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EVALUATION OF THE CURRENT STATE OF KNOWLEDGE ON EFFECTS OF MARICULTURE ON MARINE AND COASTAL BIODIVERSITY

All forms of mariculture, regardless of physical structure or economic motivation, affect biodiversity at genetic, species, and ecosystem levels. At the ecosystem level, both goods and services functions can be affected, with far-reaching consequences and long-term impacts. Because aquatic communities are interconnected, impacts to aquatic ecosystems must be considered holistically in both the short and long term. This section provides a summary of the major impacts of mariculture on biodiversity.

Mariculture can alter, degrade, or destroy habitats, disrupt trophic systems, deplete natural seed stocks, transmit disease, and reduce genetic variability. For example, coastal mangrove forests have been converted to shrimp ponds, closed or semi-enclosed waters have been impacted by nutrient inputs (or stripping), and benthic habitats have been impacted by shellfish culture and sedimentation.

However, in some circumstances, mariculture can also improve local biodiversity; for example, birds may be attracted to mariculture sites, and artificial reefs that serve as species aggregators can lead to increased biodiversity. In situ coral reforestation programmes have also had a positive impact on reef biodiversity.

Depending on energy sources used to produce biomass, mariculture could be divided into:

a) Autochthonous organic-based or "natural" trophic systems, such as kelp culture, and raft culture of mussels or oysters. Such culture practices derive their energy from solar radiation or nutrient sources already available in natural ecosystems, and tend to have fewer negative effects on biodiversity. In some cases, their impact on biodiversity may even be positive;
b) Allochthonous organic-based or "artificial" trophic systems, such as net and pond culture of fish and shrimps, derive energy mainly from feeds supplied by growers and are more likely to disrupt the natural ecosystems.

All environmental impacts depend heavily on the sensitivity of a particular ecosystem or its type. For example, some wetland habitats and ecosystems are particularly vulnerable, such as those that have been designated as threatened or sensitive either because of their rarity ortheir vulnerability to change. These ecosystems include mangroves, estuaries, seagrass beds,coral reefs, and certain benthic communities. Specific impacts depend on the varying resilience requirements of different farming practices in a given ecosystem, but these are poorly understood.

Culture systems in open waters discharge their nutrient rich wastes (faeces and uneaten feed) directly into the water and could cause increases in trophic status. Semi-enclosed ecosystems such as sheltered bays are particularly sensitive to such effects. This may lead to blooms of phytoplankton, including toxic species, and their consequent degradation can drasticallyreduce oxygen levels. Algal blooms can also cause severe shading of seafloor vegetation thatserves as nursery habitat and refuge for finfishes and benthic invertebrates. In sheltered bays the effects of such waste sedimentation on the sea bottom tend to be confined to within 50 or 100 meters of the site. However, in bays swept by strong currents the nutrients may spread widely and spark algal blooms within days, strong tidal currents tend to dilute wastes before they can cause hypernutrification or eutrophication. Both effects are culture density-dependent. Scallops and oysters, for example, individually produce up to 50-60 g and 120 g faeces in dry weight respectively each year. Some of this waste will decompose and be carried away, but most will settle under the beds. During storms, the sediment can be drawn up into water columns and cause heavy mortality by blocking the gills of the bivalves. This overlay of sediment can also shift the composition of benthic communities towards pollution-tolerant species, a clear biodiversity effect. The waste problems associated with intensive culture of high-value marine finfish have led to the beginnings of reform in industry practices.

Considering the fate of by-products of culture practices, particulate matter including organic particulate forms of nitrogen, phosphorous and sulphates typically move downward into the benthos, while carbon dioxide, dissolved organic carbon, and various soluble nutrients (e.g., ammonia and phosphate) move into the water column. Benthic communities (e.g., microbes and suspension feeders) modulate the transport pathways of by-products, as does the structure of pelagic communities. The structure and function of benthic and pelagic communities are in turn modified by these processes.

Table 1. shows the types of pollutants arising from aquaculture practices, and their common effects on biodiversity.

Pollutant	Source / Uses	Impact
Antibiotics	Hatcheries, culture ponds	Accumulation in sediments and living organisms, genetic diversity of benthic microflora
Pesticides	Cages, algal beds	Invertebrate mortality
Disinfectants	Hatcheries, culture ponds	Hypoxia, mortality
Antifoulants	Cages	Invertebrate mortality
Hormones	Hatcheries	Unknown

The potential threats to biodiversity in areas where pollutants such as chemicals, pharmaceuticals, and other additives used in mariculture are discharged have not been adequately studied. Such discharges are the result of overuse of these pollutants. Lack of access to information on appropriate use has led some aquaculture operators to misuse some chemicals (e.g., antibiotics). Salesmen or pharmaceutical companies may also encourage misapplication. Commonly used chemicals include antibiotics, pesticides, disinfectants, antifoulants and hormones (Table 1).

The organophosphate class of chemicals like dichlorvos and trichlorphon used outside the United States of America to control sea lice (parasite copepods that feed on salmonid mucus) includes nerve gases and many insecticides. Effects on the marine environment are not well studied, though are usually assumed to be negative. However, supporters of the use of the carbamate insecticide Sevin to kill burrowing shrimp (which undermine intertidal zone sediments used for oyster beds) believe that by stabilizing sediments the insecticide promotes greater biological diversity.

Chemicals are also used as antifouling agents and as disinfectants. Antifoulants such as TBT are banned in developed countries for aquaculture purposes, but are still used in some other countries, where they continue to impact on biodiversity.

Hormones are used to induce or prevent reproductive maturation, for sex reversal and to promote growth. Bath and feed-incorporated applications of hormones are obviously more of a concern than controlled injection into individual broodstock animals because they become readily released into surrounding waters where they can persist in the environment or in aquaculture products. Hormone use is not well documented and is sometimes carried out without adequate understanding of the quantities needed.

Parasites in cultured stock pose problems not only for aquaculturists but also for other organisms in the environment. In British Columbia, for example, one theory for the rise of Parvicapsula infection in migrating Pacific salmon is acquisition from a fish farm. The parasite is suspected to be linked to profound changes in migratory behaviour of salmon that leads to massive pre-spawning mortality and may be responsible for decimation of diversity at the population level.

While there is a welcome trend in capture and culture fisheries management to consider harvested species as part of an ecosystem rather than “stand- alone” targets, the effects of mariculture on aquatic ecosystems have been little studied. Given the scale of culture of some of the major species, the effects on the different hierarchical levels of biodiversity can be far-reaching.

The high value marine carnivorous species that are farmed require feeds incorporating animal sources of proteins. The most obvious effect of farming these carnivorous species such as salmon, trout, and sea bream is that more protein is fed to the fish than is later harvested for human consumption. Most of this feed comes from marine sources in the form of fish meal and fish oils, and the percentage of fish meal incorporated into fish feed has been increasing from 10% in 1988, to 17% in 1994 to 33% in 1997. However, a large proportion of fish meal is also diverted to pig and poultry feeds. The fish meal industry, partly driven by global deficits in fish oils, is actively seeking plant protein replacements. Fish protein and lipids presently come from large fisheries for small pelagic fish, such as anchoveta, Chilean jack mackerel and Atlantic herring. These fisheries comprise four of the five top global fisheries. Although plant proteins are being developed for inclusion as protein sources in fish feeds, complete replacement of fish oils in fish meals may not be possible since they have a beneficial effect on in resistance against fish diseases.

Harvesting small fish for conversion to fish meal leaves less in the food web for other commercially valuable predatory fish, such as cod, and for other marine predators, such as seabirds and seals. Pauly and co-workers (2001) identified a significant trend in aquaculture of "farming up the food chain" that they consider in combination with the global problem of "fishing down the food chain". However, this statement continues to attract debate. Increasing intensification of aquaculture, especially in Asia, and its concentration on higher-value carnivorous species, is inexorably raising dependence on capture fisheries through increased feed production. The competitive nature imposed on marine fisheries by culture fisheries merits further investigation.

Bivalve culture takes nutrients away from the marine food web, but only affects biodiversity adversely if the carbon and nitrogen removed from the water column becomes excessive, leaving less for other herbivores and phytoplankton, thereby affecting the growth and reproduction of zooplankton and other herbivorous marine animals. Bivalves do take suspended seston (particulate matter suspended in water) and change it into denser particles that fall to the bottom. Permanent extensive bivalve culture may bring about changes in the coastal food web causing eutrophication.

