 record in 2015 (1 positive sample)
- *Bonamia excitiosa*:
    - 1 record in 2016 (51 positive samples)

Non-native species of consequence

A number of non-indigenous organisms have been found on benthic surveys in Mali Ston bay, including: green algae *Caulerpa cylindracea* and *Caulerpa racemosa*, red algae *Asparagopsis armata*, a sponge *Paraleucilla magna*, a polychaete *Hydroides elegans*, nudibranchs *Bursatella leachii* and *Melibe viridis*, a bryozoan *Amathia verticillata*

Non-native species that are often present on bivalve farms in Mali Ston bay as biofouling organisms or in the surrounding area and that negatively affect or could potentially have anegative effect on farming practices are:

- *Paraleucilla magna*
- *Hydroides elegans*
- *Amathia verticillata*
- *Ficopomatus enigmaticus*
- *Pinctada imbricata radiata*
- *Arcuatula senhousia*
- *Brachidontes pharaonis*
- *Magallana gigas*

Potential vectors of introduction are both finfish and bivalve aquacutlure. In the case of finfish, the main vector would be when offering services to wash cage nets from other production sites in Croatia. When it comes to bivalve culture, the main vector to date has been translocation from other production sites in Croatia and even an attempt to introduce the pacific oyster *Magallana gigas* to the bay which was, thankfully, unsuccessful. Other potential vectors are maritime traffic which is limited to small recreational and commercial vessels (mainly up to 15 m) coming into the bay and ballast water discharged by large shipping vessels associated with the commercial shipping port Ploče located just outside the bay.

5.2. Novigrad sea and Velebit channel

Geography

The area of the Novigrad Sea and the Velebit Channel geomorphologically belongs to the areas of Ravni kotar, Velebit and the island of Pag (Figure 2), between which there are sea surfaces that are suitable for the cultivation of shellfish.

Figure 2. The Velebit Channel in Zadar County is connected by the Novi Gorge to the Novigrad Sea, which is connected to the Karin Sea via the Karin Gorge.

The Velebit Channel is part of the canal area of the northern Adriatic. It is bounded by Velebit on the east/northeast side and the island chain Krk - Rab - Pag on the west/southwest side. Thewidth of the channel varies from ten to less than two kilometers. The depths of the bottom of the channel in the largest part are between 60 and 80 m, while in the area between the islands of Raband Jablano they exceed 100 m.

The basic rock mass of the coastal slope of Velebit is limestone. The degree of tectonic fragmentation is very high, and the route of the assumed and very significant Velebit fault runs through the Velebit Channel. The pronounced collision of Velebit, as a consequence of the predominantly carbonate structure and extremely strong tectonic movements, has influenced that Velebit does not represent a hydrogeological barrier, but the majority of Lika's groundwater is drained through Velebit towards the Velebit channel. These waters emerge as numerous permanent or occasional springs along the entire Velebit channel, and as coastal sources of variable abundance in many places.

Novigrad Sea is a bay cut deep into the land. It represents an isolated, highly separated and complex hydrogeomorphological system. In the central part of the northeastern edge of the Novigrad sea is the bay of the mouth of the river Zrmanja. According to its characteristics, the mentioned water area represents an estuary, a very dynamic system with somewhat lower biodiversity compared to other bordering marine and freshwater systems. Significant spatial and temporal fluctuations of hydrographic factors are significant for them.

The Novigrad Sea with an average depth in the central part of 28 m, its surface area of 28.65 km^2 (length 11 km, width 5 km) and water mass of 0.5 km^3 is larger than the Karin Sea with an area of 5.4 km^2 and volume of 0.04 km^3. Both water areas are connected by the Karinsko tisno strait. On the other side, the Novigrad Sea is connected to Novsko ždrilo, a canyon of steep banks, which fall below the sea surface to a depth of 40 m.

The area is strongly influenced by karst surface and underground waters, and numerous hot springs spring up along the coasts. The particularity of the geographical location, the natural closure and isolation, and a significant influx of fresh water inflows. The catchment area of the river Zrmanja is 854 km^2, and the average annual flow near Jankovic buk is 40 m/s, that is, at the inlet to the Novigrad Sea, the average intake of fresh water is 37 m3/s. In addition to Zrmanja, thewater bodies of Bascica, Drago, Slapac also flow into the Novigrad Sea, and the waters flowing in from the Karin Sea, which are influenced by Karisnica and Bijela, are also important. Bascica is 19.5 km long, the catchment area is about 69 km^2, the average flow at the confluence is about 0.52 m3/s, with two artificial lakes built to irrigate agricultural areas. Novigradska Draga is about 11 km long and has a catchment area of about 58 km^2, but its flows are weak (the average flow is about 0.28 m3/s), and the water is lost in numerous sinkholes, so the water itself rarely reaches the sea in Novigrad itself.

The rivers Karišnica and Bijela are short, intermittent watercourses with a torrential character, and at the point where they merge into a common estuary and flow into the Karin Sea, there is the Tuvina wetland.

Fluctuations in sea temperatures in the bay are significant due to the Inflow of cold water in winter and strong warming in summer, reaching up to 22°C at a depth of about 5 m. During severe winter cold, the surface of the sea can freeze, even in its entire surface. In the Velebit channel in the Velebit channel (Ražanac), the sea temperature varies from 8-23.1 °C.

The salinity of the Novigrad Sea is lower than that of the open Adriatic Sea and increases with depth because the sweetened water, which is specifically lighter, sticks to the surface layer. Salinity on the surface varies between 1.46 and 35.77, and in deeper layers between 35 and 38. During the greater inflow of fresh water, the halocline is pronounced in the area of the Novigrad Sea, but somewhat less pronounced remains present in the southeastern part of the Velebit Channel. Due to the bringing of nutrient salts from the land, the transparency is usually not higherthan 5 m.