The loss or alteration of habitat becomes a biodiversity effect when it changes living conditions for other species. Seed collection from habitats such as lagoon bottom habitats using destructive gear results in habitat destruction or/and alteration. Mariculture takes up space, often very large amounts of it, not only in bays and oceans but also on nearby foreshores. The sheer occupying of acres of water can affect migratory routes and feeding patterns of a wide variety of non-target species. Salmon farms, for example, are believed by some to interrupt the free movement of wild migrating salmon and feeding killer whales. Underwater exploders and other acoustic devices intended to deter predators may also increase the stress on non- target animals.

Converting tidal wetlands for shrimp ponds and building roads, dikes, and canals threatens benthic habitat diversity in the tropics, particularly in Latin America and Asia. Tidal marshes and mangroves that serve as nursery grounds for wild shrimp and fish populations are lost, and less man- grove and marsh grass detritus enters coastal food webs. The draining of pondsfor harvest releases diseases, antibiotics, and nutrients into estuarine and coastal waters. Despite the possibly large-scale implications, the effects in the coastal zone remain poorly studied. Conversion of new habitats for brackish-water prawn farming by the transport of salt water to inland ponds, as well as conversion of productive rice growing areas to prawn ponds,changes their associated biodiversity.

The best-known example of habitat alteration arising from mariculture is the effect of shrimp farming on mangrove ecosystems, which have very high species diversity both in the water and on land and contribute about one-third of yearly landings of wild fish in South East Asia. Abandoned shrimp ponds serve as a threat to contiguous coastal habitats and their biodiversity.

The local or more widespread effects on non- target species such as the by-catch of seed collection from the wild have not been well studied. In culture systems where there are no methods for artificial control of reproduction, or where such methods exist but are beyond the means of local farmers, manual collection of fry for grow out can remove significant amounts of biomass and biodiversity. For example, the collection of one tiger shrimp larva involves the removal of 1400 other macrozooplankton individuals. Naylor and co-workers review the effects of fry collection on natural seedstock, noting that 85% of the larvae collected for milkfish farming in the Philippines, for example, are from species other than milkfish, and are discarded - a significant bycatch. Although hatchery reproduction techniques are available for some species, in poorer areas where hormonal or environmental manipulation of broodstock is impossible, wild fry are still resorted to.

In net-pen culture, crowded and stressful conditions frequently lead to outbreaks of infection. Sometimes the infections result from organisms naturally present in wild fish; in other cases, the disease organism is an exotic one. Salmon net-pen farming provides an example of the spread of exotic pathogens. In 1985, a virulent strain of the bacterium *Aeromonas salmonicida*, which causes the disease furunculosis, was believed to have been brought from Scotland to Norway, spreading to salmon farms and thence to wild salmon and killing large numbers of fish. Bivalve and shrimp farming can also cause disease transmission. Wild broodstock of Pacific white shrimp (*Penaeus vannamei*) infected with white spot disease (WSSV) have been moved to previously disease-free regions while Taura Syndrome, caused by the TSV virus, may have been spread through shrimp cultures in Latin America by the transfer of diseased postlarvae and broodstock. The impact of this introduced virus on its recipient environment is still unknown. The Japanese oyster drill (*Ocenebra japonica*) and a predatory flatworm (*Pseudosylochus ostreophagus*) were brought to American waters along with the Pacific oyster, now the mainstay of bivalve farming in North America. However, these parasites have contributed to the decline of native West Coast oyster stocks. The case of MSX in the USA, Bonamia in Europe provide further examples of poorly managed aquaculture practices. However, a considerable amount of guidelines and legislation relating to disease regulation and control have been developed, such as those of the International Council for Exploration of the Seas (ICES), Network of Aquaculture Centres in Asia-Pacific (NACA), Food and Agriculture Organization (FAO), and European Inland Fisheries Advisory Commission (EIFAC).

The genetic effects of mariculture are varied and highly significant for biodiversity. Unlike many of the other effects discussed so far, understanding genetic effects demands a high level of understanding of the genetic structure of both the farmed and wild populations, something we do not have for any species. The field of fish molecular genetics is just starting to expand rapidly as new analytical techniques become available. For now, predicting the genetic effects of mariculture will remain difficult, and many prognostications may turn out to be wrong. The genetic effects of cultured marine animals are either inadvertent (through escapes of cultured animals) or deliberate (enhancement or sea ranching).

Studies of hatchery populations suggest that such loss of genetic diversity is common (for fish and for invertebrates). Such reduced interpopulation variation is not necessarily bad for cultured populations, but can have a long-term impact on species survival if the farmed stocks intermingle with wild neighbours. This situation occurs when the species being farmed is a local one, and might be called “inadvertent enhancement”. It is best studied in salmonaquaculture. It is known that the use of a smaller number of individuals for breeding programmes would result in inbreeding, crossing of two or more locally adapted populations leads to outbreeding depression because a high level of local adaptation occurs in each population. Though this outbreeding depression usually does not affect fitness in the first generation of progeny, subsequent progeny generations are affected by a reduction in fitness, as has been demonstrated with Pacific salmon in one recent study. The escape of fertile hybrids of closely related species that is being presently being carried out in sturgeon breeding programmes could bring about genetic changes, the effects of which are yet unknown. Another undesirable effect on biodiversity at the genetic level could be the loss of co-adapted gene complexes through repeated inbreeding.

The production of sterile fish is often advanced as a mitigating technology. However, although sterile fish cannot establish wild populations or inter- breed with wild fish, they can still compete with wild fish for food, spread disease, and disturb wild nesting sites. Escaped or released fertile tetraploids may attempt to breed with wild fish and disrupt overall spawning success. Gene transfer (not yet used in commercial mariculture) may have ecological effects if the introduced DNA causes major change in the ecological role of the transgenic fish (by, for example, increasing its size or its ability to use new food sources). Transgenic fish given a gene to speed growth, for example, could out-compete wild fish for food or spawning sites, while fish engineered for cold-tolerance might intrude on the ranges of more northerly species. Unanticipated pleiotropic (multiple) effects may also appear.

Most animals farmed on land are highly domesticated, and without human protection they would likely fail to survive in the wild. Organisms used in aquaculture on the other hand are still relatively wild, and may easily survive and reproduce outside their natural ranges.83 Because much of the world's aquaculture relies on species outside their native range, escapesare a constant biodiversity concern. In the short term, escapes of hatchery species may swamp wild populations through sheer weight of numbers. Skaala (1995) stated that the number of Atlantic salmon (*Salmo salar*) escaping from fish farms in Norway exceeded the number of wild fish harvested in Norway. A comparison of wild and farmed Atlantic salmon showed that farmed fish had higher growth rates and were more aggressive than wild fish, thus posing a threat to native populations that were already depleted by environ- mental factors.

Many alien marine species resulting from escaped cultured stocks have become firmly established far from their native ranges and are culturally accepted as "just more biodiversity". However, when self-sustaining populations of escapes become established, they could interact with native communities in a number of ways, including predation, competition and even elimination of native species. Japanese oyster and Manila clam, for example, are treasured by recreational fishermen on the Pacific coast of North America as well as in Europe. The risk is probably greater with escape of species occupying similar niches to local ones, because they are more likely to interact with native populations and affect their survival. The ability of natural populations to recover from introgression of farmed genes has been very little studied.

AVOIDING THE ADVERSE EFFECTS OF MARICULTURE ON MARINE AND COASTAL BIODIVERSITY

While mariculture has a variety of adverse effects on biodiversity, many of these effects can be mitigated or eliminated. In some cases, it is even possible to produce some positive biodiversity related effects. It is important to mention that mariculture based on allochthonous feed (most finfish and crustaceans) could have larger and more significant adverse effects than mariculture based on authochthonous feed (filter feeders, macroalgae, deposit feeders). The areas offering the most promise for avoiding adverse biodiversity effects of mariculture include reducing waste by better management, changes in nutrition (reformulation of feeds, reduction in use of animal protein, improving utilization) and technological improvements such as "enclosed systems". In such enclosed tanks or ponds, it is possible to treat the effluent in order to avoid outflow of chemicals, antibiotics, diseases, as well as excess nutrients. AnnexII below describes problems, impacts, main mitigation tools, and the results of mitigation.