The areas at the foot of Velebit and the Velebit Channel are known for strong and stormy winds, most often gale force winds. On the coastal side of the coastal mountains, the gale is the descending wind, most often in the NNE, NE and ENE direction, depending on the mountain obstacle." At Karlobag station, the wind is usually 1-3 Beaufort winds. Wind with a strength greater than 4 Beaufort blows in 15.8% of cases and then it is in the NE direction. The largest storms occur in the cold part of the year due to the distribution of baric systems, i.e. the movement of cyclones and anticyclones over Croatia, with the most common distribution of baric systems such that the area of high pressure is located over the continental part of Croatia, andthe low pressure area is located on the Adriatic.

Anthropogenic activity

In the area of the Novigrad Sea and part of the Velebit Channel in Zadar County, there are seven local government units: Starigrad, Jasenice, Obrovac, Novigrad, Posedarje, Ražanac and Pag (Figure 3).

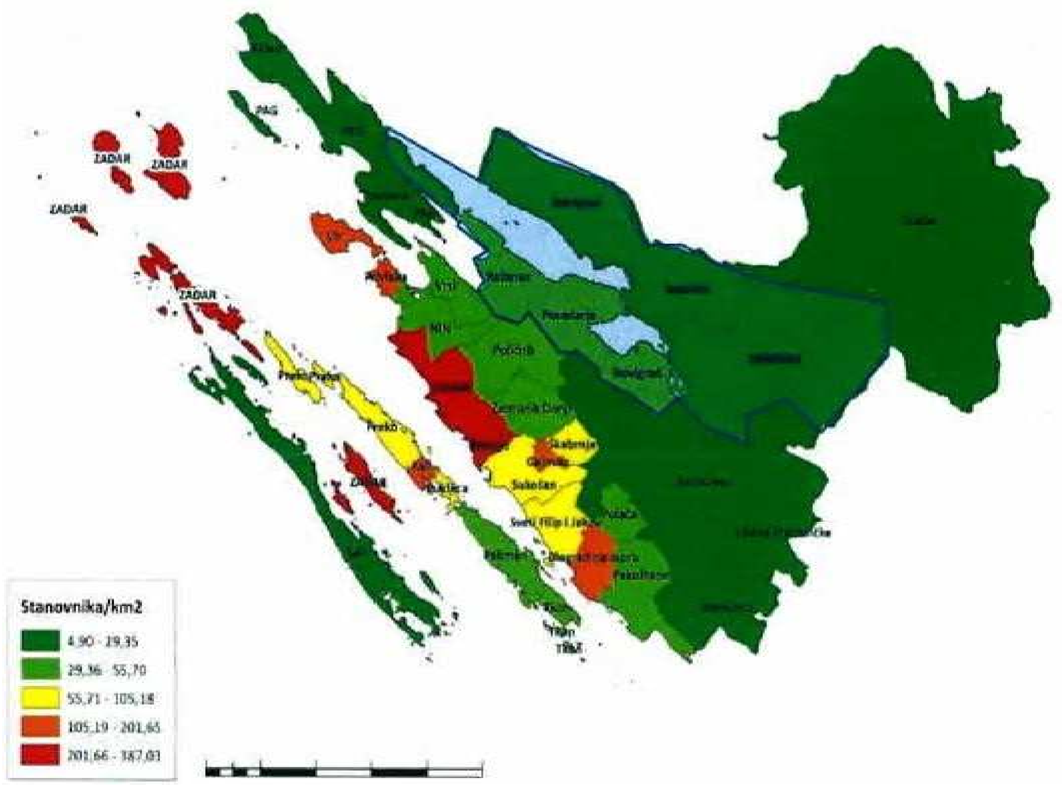

Figure 3. Zadar County territorial structure (cities and municipalities) The shaded part includes the municipalities that surround the area of the Velebit Channel and the Novigrad Sea.

In the population census from 2011, slightly more than 19 thousand inhabitants were recorded in the area of seven local self-government units (Table 2.)

Table 2. Number of inhabitants by municipality (population census 2011) in the area of Zadar County that have shores in the area of the Novigrad Sea and the Velebit Channel.

Municipaly	Habitant number 2011
Starigrad	1186
Jasenice	1398
Obrovac	4323
Novigrad	2375
Posedarje	3607
Ražanac	2940

Pag	3846
TOTAL	19675

Tourism is developing in this area. In 2022, 239,586 tourist nights were recorded in the area of the Novigrad Sea Tourist Board. Over 8,500 tourist beds were registered in the area of "Rivijere Paklenica", where approximately four hundred thousand overnight stays were realized in 2021, and 247,265 overnight stays were realized in the area of Ražanac in 2022. In the area of the Novigrad Sea and the Velebit Channel in Zadar County, there are the following ports open to public traffic: Novigrad, Posedarje, Starigrad, Seline, Ražanac and ports in the function of mariculture: Seline and Novigrad.

Production quantities

In the area of the Novigrad Sea and part of the Velebit Channel in Zadar County, permits were issued for the cultivation of shellfish in a maximum quantity of 1,560 tons, on an area of 29 hectares.
https://ribarstvo.mps.hr/UserDocsImages/akvakultura/Registar%20dozvola%20u%20akvakul tu ri%2008022023 .xlsx

History of bivalve farming and translocation of bivalves

Throughout history, there have been several attempts to cultivate shellfish (mussels) in the Novigrad Sea, but permanent cultivation was not established until the beginning of this century. Spiridon Brusina wrote about the quality of Novigrad mussel as early as 1873. Between the two world wars, oyster and mussel farming experiments were carried out in the Novigrad Sea, but on a small scale and without much success. In 1949, concrete installations for the cultivation of mussels were built, which collapsed under the weight of mussels in 1951 due to insufficient expertise in construction and the sudden development and growth of mussels.