Better management practices for non-enclosed systems, include:

a) Most importantly, proper site selection. The location of cages, pens, rafts, etc., should ensure proper water circulation to satisfy both the needs of mariculture and the flushing of nutrients and wastes;
b) Secondly, optimal management, including proper feeding to decrease conversion ratios. Proper feeding requires proper training and a good knowledge of the behaviour of organ- isms to be fed. Often workers feeding finfish or crustaceans have poor knowledge of what they do, and the basis of feeding practices. This is true in particular in developing countries. It should be noted that cheap labour often works against biodiversity simply because the lack of proper management knowledge and training investment.

Other mitigation measurements include culturing different species together (polyculture) to make better use of available resources (such as salmon and bivalve culturing or salmon and macroalgae) and coupling mariculture with other activities such as artisanal fisheries and sport fishing. However, all such forms of mitigation are effective only if chemicals and antibiotics are avoided in intensive production.

Enclosed, and especially re-circulating systems require, for many forms of aquaculture, high technological development and capital investment, making the use of such technology impossible for many species and countries. However, future development of mariculture should proceed in this direction in order to minimize impacts of every kind. This is particularly true for the production of fin fish and crustaceans.

Other impacts such as dependence on wild seed, reducing the use of chemical additives and treatments that promote ecosystem changes, and reducing disease transmission between cultured and wild stocks can be avoided with better management practices and other technological improvements, which are discussed here in more detail. For all of the foregoing strategies, development of appropriate policies and legislation is an overarching necessity. Responsible mariculture (codes of conducts, licence permits), policies and regulation should reinforce mitigation measurements.

Mariculture could also be considered as having positive effects when, under certain circumstances, it provides seed for sea-ranching and recovery of wild stocks, endangered species, or even improves productivity and biodiversity.

Effects of mariculture on the environment - Adriatic Sea

Aquaculture is the part of fisheries that deals with the cultivation of aquatic organisms, including fish, mollusks, crustaceans and seaweed. Today, fisheries worldwide are experiencing the peak of aquaculture development as demand for food from marine and freshwater organisms increases and wild sources are limited. With an average growth rate of 6-8% per year, aquaculture is one of the fastest growing food production activities in recent years. Half of the world's supply of aquatic organisms for human consumption now comes from aquaculture.

Most of the world's fishery stocks are currently at or below sustainable exploitation levels. At the same time, global consumption of edible fish has doubled in recent decades, with a corresponding development of aquaculture, which is becoming increasingly important for the supply of fish and other food from the sea.

One of the challenges in existing aquaculture activities and their expected further development on land and sea waters is the availability of space and environmental sustainability, especially in relation to protected areas and the corresponding procedures for issuing permits. This is particularly true for the Natura 2000 network, a network of sites for the conservation of the rarest and most endangered species and habitat types in Europe under the two EU directives, the Habitats Directive and the Birds Directive, which are the foundations of European biodiversity policy.

Aquaculture is the cultivation or rearing of aquatic organisms using techniques that increase the production of these organisms beyond the natural capacity of the environment. Aquaculture in the EU is divided into three subsectors, which have different development histories and characteristics. These are: Shellfish farming, marine fish farming and freshwater aquaculture.

Croatia has a long tradition in aquatic organisms farming. Thus, mariculture, organized oyster farming in Maloston Bay, was mentioned as early as the 16th century, and Croatia was a pioneer in the farming of sea bass and sea bream in the Mediterranean. The first tuna farming in cages in the Mediterranean was started in the Adriatic Sea, and we are still one of the leading countries in the farming of this species. Sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), tuna (*Thunnus thunnus*), mussel (*Mytilus galloprovincialis*) and oyster (*Ostrea edulis*) are nowadays the most important farmed species in Croatian mariculture, which is developing intensively and showing a constant increase in productivity and employment.

Aquaculture in the sea and coastal areas of the Republic of Croatia includes the cultivation of shellfish, mainly mussels and oysters, the cultivation of which is mainly based on concession areas in nature and the use of nutrients from the environment. Marine fish farming takes place mainly in coastal areas, where farming facilities are located in coastal lagoons, and there are plans for farming in tanks and basins on land and farming in the open sea.

The environmental impacts of mariculture can be significant. Increased emissions of organic matter, the source of which is the excreta of farmed organisms and uneaten food, emissions of pharmaceuticals and other substances used in farming, escape of potentially invasive species from farms, and the possibility of disease transmission from farmed organisms to wild populations and vice versa can negatively impact surrounding ecosystems. A prerequisite for sustainable aquaculture development is coordinated spatial planning and zoning of river basins and marine areas, as well as provision of the necessary locations for farming and supporting infrastructure. Sustainable aquaculture relies on scientific research and the application of research results in practice to improve farming techniques and reduce negative impacts on the environment. By developing feeding management, regulating the use of medicines, and modernizing and rebuilding existing infrastructure, economically, socially, and environmentally sustainable aquaculture can be achieved.

Impacts on nature and wildlife and mitigation measures

Different aquaculture production systems can have different impacts on the natural environment, such as habitat loss or degradation, species disturbance and displacement, and changes in local communities. The impacts of different aquaculture systems depend on a number of factors, including the hydrographic conditions at the fish farm location, species of organisms farmed, and production methods, management practices, and more. All of these factors must be considered when assessing potential risks, taking into account the sensitivity or vulnerability of the ecosystem to potential stresses from aquaculture activities.

Table 2. shows the various influences that must be considered when evaluating each production system. It is important to emphasize that the potential impacts listed may not always occur or may not be relevant to the conservation objectives of a particular site. Case-by-case consideration is required to determine the specific potential impacts that depend on environmental and operational conditions, as well as the mitigation measures and appropriate management practices that must be employed to avoid or minimize such impacts.

Table 2. List of impacts to be considered for different types of mariculture.

Type of aquaculture / Possible impacts	Cage Farming	Molluscs Rafts and Lines	Intertidal Molluscs Farming	Bottom Molluscs Farming	Onshore Tanks	Onshore Pools	Lagoon Farming
Sedimentation	X	X	X	X		X	X
Biogeochemical changes in water	X	X			X	X	X
Intake of chemicals	X				X	X	
Impact of infrastructure			X	X	X	X	X
Harassment	X	X	X	X	X	X	X
Predator control	X	X	X	X	X	X	X
Crossing between species	X				X	X	
Transmission of pathogens	X		X	X	X	X	X
Foreign species*	X	X	X	X	X	X	X

Also, various guidelines and application of best practices that contribute to biodiversity conservation provide a number of concrete suggestions for conservation measures that should be adopted for all aquaculture systems:

- in marine cage farming, controlling and limiting breeding density can reduce potential impacts from organic waste particles, while improving feed digestibility and food waste reduction systems can also mitigate these impacts;
- appropriate placement of rafts and ropes for shellfish aquaculture in areas with good water exchange, as well as appropriate sizing of aquaculture facilities using predictive models that allow assessment of the footprint of benthic loading, can reduce the most significant potential impacts of these systems;
- for systems with onshore marine tanks, a potential mitigation measure is to allow the source water to be microfiltered, treated, and purified by a treatment system prior to discharge to the lagoon-sea connection channel to allow microalgae to take up nutrient particles.

Proper implementation of relevant national and EU regulations can prevent or minimize most potential aquaculture pollution and impacts. In addition, aquaculture operators are voluntarily making significant efforts to implement good management practices (e.g., code of conduct, monitoring, certification), and the EU is promoting organic aquaculture. The introduction of foreign species for aquaculture is regulated by Regulation No. 708/2007.

Aquaculture, apart from a possible harmful effect (Petar Kružić: Mariculture is necessary, but it's a dirty industry; www.agrokultura.com), can also have positive effects on the environment, including Natura 2000 sites (Quidance on Aquaculture and Natura 2000; https://ec.europa.eu/environment/nature/natura2000/management/pdf/guidance_on_aquaculture_and_natura_2000_en.pdf), by providing ecological benefits and services and supporting suitable habitats for species of EU and HR interest.

For example, shellfish aquaculture provides ecosystem services by removing inorganic nutrients from polluted ecosystems (bioextraction, environmental bioremediation). Mussel farming serves as a method of water quality management in areas with diffuse nutrient input (e.g. BlueBio project MuMiFaST, https://bluebioeconomy.eu/mussel-mitigation-feeds-and-supply-system-technological-development/). Currently, there is no integrated multitrophic aquaculture (IMTA) with algae farming in the Republic of Croatia. Fish farming is mostly combined with mussel farming, where mussels are farmed to compensate for nutrient enrichment from fish feeding, e.g., the fish and mussel farm in the Budava Bay and earlier in the Lim Bay (Cromaris d.d. and Istrida d.o.o.; SUO Fish farms in the Limski Bay and Budava, IOR, Split, 2008, 2010).