The preparation of the "Study on the use and protection of the sea and the undersea in the area of Zadar County" preceded the spatial plan of the ZŽ in 2003, when the breeding areas for shellfish in the ZŽ were defined on the basis of the study. Shellfish cultivation has been established for the last 15 years, and considering the utilization of locations (about 10%), it can besaid that it is only in its infancy. Given that mussels and other shellfish from this area are in demand on the market, it is necessary to accept the challenge and strengthen production with the necessary infrastructure and innovative solutions.

Bivalve farming technology currently in use

Bivalve aquaculture in this area is performed on floating longlines. Mussels are collected from natural spawn that settles on the lines and buoys of the farm. Tubular nets, i.e. mussel stockings, are filled with the collected spat and placed on floating longline systems. After a few months the stockings are usually rearranged repeatedly (up to two times) until the mussels reach a marketable size. In growing conditions in this area, a yield of up to 20 kg per meter of a single line system can be achieved. Experimental cultivation of oysters and scallops in lanterns has recently been giving encouraging results.

The biggest technological challenge in the Novigrad Sea is adaptation to large and sudden inflows of fresh water, which, due to the volume and length of time, occasionally causes significant mortalities of mussels on culture installations.

Ecosystem and nature protection

The entire Novigrad Sea is in the NATURA 2000 protection regime (https://www.bioportal.hr/gis/} in which, according to the National Classification of Habitats, several types of marine habitats are present:

- G.2.4.1. Biocenosis of upper rocks of mediolittoral
- G.2.4.2. Biocenosis of bottom rocks of mediolittoral
- G.3.1. Infralittoral sandy muds, sands, gravels and rocks in euryhaline and eurythermal environment
- G.3.2. Infralittoral fine sand with more or less mud - Euryhaline and eurytherme biocenosis Association with *Cystoseira barbata* - Biocenosis of well sorted fine sands(Association with *Cyrnodocea nodosa* on well sorted fine sands)
- G.3.5. Posidonia oceanica meadows(?)
- G.3.6. Infralittoral hard beds and rocks
- G.4.2. Circalittoral sands

The marine habitats of *Posidonia* in the Novigrad Sea were mapped based on ecological modeling, however, they were not confirmed by findings. In the research conducted by Antolić et al. (2008) a total of 196 taxa of benthic organisms were collected and determined: 88 taxa of benthic algae (44.9%), 1 species of sea grass (*Cymodocea nodosa*) and 107 taxa of benthic invertebrates (54.6%). One of the specificities of the vegetation of the researched area is the existence of a well-developed settlement of the Adriatic endemic *Fucus virsoides* on the mediolittoral bionomicstage, which is a consequence of the strong influence of the fresh waters of the Zrmanja River on the entire area. In the area of the Novigrad Sea, 35 species of bivalve molluscs have been identified, among which the abundance is dominated by mussels, and the presence of oysters andscallops, which represent the target species for aquaculture in this area, has also been established.

The Velebit channel is located under the Velebit mountain, which has been declared a nature park in its entirety, and the Paklenica National Park is located in the Starigrad Paklenica area. There are no specially declared protected areas in the sea on the part of the Velebit Channel that is in the Zadar County. The marine habitats in this part are similar to those in the Novigrad Sea, and according to the national classification of habitats, the following are listed:

- G.2.4.1. Biocenosis of upper rocks of mediolittoral
- G.2.4.2. Biocenosis of bottom rocks of mediolittoral
- G.3.2. Infralittoral fine sand with more or less mud
- G.3.5. Posidonia oceanica meadows
- G.3.6. Infralittoral hard beds and rocks
- G.4.1. Circalittoral muds
- G.4.2. Circalittoral sands

In the area where the river Zrmanja flows into the Novigrad Sea, marine diatoms dominate (average of 105 cells L-1), while dinoflagellates (average of 104 cells L-1) and coccolithophorids (average of 102 - 104 cells L-1) somewhat less represented. The conducted research indicates a fairly narrow area of increased productivity, which is most productive in the Novigrad Sea and relatively sharply decreases in the Velebit Channel from the Novi Gorge to the West.

Monitoring programs

The "Program for monitoring the state of the environment and pollution of the coastal and marine areas of Zadar County" is implemented in Zadar County.

Sampling stations in the area ofthe Velebit Channel and the Novigrad Sea are shown in Figure 4.

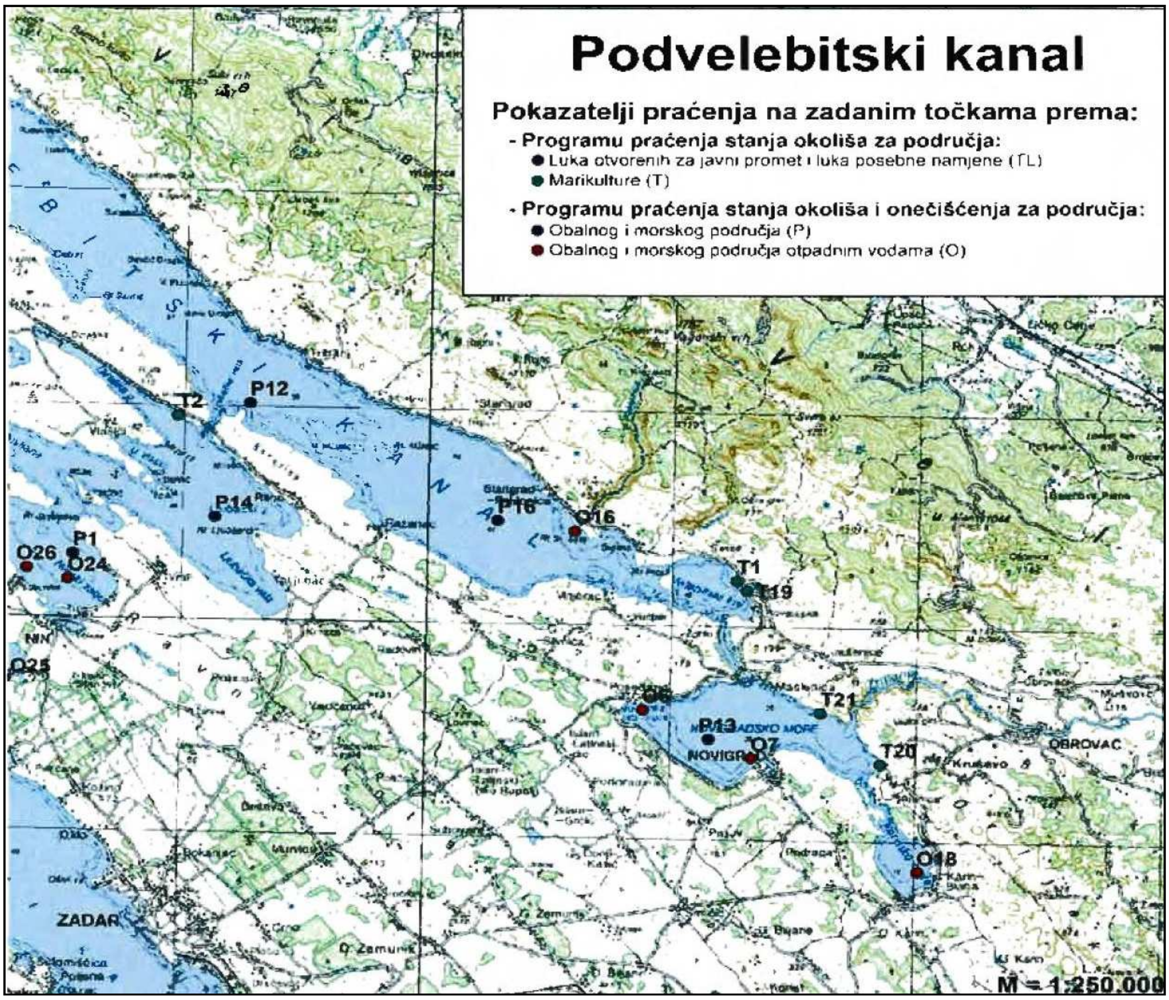

Figure 4. View of the points in the Velebit Channel and the Novigrad Sea where the "Program for monitoring the state of the environment and pollution of the coastal and marine areas of Zadar County" is implemented

The monitoring plan is carried out for the purpose of integral management, so the measurements are carried out in the summer, at the time of the greatest potential conflict between different users of the maritime property.

In this monitoring program, the results of the state of the water body are evaluated according to the Regulation on water quality standards NN no. 96/19. and include:

A. Indicators in the water column:

1. General indicators
2. Thermohaline properties
3. pH value
4. Dissolved oxygen and oxygen saturation
5. Nutrients
6. Mineral oils (alkanes ClO - C40)
7. Chlorophyll
8. Microbiological indicators (Intestinal Enterococci and *Escherichia coli*)

B. Indicators in the sediment:

1. Metals
2. Organic pollutants
3. Mineral oils
4. Redox potential
5. Nutrients

For the area of the Novigrad Sea and the Velebit Channel according to the standards of the Regulation on Sea Quality for Swimming NN no. 73/2008 and the EU directive on the management of bathing water quality (No. 2006/7 /ECL at the measuring stations in 2021, the quality of the sea is excellent.

Eutrophication is assessed as part of this state monitoring with an index for describing the trophic state of the water column (TRIX), which is defined by the combination of the logarithms of four variables: chlorophyll a (ChA), oxygen as an absolute percentage of saturation deviation (absD%O), mineral nitrogen (minN) and total phosphorus (TP). Determination of the trophic state via TRIX is applied mainly to the surface layer of the water column from 0 to 10 m deep.

The results of the evaluation of the TRIX index in 2021 are shown in the Table 3 according to the Regulation on water quality standards "Nar. Nov." no. 96/19, the limit values of the indicators of eutrophication in the condition category at the measuring stations on the calculated TRIX index can be evaluated as very good (2 - 4), good (4 - 5), moderate (5 - 6) and bad (6 - 8).

Table 3. The calculated values for TRIX at the measuring stations of the Podvelebit Channel and the island of Pag in 2021 show a low degree of eutrophication.

		P1	P2	P11	P12	P13	P14	P16
TRIX	dno	3,4	2,2	3,6	2,5	3,8	3,4	3,3
	pov	3,1	2,9	3,0	2,6	3,5	2,4	2,1
		TL17	TL25	TL26	O6	O7	O8	O9
TRIX	dno	2,4	2,9	2,7	2,9	3,2	3,4	3,4
	pov	2,7	3,1	2,5	3,5	3,6	3,6	3,5
		O10	O16	O18	O22	O23	O24	O25
TRIX	dno	2,6	3,2	3,7	2,3	3,6	2,3	3,0
	pov	3,3	3,7	4,2	2,7	2,8	2,5	4,1
		O26	T1	T2	T19	T20	T21	
TRIX	dno	2,0	2,4	2,8	3,1	3,0	3,4	
	pov	3,3	2,7	2,4	3,3	2,4	3,7	

(taken from: Report on the examination of indicators of monitoring the pollution of the coastal and marine areas of Zadar County during 2021: https://www.zadarska-zupanija.hr/images/dokumenti/Izvje%C5%Altaj_M M_2021-RP Karte_potpis. pdf)

In accordance with the EU legal framework in Croatia, on the basis of the Act on Food Hygiene and Microbiological Criteria for Food (NN, no. 83/22), the "Plan for monitoring the quality of sea and shellfish in production areas and areas for the re-laying of live shellfish" is implemented, which is adopted for each calendar year.
(http://www.veterinarstvo.hr/UserDocsImages/HranaZivPod/2023-0l-04.
monitoring.skoljkasi.2023.pdf)

This plan also includes the production areas of Modrič-Selina (Velebit Channel) and Novigrad Sea, where the quality parameters are monitored as shown in the Table 4.