Overview of indicators - Impact of mariculture on ecosystems

The authors Cvitković et al. (2005) in the report - Impact of mariculture on ecosystems (data source Institute for Oceanography and Fisheries, Split, http://baltazar.izor.hr/azopub/indikatori_podaci_sel_detalji2?p_ind_br=1E10&p_godina=2005) as part of the MSFD indicator, summarizes:
In mariculture fish farming, the greatest environmental impacts occur during the cage feeding phase. As mariculture fish farming, particularly tuna farming, has proliferated over the past decade, there has been increasing controversy over the extent of its impact on the environment and whether it is justified from a profit perspective. Fish farming results in a significant input of organic matter and nutrients into the environment, which affect the ecosystem in a similar way to eutrophication and are studied using similar parameters (Table 3).

Table 3. Common parameters investigated in studies on the environmental impact of caged fish farming.

SEA SURFACE AND WATER COLUMN	SEDIMENT	BENTOS COMMUNITIES
Smell Fat stains Transparency Suspended matter Temperatures Salinity Oxygen pH Nutritiens	Granulometry Carbonates Redox potential Organic matter Organic carbon Total nitrogen Inorganic phosphorus Organic phosphorus	Composition Distribution

The impact on the environment depends on the type of farming organisms, the method of cultivation, the amount or density of livestock, the type of food, the hydrographic characteristics of the site, and farming skills and practices. According to global studies, of the total amount of phosphorus, carbon, and nitrogen ingested, about 85% P, 80-88% C, and 52-95% N are believed to enter the environment through uneaten food, fish excreta, and feces. Various global studies have shown that about 23% of carbon, 21% of nitrogen, and 53% of phosphorus from feed introduced to the farm enter sediments, with significant impacts within 1 km of the farm. Nitrogen, phosphorus, and carbon are elemental substances whose accumulation in the sediment determines the fate of the benthic organisms of the sediment bottom. Model studies of tuna farming have shown that on the Croatian coast the zone of influence on the sediment is within a radius of up to 500 m from the center of the farm (Table 4).

Table 4. Inputs (t/year) of nitrogen and phosphorus to the Adriatic Sea from whitefish and tuna farming calculated with the model. These amounts correspond to the equivalent uptake of nitrogen and phosphorus in the municipal wastewater of a city with 387,500 inhabitants (nitrogen) and 179,500 inhabitants (phosphorus).

	WHITE FISH	TUNA	TOTAL
Dissolved nitrogen	364	975	1339
Particulate nitrogen	28.65	27	55.65
Total nitrogen	392.65	1002	1394.65
Dissolved phosphorus	15.7	20	55.7
Particulate phosphorus	15	1.1	16.1
Total phosphorus	50.7	21.1	71.8

Nitrogen and phosphorus levels, and thus impacts on the benthos, decrease exponentially with distance. However, below the cages and in close proximity, the impacts of caging fish on benthic organisms are significant. The greatest risk of caging occurs when rare and endangered organisms live in the area affected by the farm or when there are rare formations that create certain organisms, such as a coral reef. In practice, however, one of the greatest threats of cagefish farming to the environment is its impact on Posidonia oceanica populations. Posidonia oceanica is an endemic Mediterranean marine flowering plant that grows almost from the surface to a maximum depth of about 45 m, forming dense underwater meadows (Figure 1). The insufficient amount of available light is the main reason why the plant cannot develop at greater depths. In coastal areas, it is most prevalent at depths of 25 to 35 m due to lower transparency. Posidonia settlements are the most species-rich areas in the Mediterranean. This plant, together with epiphytic algae, is the main producer of oxygen and organic matter in the coastal area. Its meadows protect the coast from erosion by retaining sediments and reducing wave energy.

Figure 1. The natural settlement of *Posidonia oceanica*, which represents the area with the highest biodiversity in the Mediterranean Sea, and the drastically degraded settlement as a consequence of tuna farming in the immediate vicinity (Cvitković et al., 2005).


Any destruction of their meadows is an almost irreversible process for the human understanding of time. In fact, the stems (rhizomes) of Posidonia grow on average about 1 cm per year, so it can take several centuries to restore a settlement only ten meters in diameter. A study of the impact of cage farming of mullet and sea bass in Spain (El Hornillo Bay), where production reached 700-800 tons of fish in 30 cages (each 20 m in diameter) on an area of 7 ha, showed that within ten years 11.29 ha of Posidonia meadow were completely destroyed and another 9.86 ha were significantly damaged.

Impacts to the Posidonia colony may also be physical, such as the farm's anchor blocks and chains mechanically damaging the colony. Physical shading caused by the construction of the farm and reduced transparency of the sea near the farm due to eutrophication may negatively affect photosynthetic processes in the deepest parts of the meadow. Due to increasednutrients in the sea column, the development of epiphytes on Posidonia leaves (organisms that grow on the leaves) also increases, further reducing the amount of light available to the plant. Increased development of algae due to nutrient salts is accompanied by increased development of herbivorous organisms, particularly sea urchins, which then also gnaw Posidonia leaves to a significant extent. Increased sedimentation buries slow-growing rhizomes (stems). The development of anoxic conditions in the sediment and the generation of toxic gases negatively affect physiological processes in the plant. In any case, caging fish in close proximity to or over Posidonia meadows is certain to cause significant and nearly irreversible damage to the plant. Since *Posidonia oceanica* is widespread in the Croatian coastal area, there are few farms that are not in close proximity to the plant. This has resultedin a localised impact on meadows, although the overall extent of the impact is unknown.

Research on impacts to rocky meadow algal communities conducted in Croatia has shown that complex changes occur within the algal community in the vicinity of fish cage farms. Altered ecological factors allow the development of algal species that are atypical for these areas, and certain species develop in unusually large amounts. For example, the increased amount of organic material can lead to the development of nitrophilous algal species such as the green alga *Ulva rigida* (sea lettuce) or the brown algae *Colpomenia sinuosa*, *Scytosiphon lomentaria*, and members of the order *Ectocarpales*, which are typical organisms for areas polluted with organic material. In some areas, the unusual development of benthic diatoms has also been noted, forming filamentous coverings up to 1.5 m in diameter on the seafloor at depths of 10 to 20 m and completely covering sessile, immobile organisms. Their unusual development was also noted in the intertidal zone, where they formed slimy brown isthmuses. Within the algal settlement and near the fish farming cages, there is often an extraordinary increase in the number of sea urchins *Paracentrotus livid*us and *Arbacia lixul*a. These sea urchins not only reproduce, but also spread to greater depths than in their usual range. Numerous urchins often completely overgrow the algae, and if they are near a Posidonia meadow, they spread within the settlement of this flowering plant and do considerable damage there. It is not clear why urchin numbers often explode near farming sites, but the reasons may be complex.

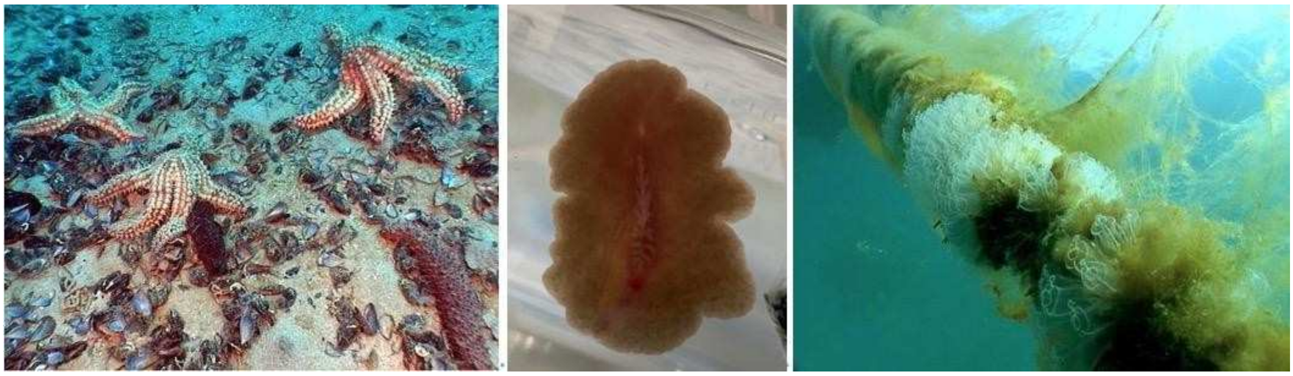
The effects of caged fish farming on benthic organisms have also been observed in Croatia on sedimentary bottoms where a *Posidonia oceanica* meadow has not developed. Such studies are usually conducted at depths of 35 to 50 m. However, due to the difficult and relatively dangerous diving at these depths, such surveys are conducted only sporadically. The greatest impacts are observed just below the cages. A survey of a tuna farm located far from shore at a depth of 54 m and in an area with strong currents showed that the seafloor changed significantly despite all the favorable conditions. The sediment became muddy and was full of fish food debris (bivalves and bones). Immediately above the seafloor was a large amount of suspended sediment in the seawater column, so that almost no sunlight visible to the eye reached the bottom, even though there should be good visibility at this depth during the day. A considerable amount of uneaten food that fell to the bottom favored the development of numerous scavengers such as solitary crabs of the genus *Pagurus*.