Table 4. Parameters and sampling plan for the production of Modrič-Selina and Novigrad sea for the year 2023.

Sea water	Frequency
Phytoplankton composition	from December 1st to March 31st every two weeks, from April 1st to November 30th once a week
Shellfish meat	**Frequency**
Benzo(a)pyrene and the sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene	Half-yearly (IV., X. month)
Metals {Cd, Hg, Pb)	Half-yearly (IV., X. month)
E.coli	Monthly (on the third Monday at month)
Biotoxins (PSP, LT, ASP)	Per week

Based on the Order on measures to protect animal health from infectious and invasive diseases and their financing (https://narodnenovine.nn.hr/clanci/sluzbeni/2021_12_145_2506.html), control is carried out every year in 2022 Aquatic Animal Health Monitoring Program. Until now, Animal health monitoring programs included surveillance for:

- Infection by *Bonamia ostreae* once per year
- Infection by *Marteilia refringens* once per year during highest sea temperatures is analyzed in mussels

Microbiological/sanitary conditions

The production areas of the Novigrad Sea and the Velebit Channel have been classified as A category for the entire time since they were declared production areas (2009), which means that they can be sold to the end customer without purification and/or re-laying in shellfish re-laying areas.

Bivalve pathogens

The presence of parasites *Bonamia ostreae* and *Marteilia refringens* was not determined in the area of the Novigrad Sea and the Velebit Channel.

5.3. River Krka estuary

Geography

The river Krka estuary is located in the Šibenik-Knin county in the central part of the Croatian coastline. It is a 22 km long sunken river valley with an average slope of 1.6 m/km. It is defined as the body of water extending from the base of the Skradin waterfalls (Skradinski buk) until the estuarine mouth. The estuary is relatively narrow, except for two wider parts: Prokljan Lake and the Šibenik area. The depth gradually increases from 1-2 m below the waterfalls to 43 m near the estuarine mouth. The maximum tidal range is around 40 cm at the mouth and 30 cm at the head of the estuary.

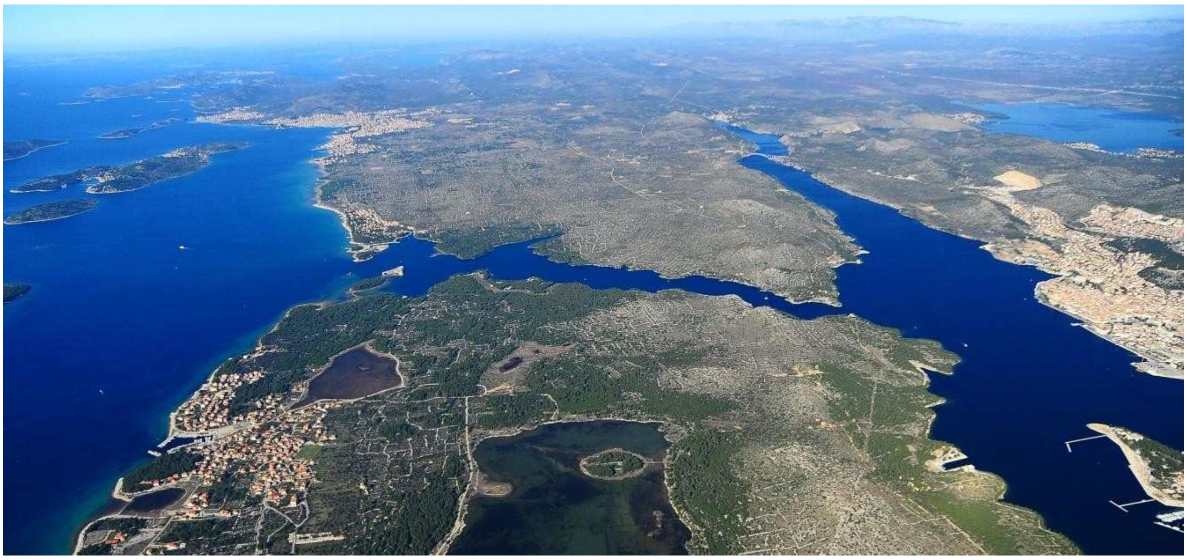

Figure 5. The lower Krka estuary from St. Ante Channel (center) that exits to the Adriatic sea to Prokljan lake (upper right) (source: https://priroda-skz.hr/hr/zasticena-podrucja/znacajni-krajobrazi/kanal-luka/o-lokalitetu/)

The river Krka is 49 km long before it enters the estuary and has a karstic watershed of approximately 2500 km^2. The freshwater lake Visovac and a series of moss-covered travertine barriers and waterfalls precede the estuary and retain much of the already low sediment load. The mean daily flow of the river varies within the year between 2 and 415 m^3/s, with a ten-year mean of around 55 m3/s (data for 1981 to 1990). A smaller river, Guduča, enters the estuary at Prokljan lake, which is also under the influence of numerous torrential streams.

When these numerous freshwater inputs come in contact with seawater the water column becomes strongly stratified by salinity. Although these water masses mix partially in spring and autumn, a sharp halocline exists between the surface/ brackish and lower/marine layer for most of the year. The movement of freshwater along the surface pulls along the top layer of underlying seawater returning it to the sea, which results in a circulation and exchange of seawater through the lower layers of the bay. The water body of the estuary is completely exchanged 2-3 times per year.