In the neighboring farm about 2 km away at a depth of about 52 m, the composition of the predominant benthic species was completely different. In addition to individual crustaceans, detritophagous carp were extremely abundant here, and the sediment was sandier. This suggests that ocean currents played a greater role along this bottom, and suggests that small differences in environmental factors can lead to a completely different environmental response. Decomposition of a large amount of organic particles and uneaten food on the seafloor consumes more oxygen, which can lead to the development of hypoxic and anoxic conditions (conditions without oxygen) and the generation of toxic gasses such as hydrogen sulfide. The development of anoxic conditions and hydrogen sulfide is also indicated by the white coating of the sulfur bacterium *Beggiatoa* sp. that often occurs in the area affected by the farm.

Plants often exhibit heavy growth of invertebrates and algae. The invertebrates are mainly filter feeders mollusks such as mussels *Mytilus galloprovincialis*, tunicates, bryozoans and polychaetes. Their removal results in additional organic loading to the seafloor. Although cleaning of the cages is done at regular intervals, a large amount of organic material accumulates on the bottom of the cages in a short period of time. The occasional shedding of these organisms, especially the shells, favors the development of carnivorous organisms on the bottom below the cage, such as the starfish *Martasterias glacial*is (Figure 2).

Figure 2. Unusual numbers of starfish (*Martasterias glacialis*) feeding on the mussel *Mytilus galloprovincialis*, which reaches the bottom by falling from the structure; the appearance of the

opportunistic predator flatworm (*Imogene mediterranea*); the abundant development of brown filamentous algae (*Ectocarpus* sp.) and ascidian tunicate (*Clavelina lepadiformis, C. oblonga*) on the ropes of breeding cages for farming sea brem (*Sparus aurata*).



Restoration of damaged algal communities and sedimentary bottoms with the marine flowering plant *Posidonia oceanica* is much faster than restoration of settlements without it. However, research on restoration of such communities is extremely scarce, and there are no clear data. Based on some studies on algae of the genus *Cystoseira*, which are the most important macroelement of our benthic vegetation on rocky bottoms, and studies on the recovery of communities on the muddy bottoms of the northern Adriatic Sea after the anoxia period, we can assume that it would take about 10 years for such communities to recover after the end of the influence of the farms.

Caging leads to the closure of traffic routes, and there is also a conflict of interest with tourism. Greasy stains, intense fish smell, numerous sea urchins, slimy coatings of benthic diatoms in the intertidal zone, and numerous seagulls polluting the coast with their excrements are the reasons that prevent the normal development of tourism in the area of fish cage farming.

The almost irreversible destruction of Posidonia settlements must be avoided, and the impact on the algae and sediment communities, the conflict of interest with tourism and the economic benefits that fish farms have for the local community must be in the most favorable relationships of sustainable development.

EFFECT ON NATURAL POPULATIONS OF ORGANISMS

Cage farming undoubtedly generates a great deal of waste that can impact the marine environment, calling into question the long-term sustainability of the farming process itself. Of the total nutrients fed in marine fish farms, it is estimated that only about 30% of the nitrogen and up to 40% of the phosphorus is absorbed into the fish biomass (Neori and Krom, 1991). Improvements in food composition and diet have significantly improved these ratios in favor of the environment over the past 15 years (Katavić, 2003; 2006).

Uneaten food, excreta, and feces are the main wastes that end up in the marine environment during the farming process. Food loss is estimated to be as high as 10% when fed entirely and as high as 40% when fed freshwater. Suspended organic matter typically accounts for 10 to 12% of ingested feed (calculated on a dry weight basis). Fecal matter and uneaten feed significantly increase carbon, nitrogen, and phosphorus levels in sediment, especially in the immediate vicinity of the cage (Katavić, 2003; 2006). In addition, caging has major negative impacts on natural organism populations, especially on wild fish populations. Namely, the impacts occur when individual fish manage to escape from the cage and mate with individuals from the natural population, resulting in recombination of genetic material between these two cultivars. The recombined individuals can have positive and negative effects on the population as well as on the entire ecosystem. Recombination only accelerates the natural path of species evolution, which in most cases leads to the extinction of one fish type and the dominance of another. To get around this problem, it is proposed to create monosex and triploid populations so that interbreeding cannot occur in the first place, because in nature all odd polyploids are mostly sterile and all even ones are mostly fertile.

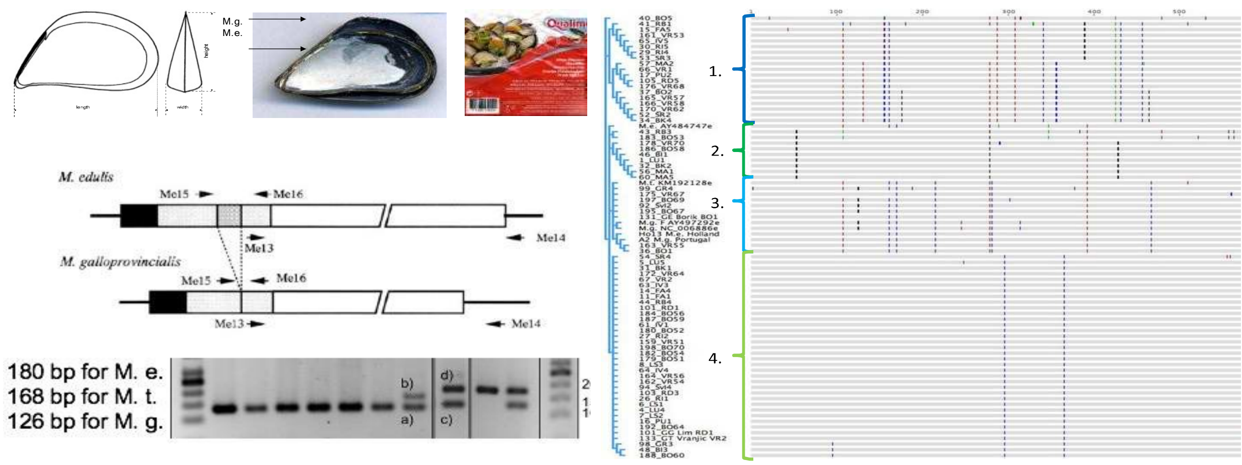
Research on fish defections in the Republic of Croatia

Katavić et al. (2013) conducted research in cooperation with the Croatian Sports and Fishing Association. They made a study in which they determined the genetic structure of original and natural populations of species of interest for sport-recreational fishing and mariculture along the Adriatic coast (goblin, toothfish, sea bass and bream). The results showed considerable genetic similarity between natural populations of sea bass from Piran Bay and breeding populations of Italian origin sampled also in Piran Bay, suggesting genetic interactions of defectors with local populations. The results also showed considerable genetic similarity between the breeding population from the island of Ugljan, whose broodstock is of Adriatic origin, and the wild population from the waters of Makarska, which indicates quality genetic management of the broodstock with a preserved high level of genetic diversity and a low rate of inbreeding. their results show a genetic difference between the wild populations of the central and northern Adriatic, which indicates a reduced gene flow between them, and a strengthening of the genetic structuring of populations in different parts of the Adriatic.