While the lower part of the Krka river and the upper part of the estuary are located within a national park, the city of Šibenik is located in its lower reach, where the majority of the county's population lives. The bivalve aquaculture production site spans from just upstream of the city of Šibenik up to Prokljan lake.

Anthropogenic activity

The Estuary is close to the urban settlements of Šibenik (municipality; 51.5 inhabitants/m2), Drniš (municipality; 17.7), Knin (municipality; 35.1) and Skradin (municipality: 17.9 inhabitants/m2), as well as smaller settlements such as Zaton and Raslina, which are a part of the Šibenik municipality and Bilica which is its own municipality (99.2 inhabitants/m2).

According to the data provided by Vodovod i odvodnja d.o.o of Šibenik-Knin County, the parts of the city that are closest to the agricultural fields (Crnica, Dolac, Mandalina) are connected to the public drainage system of the city of Šibenik, which has an outlet behind the island of Zlarin. Almost all remaining wastewater channels have been directed into a central collector which further reduces eutrofication of the estuarine system The town of Skradin has a wastewater treatment plant with 2 degrees of purification, and the annual discharge of treated wastewater is about 70-80 thousand m^3. However, surrounding settlements (Bilice, Raslina and Zaton) still discharge wastewater into black or septic tanks which can overflow into the surrounding water systems.

In addition to communal wastewater, a number of existing or past industrial facilities have left and continue to leave a mark on the water body of the lower Krka estuary (Šibenik bay). In the past, a strong metal and metal processing industry was present, of which the most important were the Electrode and ferroalloy factory (TEF) and Light metal factory (TLM), which continued their work up until 1990. In the last three decades, these industries have been abandoned and theeconomy has turned to tourism instead. To this day, heavy metals and other toxic compounds such as polycyclic aromatic hydrocarbons (PAH) remain present, although mainly in the sediment, with increased levels of tributyltin (TBT) which is mainly associated to nautical tourism and shipping being found in surface waters.

Nautical tourism is present along the whole estuary, with marinas and moorings of various sizes in the towns of Skradin, Bilice and Rasline upstream from the aquaculture production sites, resulting in frequent passage on numerous boats and yachts in relatively close proximity to the farms. Some farms have capitalized on this and offer on-site purchase and/or tasting of farmed bivalves.
The Port of Šibenik is located on the southern edge of the city of Šibenik across from Sv. Ante channel (St. Anthony), i.e. downstream from the bivalve aquaculture farms. The port is 10km long, 300-1200 m wide, with depths of 8-40 m. It has a terminal for incoming bulk cargo with an annual capacity of 1 million tons (ship size up to 30 000 tons), an outgoing bulk cargo terminal with annual capacity 400 thousand tons (ship size up to 30 000 tons), a wood terminal (sawn timber and wood products) and a passenger terminal which, in addition to local ferry lines, annually welcomes around 100 cruisers, some of which remain on anchor within the lower part of the estuary.

History of bivalve farming and translocation of bivalves

The history of bivalve farming in the Krka estuary is relatively new compared to other locations on the Croatian Adriatic coast. After initial research of natural mussel populations in the estuary was performed in 1979, experimental farming of this species began in 1980. Promising results obtained through these initial attempts led to the establishment of two commercial farms in 1983with an annual production capacity of 100 tons. In the span of the following five years, the aquaculture potential of the Mediterranean scallop (*Pecten jacobaeus*) and variegated scallop (*Mimachlamys varia*) was explored. Results confirmed their potential for commercial aquaculture and led to the development of farming technology for these species. Soon after, new marine spatial plans that included areas for aquaculture of marine organisms were accepted and potential zones for farming were elected to be complimentary to current users of the marine space, taking care to separate such sites from points of wastewater discharge from settlements and industry facilities, marinas, hotels, underwater cables and other infrastructure. After some further research, environmental impact studies and deliberation from governing bodies, the following sites were determined in 2003: Prokljan strait (fish farming capacity up to 400 t), lower Krka estuary (fish and bivalve farming capacity up to 210 t and 2100 t, respectively). At this time, farming was already in progress at 20 localities, with bivalve farming being the predominant activity. In 2007, amendments and additions to the spatial plans of the City of Šibenik confirmed the continuation of farming at existing sites in accordance to previous plans, with the added possibility of increasing production capacity by increasing the surface of farms if implemented environmental monitoring does not report any negative changes to the environment. Otherwise it would be necessary to perform dedicated environmental impact studies which would determine the production capacity of the individual sites. This was indeed the case, but the environmental impact study confirmed the possibility of expansion of existing farms, but no construction or additional use of the coast was allowed. It was also determined that Martinska bay would be the location of a new bivalve depuration facility, with a maximum footprint of 4 000 m2 and locatedat least 70 m from the coastline. However, this project was never realized. Today, a bivalve processing and depuration facility is under construction in Sv. Petar bay (St. Peter) in the Mandalina area of the lower Estuary.

Until the end of 2007, a total of 38 concessions for farm sites were assigned for commercial use with an estimated maximum production potential of 2000 tons. These were awarded to 14 concessionaires, of which 8 were artisans and covered 47.5% of total potential production, 4 professional fishermen (35% of total potential production) and two were registered companies (17.5% of total potential production). However, it was determined that a total of 507.5 t of mussels were currently on aquaculture farms in the lower Krka estuary (Krka river mouth), of which 342 t were market size and 165.6 t were spat, and 15,000 pieces of oysters. Thus, in 2008, 25.4% of the possible total production was utilized and only 17.1% of market-size mussels were grown on the 38 concessions.

Production quantities

In the last five years of available production data (2017-2021; Table 5.), production has fluctuated and declined somewhat, with a significant dip in 2020, although this could likely be attributed to specific market conditions during the COVID-19 pandemic.

Table 5. Aquaculture production of bivalves in the Krka estuary

	2017	2018	2019	2020	2021**

Oysters (pcs.)	39,436	22,869	31,795	9,930	18,091
Oysters (kg)*	2,761	1,601	2,226	695	1,266
Mussels (kg)	109,267	85,232	92,260	37,183	41,392
Total (MT)	112	87	94	38	43

*market size of oysters is estimated at 70 g for this calculation
**Preliminary values

Based on local data collection surveys performed for the ARGOS project in September 2022, a total of 12 registered bivalve farms are currently located in the Krka estuary, with 8 of these being artisanal, 3 registered companies and one cooperative of farmers. A total of 57 concessions are used for farming bivalves, totaling at 280,168 m2 of used surface, ranging from 1,100 to 23,484 m2. If the total production potential of these concessions is considered, it is clear that the realized capacity for production is lacking.

Bivalve farming technology currently in use

The dominant farmed species are Mediterranean mussels (*Mytilus galloprovincialis*), but farmers are generally registered for farming mussels and European flat oysters (*Ostrea edulis*). Oysters are only farmed sporadically. Species of scallops that have been given attention in the past have, for the meantime, been abandoned.

The dominant technology currently in use are floating longlines for suspended culture of bivalves. The floating longlines are placed in one of two ways: (i) individual mutually independent 100 m longlines attached to buoys every 7 m and set up parallel to each other, each with dedicated mooring systems and (ii) a modified version of this that uses a double set of longlines connected to a single row of buoys, but to each end of the buoy.

Cultivation of both oysters and mussels in the Krka estuary is based on collecting spat from nature. Mussel juvenile collecting is carried out by placing collectors close to the surface or using the load-bearing ropes of the longlines themselves. Mussel collectors are usually prepared in March before spring or in September before the autumn reproduction seasons, with peak settlement on collectors occurring in Spring. They consist of thicker ropes (diameter approx. 40

mm, length 2 m) that are tied to the longlines every 0.5 m. Once mussels have reached a size of 30-40 mm, they are transferred from collectors to mussel stockings.

Once loaded into mussel stockings, they are suspended from the longlines every 0.5 m. The stockings are initially stocked at a density of 2.5 to 3 kg per m of stocking. Half way through the cultivation period, the mussels are harvested, sorted and stocked into stockings of larger size at 15 to 18 kg per m of stocking.

The entire culture period from settlement on collectors to harvesting for market takes around 15 months.

As was commented on by farmers during the local data collection activity of project ARGOS, the practice of lowering mussel stockings into deeper water (around 3-5 m) during the freshwater inflow season is commonplace.

A third shellfish production technology has been introduced relatively recently, but has not proven efficient for the environmental conditions in the Krka estuary. This method uses a long floating PEHD pipe instead of the floating longline with buoys. The pipe has a net attached to it that hangs freely into the water column and is used as a collector and culture substrate for mussels in one. Although having good results in larvae settlement and growth, the inability to lower the system during strong freshwater inflow is limiting and results in complete mortality of the cultured stock.

Nature protection

The Krka estuary contains two areas that are protected at the national level under the category of Significant landscapes: The lower course of Krka (Skradin to Šibenik bridge) and the Channel-Port (Šibenik bridge to channel of St. Ante). Both of these sites and also Prokljan lake are covered by the Natura 2000 ecological network under the single name of Krka estuary (Ušće Krke) HR3000171 which has a total area of 4423.84 ha (marine area 603.41 ha, land area 3820.43 ha) and mostly belongs to habitat types 1130 - estuary and 1110 - sandbanks which are slightly covered by sea water all the time (coverage in ha has not been determined). Upstream from this area is the National park Krka.

Monitoring programs

Since 1976, the Krka estuary has constantly been covered by ecological monitoring programs. From 2009 to date, the monitoring program that has been enforced is the “Systematic research ofthe quality of transitional and coastal waters”.

Several general and dedicated monitoring programs have been put in place in order to assure the quality of food items produced by aquaculture, as well as the presence of shellfish diseases and different ecological parameters of the production site. These include:

- Sea and shellfish quality monitoring plan
    - bivalve tissue of farmed mussels are examined for *Escherichia coli* once per month in 6 production sites
    - biotoxins in mussels at two sampling points once per week
    - qualitative and quantitative phytoplankton composition in the water column twice per week from December to March and once per week from April to November at two sampling points
    - heavy metals (Cd, Hb, Pb), benzo(a)pyren and the sum of benzo(a)pyren, benzo(a)antracen, benzo(a)fluoranten and chrysene in mussel tissues twice per year (April and October) at 2 sampling points
- Systematic study of quality of transitional and coastal waters (samplings performed 4 times per year, every two years)
    - phytoplankton (composition, blooms, toxic species)
    - zooplankton (microzooplankton, mezozooplankton, copepods, alien species)
    - benthos (composition of communities, alien and invasive species)
    - fish (composition of communities, endemic species)
    - microbiology (marine microorganism composition)
    - chemical oceanography (indicators of eutrophiction, heavy metals, TBT, PAH)
    - physical oceanography

Microbiological/sanitary conditions

Given that the stratification of the water in the Estuary is influenced by oceanographic, hydro-logical properties and the synoptic situation, changes in these systems cause specificity in microbiological characteristics. Therefore, the microbiological situation in the water largely depends on the inflow of fresh water and the anthropological influence on the area. In addition to river tributaries, wastewater is also part of the tributaries to the estuary and is the primary source of bacteriological pollution. Due to its physicochemical properties, wastewater spreads over the surface of the sea at pretty large distances from the source of pollution, depending on surface currents and wind.

The Krka estuary is divided into six production zones for bivalves (Šibenik I, Šibenik II, Šibenik III, Šibenik IV, Zaton, Strmica), which are monitored monthly for Escherichia coli levels in shellfish tissue using mussels as the reference species for all production sites. By default, they are considered as sanitary class A production sites meaning that harvested bivalves can be placed directly on the market without requirements for depuration.