In the research conducted as part of the thesis (Relić M., 2015, Differences between wild and by selective breeding modified farmed sea bass *Dicentrarchus labrax* L. 1758 on the west coast of Istria), the distinguishing phenotypic characteristics of wild and farmed sea bass werestudied. A total of 200 specimens were processed, 100 wild and 100 farmed, collected in autumn and winter 2014/2015 in the above mentioned area. The tied network method and morphometric indicators were used to determine the origin of the individuals. The results showthat the biggest difference between wild and cultivated sea bass is in the head area. Wild seabass have a higher head index, which was also evident from the results of processing the dataof the tied network method. In addition to the difference in head area, the results also showeda difference in tail shaft measurements. Based on these differences, the origin of all 200 individuals was determined. Based on the variables described, discriminant analysis classifiedthe cultivated animals with 100% accuracy according to the original distribution and cross- validation, meaning that there were no fish escapes determined. Through personal communication with sport fishermen, the above research results were confirmed, and it was found that it is relatively easy to distinguish farmed and wild sea bass, with farmed specimensbeing shorter and stockier than wild specimens of the same weight. Wild sea bass are much longer and slimmer, and this difference is most obvious in fish of the consumption size ca 500 g.

Regarding the characterization of farmed and wild populations of bivalves, the study by Hamer et al. (2012), using morphometric and genetic methods (nuclear marker Me15/16), found that the species *Mytilus galloprovincialis* predominates along the Croatian Adriatic coast. Further research on characterization of maricultured mussel stocks in Istria County confirmed that there are no hybrid heterozygotes (*M. galloprovincialis* - *M. edulis*). As mtDNA barcoding analysis does not give clear results in the case of complex species of *Mytilus edulis*, since *M. edulis, M. galloprovincialis and M. trossulus* can still produce offspring when present at the same site. The nuclear marker PreCoID was developed to distinguish between these three mussel species and the Adriatic populations (Figure 3).

Figure 3. Me15/16 and barcoding analysis of the PCR mtDNA COI product of the Adriatic mussels.



Preliminary genetic analyzes of Adriatic mussels indicate the similarity of populations-individuals sampled in the Croatian part of the Adriatic (Hamer et al. 2012; Wenne et al. 2022) and the deviation of the size of the PCR fragment by analysis of the PreCoID nuclear marker of mussels sampled in the area of Venice. What can it mean that in the mussel breeding areas of Chioggia, for example, the specimens or genes of the Atlantic mussel species *M. edulis* has been introduced?

Based on the literature and our own research, we can conclude that mariculture can have a significant impact on the environment, depending on the species chosen and the method of cultivation. In general, conservation needs to preserve an area, including biodiversity, can be reconciled with sustainable aquaculture development by preparing high quality environmental impact studies, limiting the number of cages per farm, proper site selection, and conducting adequate monitoring.

A case study of recent invasive species appearance in Istrian shellfish production areas

The Region of Istria activities with the 1 set of pilot guidelines for biodiversity protection related to marine aquaculture science aimed to evaluate the current state of knowledge on effects of mariculture on marine and coastal biodiversity with a local case study of recent invasive species ascidian tunicate (*Clavelina oblonga* Herdman, 1880) appearance in Istrian shellfish production areas by proposing a local/regional protocol for aquaculture threats monitoring.

Shellfish/fish farmers

The regional aquaculture sector consists mainly of mussel farming *Mytilus galloprovincialis*, oyster farming *Ostrea edulis* (6 production areas) and fish seabream *Sparus aurata* - seabass *Dicentratchus labrax* (1 production area) on the west and south coast of Istria.

From the point of view of shellfish/fish farmers, the establishment of the monitoring protocol is really necessary in order to survive on the market, to maintain production and profit, and to reduce the cost of additional, increased staff and resources.

Mariculture operators

From the regional context e.g. Region of Istria, Administrative Department for Agriculture, Forestry, Hunting, Fisheries and Water Management; Center for the Development of Fisheries and Aquaculture of the Istrian County; LAG/FLAGs are interested in keeping good environmental quality and socio-economic sustainability with possible increase of healthy food production for local consuming and tourism purposes.

Scientific institutions

A list of regional scientific institutions (Center for Marine Research of the Ruđer Bošković Institute; Center for Invasive Species of the Institute of Agriculture and Tourism; Department of Marine Biology and Oceanology of the Faculty of Natural Sciences in Pula) with contacts forthe implementation of the Protocol through systematic data collection and applied research onthe occurrence of invasive species and threats in aquaculture was prepared.

The mentioned regional scientific institutions (scientists), besides basic research interest, have the possibility to cooperate with mariculture operators and shellfish farmers through joint applied projects/research.

Description of the content of the guideline/protocol

In 2015, a new invasive species of the ascidian tunicate *Clavelina oblonga* was observed in the Bay of Trieste and in the Savudirjska Vala (Mioković, 2016) (Figure 5.3.4-1).

Figure 5.3.4-1. In 2015, a new invasive species of the ascidian tunicate *Clavelina oblonga* was observed in the Bay of Trieste and in the Savudirjska Vala (Mioković, 2016)

Few years later, it was obvious that the intensive *C. oblonga* fouling is affecting only mussel production areas and spreading at shellfish farms along western and southern coast of Istria (Majnarić et al., 2022; Hamer et al., 2022) (Figure 5.3.4-2). With some exceptions, shellfish farmers avoid addressing this issue for fear of negative information and potential negative impact on the sale and distribution of their products.

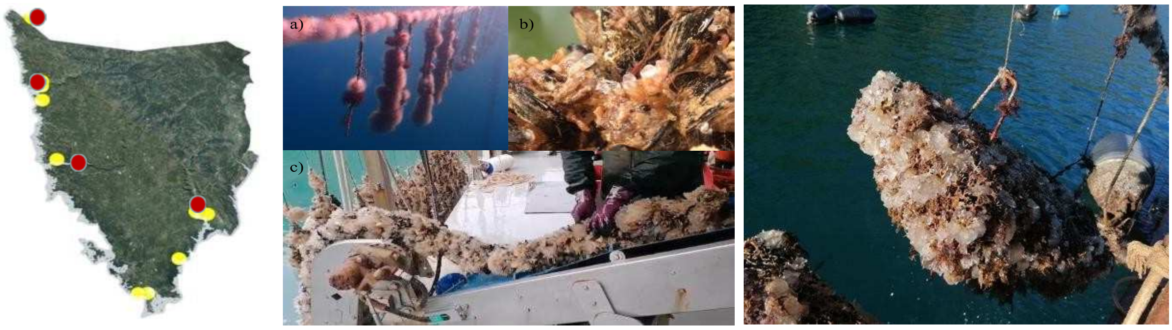


Figure 5.3.4-2. Intensive *C. oblonga fouling* is affecting only mussel production areas - shellfish farms (e.g. Lim Bay) and it is spreading along western and southern coast of Istria.

Apart from individual cooperation between shellfish farmers and regional scientific institutions and mariculture operators, there is generally no official information and no systematic data collection on the occurrence of invasive species and other threats in aquaculture in the Istrian region.

As the first step in the creation of the protocol was the definition of shellfish farmers / contact persons as unofficial representatives of the six main shellfish production areas (Savudrijska vala, Vabriga, Lim Bay, Medulin Bay, Budava and Raša Bay) in the Region of Istria.

Then the regional mariculture operators (Region of Istria, Administrative Department of Agriculture, Forestry, Hunting, Fisheries and Water Management; Center for the Development of Fisheries and Aquaculture of Istria County; LAG /FLAGs) and scientific institutions (Center for Marine Research of the Ruđer Bošković Institute; Center for Invasive Species of the Institute of Agriculture and Tourism; Department of Marine Biology and Oceanology of the Faculty of Natural Sciences in Pula) were identified.

Instead of a direct link between farmers and scientific institutions, the newly established Center for the Development of Fisheries and Aquaculture of the Istrian County is envisioned as an important intermediary.

In drafting a protocol on actions to be taken in the event that shellfish farms are affected by threats, such as the recent occurrence of the invasive alien species *C. oblonga* in the shellfish farming areas of the Region of Istria, we took into account local public bodies and institutions, but with the interests of shellfish farmers in mind.

The protocol includes the logical transfer of the relevant information to the decision makers in terms of reporting, recording and documenting the threat, the damage caused, the additional costs for the farmers, the proposed measures, the subsidy and/or compensation from the relevant institutions and the available funds.