However, there are sporadic incidences of individual production site lockdowns due to increased levels of *Echerichia coli* in analyzed samples, i.e. unfavorable sanitary conditions (Table 6).

Table 6. Number of zones and percentage of total number of zones in parenthesis with incidences of *Echerichia* positive findings in Šibenik estuary per year out of a total of 6 production sites.

Species	2019	2020	2021	2022
Mussel	1 (17%)	3 (50%)	1 (17%)	0 (0%)

Ecosystem

Krka and several other tributaries bring with them a small number of particles. Due to the strong stratification, the retention time of these particles is short, thus no unfavourable conditions are created in the environment. Thus, the water through the Estuary is mostly clear, and according to studies, the eutrophic layer is up to 20 m. Based on measured concentrations of heavy metals and biogeochemical markers from the early '90s, the river water entering the estuary is comparable to the world's most pristine river systems.

In estuaries, mixing two basic types of water, sea and fresh, creates the so-called transition water i.e. brackish water. Because fresh water is lighter than seawater, a two-layer flow and a mixed layer of brackish water in the middle are created by mixing these two layers. The brackish water is characterised by a fast increase in salinity with depth, also known as the halocline layer. As the brackish water passes through the estuary, it thins out and becomes salty, and it is very rich in nutrients.

As the number of phytoplankton communities and their abundance depends on the environmental parameters, their quantity in the Estuary is variable depending on the season. It is greatest during the spring and autumn fluctuations of nutrient salts in the water column. The lowest abundance occurs during the summer stratification of the water which prevents the mixingof nutrients and the creation of new supplies of food for shellfish.

The highest density of phytoplankton, freshwater and marine species is found in the upper layer of the halocline, and its number decreases sharply with depth. As freshwater phytoplankton passes through the estuary and touches seawater at the lower limit of the halocline, it decomposes and creates practical and nutritious organic matter for bivalves. The halocline is the accumulation layer for freshwater and marine phytoplankton and a site of intensive bacterial activity.

On the lower edge of the halocline a temperature maximum may be found. During summer the maximum attains the highest recorded temperature in the Adriatic Sea (31.5°C).

Based on annual nutrient flow estimates , the dominant nitrogen and silica source in the estuary is the Krka river. The dominant source of phosphorus in the upper estuary is sinking and decomposition of freshwater phytoplankton, while the dominant source in the lower estuary is the antropogenic inflow of the city and harbour of Sibenik. Growth of phytoplankton in the entire estuary is primarily P-limited with the exception of the Šibenik area, close to nutrient inputs. Only marine phytoplankton develops blooms in the estuary and not freshwater phytoplankton.

The river Krka is characterised by two types of transitional waters:

i. oligohaline estuary with fine sediment (HR-P1_3) and

ii. meso- and polyhaline estuary with fine sediment (HR-P2_3).

In the area, there is:

- one water body classified as HR-P1_3: P1_3-KR that covers 1.32 km2
- one water body classified as HR-P2_3 bearing the codes P2_3-KR and P3_3-KR, covering aras of 15.20 and 5.87 km2, respectively.

In the zone relevant for aquaculture, the dominant habitat type is G.3.2. Infralitoral fine sands with more or less silt. The coastline along the whole of this area is rocky (F.4. Rocky seashore/G.2.4. Mediolittoral hard bottom and rocks). These transition to habitat type G.3.6. Infralitoral hard bottom and rocks below the tide line. In deeper parts of this area the main habitat is G.4.2. Circalittoral sands which becomes G.3.5. *Posidonia* settlements.

Among target species that could be of interest in the Krka estuary, we can list:

- *Mytilus galloprovincialis* - the main cultured species and common species of the rocky shore
- *Ostrea edulis* - important commercial species present on rocky shore and cultured to a small extent
- *Ruditapes decussatus* - common bivalve species in sediments
- *Venus verrucosa* - common bivalve species in sediments
- *Cerastoderma glaucum* - common bivalve species in sediments
- *Pinna nobilis* - the species has almost disappeared from the Adriatic Sea - a highly endangered species
- *Poisodnia oceanica, Cymodocea nodosa, Zostera marina, Zostera noltei* - important ecological role of seagrass meadows
- *Hippocampus hippocampus* and *hippocampus guttulatus* – sea horses, endangered fish species
- *Knipowitschia panizzae* - species of goby, endangered
- *Ninnigobius canestrinii* - species of goby, endangered
- *Pomatomus saltatrix* - populations of this predatory fish have recently increased
- *Synodus saurus* - previously rare species of fish in Adriatic found in Prokljan lake
- *Petromyzon marinus* - lamprey, endangered fish species

- *Caulerpa cylindracea* – non-native, highly invasive species
- *Paraleucilla magna* – non-native species present as biofouling on farmed bivalves
- *Ficopomatus enigmaticus* - non-native species present as biofouling on farmed bivalves
- Toxic phytoplankton species – require constant monitoring to prevent poisoning of consumers

Bivalve pathogens

The World Organisation for Animal Health has been collecting data in Croatia since 2005. However, the sampling points have not included the Krka estuary so information on the presence of *Marteilia refringens* and *Bonamia excitiosa* or *Bonamia ostreae* are not available for thislocation.

Non-native species of consequence

Non-indigenous organisms of potential concern that have been found on and around bivalve farms in the Krka estuary include:

- *Paraleucilla magna*
- *Ficopomatus enigmaticus*

Potential vectors of introduction of these species have been considered shipping and aquaculture. However, more surveys and constant monitoring should be performed as there are likely others species present that have not been registered. Furthermore, there may be species present here that are not native or common in other production sites that may behave invasively.

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