Recent threats and occurrence of invasive species in aquaculture

In the Adriatic Sea, tunicates are a common occurrence. Some non-native species are invasive and can cause both ecological and economic losses. An intensive ascidian invasion *C. oblonga* fouling was observed in the summer of 2020-2022 at shellfish farms on the western and eastern coasts of Istria, Northern Adriatic, Croatia (Figure 5.3.4-2).

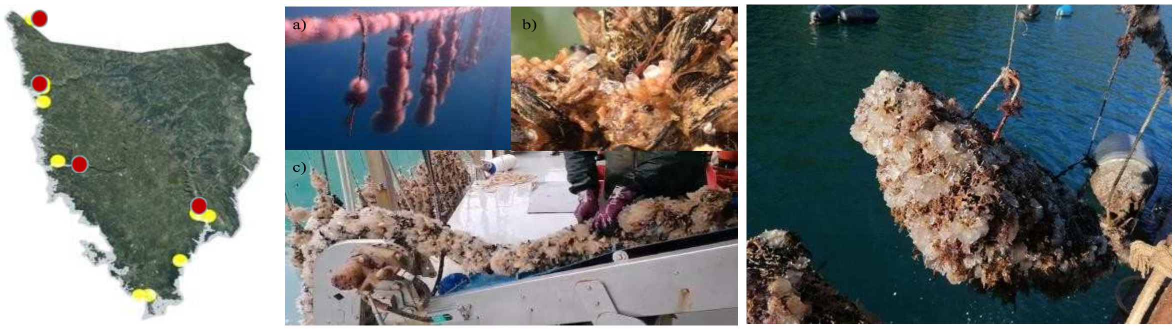


Figure 5.3.4-2. Intensive *C. oblonga fouling* is affecting only mussel production areas - shellfish farms (e.g. Lim Bay) and it is spreading along western and southern coast of Istria.

The major impacts reported were associated with the bivalve industry, while aquaculture facilities create an ideal environment for ascidians, with abundant suspended food, unlimited locations and surfaces for attachment. *C. oblonga* physically interferes with the opening of mussels valves, competes for food resources, impedes food and oxygen procurement by reducing water flow and increases the weight on load-bearing infrastructure (holding nets, cages, ropes and buoys).

According to our own observations and research in the area of the Vabriga, Savudrijska vala, Lim and Raša bays (2020-2022), we can say that colonies appear during May, and are maximally developed in September-October. We conducted surveys in October 2022 to estimate the approximate invasion intensity and sampling for experimental exposure to reduced salinities. For instance, in Lim Bay *C. oblonga* colonies occupied on average ca. 25-100% of space on the mussel nets and 10-50% of other shellfish farm infrastructure, depending on locations.

Further, during the winter, the colonies gradually disappear. The growth of this tunicate is correlated with the growth of temperature and chlorophyll a in seawater. This is still a tropical species and in its natural habitat temperatures do not fall below 10°C, but this group often undergoes regression during the winter months and survives in the form of buds from which zooids sprout again when living conditions become favorable.

Invasive alien species *C. oblonga* possible eradication measures in mariculture

It is known that once the farmers in the Lim Bay moved the mussels to the very beginning of the bay and left the installations exposed to the brackish sea water for the treatment to destroy the fouling organisms. Our experimental exposure of sampled *C. oblonga* colony zooids to different salinities showed that throughout the observation period (14 days of exposure to lower salinities and 14 days of recovery) there was no sexual reproduction, while salinities 20 and 11 caused disruption of feeding and feeding itself, resulting in color change and necrosis of the tissues and eventual disintegration and fragmentation of the body (Figure 5.3.4-3).

Figure 5.3.4-3. Susceptibility of invasive tunicates *Clavelina oblonga* to reduced seawater salinities exposure as a possible environmentally friendly eradication measure: different salinities treatment of A) 37, B) 30, C) 20 and D) 11 (upper row), and after 14 days recovery at 37 salinity (lower row).

In general, based on our preliminary results and the available literature, we can approximate and conclude that translocating mussel/shellfish installations with the tunicate *C. oblonga* and exposing them to reduced salinity <20 for 7-10 days would be a possible natural way to eradicate this invasive species (Majnarić et al., 2022).

Appearance of other threats (unusual abundance or nonnative species)

Due to the appearance of the invasive species of ascidian tunicate *C. oblonga*, as a related species, the flatworm *Imogine mediterranea* appeared in unprecedented numbers, which is obviously favored by intensive tunicates fouling. In cooperation with the scientists, it was established that the mentioned flatform also attacks healthy mussels, i.e. it does not feed only on weakened mussels due to intensive fouling, which represents an additional threat (Hamer et al., 2022) (Figure 5.3.4-4 and 5.3.4.5).

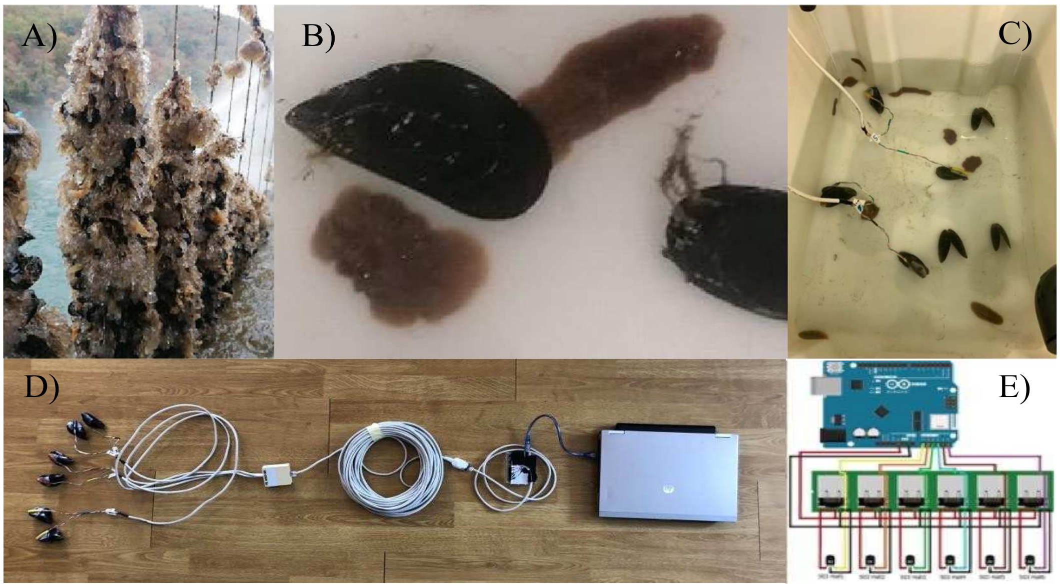


Figure 5.3.4-4. A) Mussel shellfish farm infrastructure threatened by *C. oblonga* invasion; B) predation of mussels by flatworm *I. mediterranea*; C) experimental setup; D) VGMM with 6 sensors/mussels; E) Arduino microcontroller and VG Hall sensors connection schema.

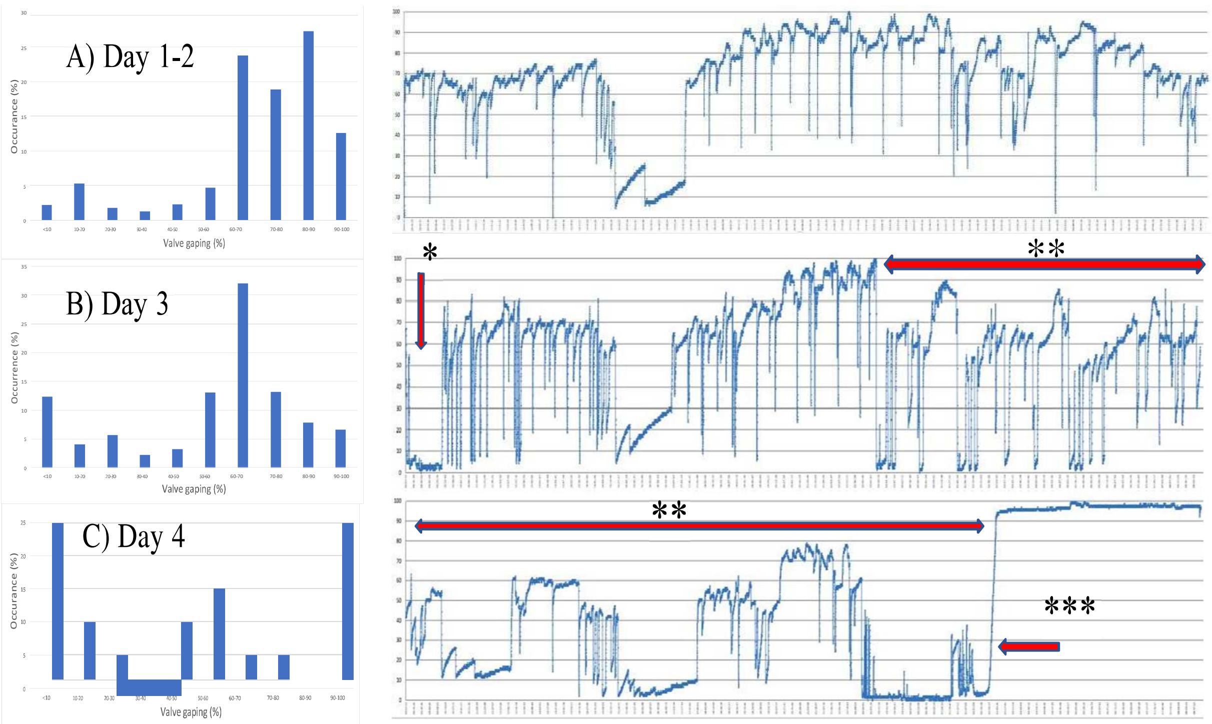


Figure 5.3.4-5. Real time observation of mussel predation by flatworm *I. mediterranea* using valve gaping mussel monitor: A) Day 1-2, mussel acclimation; B) Day 3, beginning of mussel exposure to flatform (*); C) Day 4, first attack and mussel response to inner predation (**) and mussel death (***). Bar graphs represent cumulative daily mussel VG percent occurrence (24 h, 9:00 – 9:00 h).

Outcomes at environmental and socio-economic levels

While mariculture has the potential to provide a sustainable source of seafood and reduce pressure on wild fish populations, it can also have negative impacts on the marine environment and biodiversity (Report on effects of aquaculture on marine ecosystems: overview of indicators, IOR Split, 2005; http://baltazar.izor.hr/azopub/indikatori_podaci_sel_detalji2?p_ind_br=1E10&p_godina=2005).

One of the negative effects of aquaculture on marine ecosystem is possible spreading of invasive alien species e.g. recent abundant occurrence of ascidian tunicate C. oblonga in mariculture production areas in Region of Istria. The introduction of invasive species most often takes place through ballast waters, fouling on ships, then the construction of ports, marinas or sea passages, or certain activities in mariculture and scientific research.

Outcomes at environmental level

The intense presence of overgrowth-biofouling communities in aquaculture increases production costs while decreasing product value, as well as the implementation of regulatory restrictions to reduce the risk of spread to unaffected areas. Further, fouling community dominated by *C. oblonga* led to the loss of alternative community states and reduced species diversity.

It appears that *C. oblonga* is currently in the dangerous stage of introduction in western and eastern Istrian aquaculture areas, and its further spread is expected. Such colonization may potentially cause severe impacts depending on local environmental conditions. Until now its occurrence in natural habitats outside aquaculture areas was not observed in the region.

In coordination with shellfish farm owners and Region of Istria, further monitoring of *C. oblonga* occurrence, distribution and abundancy is organized, as well as salinity and temperature continuous measurement using data loggers. Because it was determined that there is a lack of information and no systematic data collection on the occurrence of invasive species and threats in aquaculture in the Region of Istria.

Outcomes at socio-economic levels

While observed C. oblonga biofouling decreases mussel growth and production efficacy, at the same time it increases production costs by demanding additional mechanical cleaning efforts and time. There is still a lack of information on the exact damages, costs and lost profit in mariculture caused by the recent huge threat - the spread of the invasive species of the tunicate, *Clavelina oblonga* in shellfish farms in Region of Istria.

Beside invasive alien species awareness and negative impact, intensive fouling of this ascidian tunicate is also possible opportunity for local mariculture industry, by using this biomass for bioactive compounds bioprospecting, cellulose and feed production.

By implementation of the Protocol on actions in the case that the area of interest is affected by the threats in aquaculture in the Region of Istria mutual benefit for all operators is expected, including common applied projects/research application (Figure 5.3.4-6).

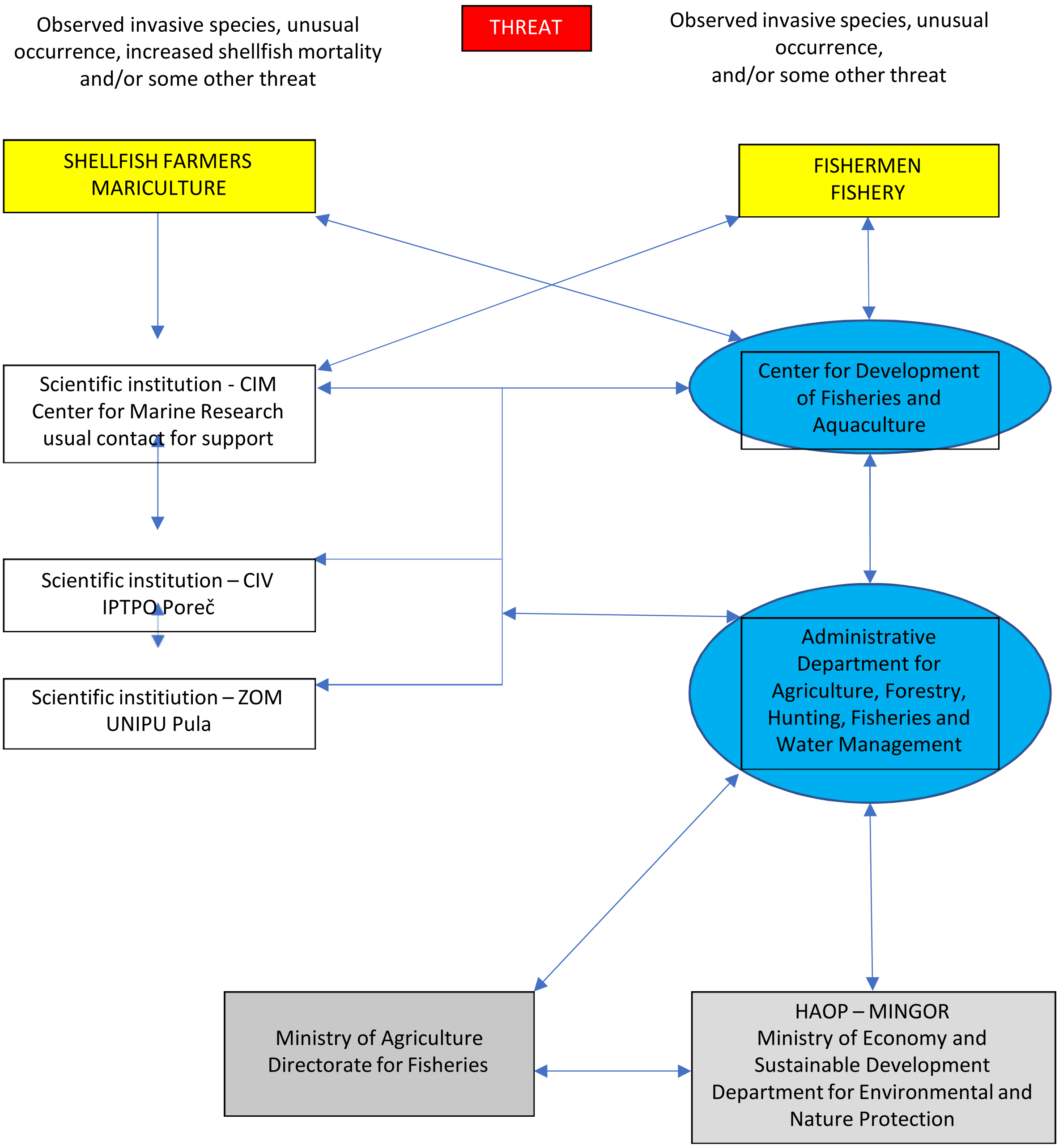


Figure 5.3.4-6. Schematic representation of the Protocol - how to proceed in the case that the

area of interest of the Region of Istria is affected by threats in aquaculture.

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