



Integrate Aquaculture:
an eco-innovative solution to foster
sustainability in the Atlantic Area

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Land-based IMTA in S-European Atlantic Area – definitions, characteristics and possibilities

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1 Introduction

Slide 2
Contents

In this part of the course on Integrate Multitrophic Aquaculture (IMTA), we will be concentrating on land-based IMTA systems. Specifically, it is intended to focus on the application of the IMTA concept to earthen ponds systems. These systems, remains of former salt evaporation ponds and many of them later converted to fish farms, are common along the coasts of Portugal and the Atlantic coast of Spain, but can also be found in other countries in Europe and other parts of the world. In this first course, elaborated by CTAQUA, we will provide a short introduction to these systems, their geographical and historical aspects, and general functioning. In the second part of this contribution, we will focus on the pilot experiment CTAQUA is carrying out in the earthen ponds, explaining the set-up, research method, and expected results in this pilot study.

2 Definition IMTA and land-based IMTA

Slide 3
Definition

At first let us shortly repeat what we understand under the concept of IMTA. You will find a more elaborate explanation in the presentation of the other partners.

An IMTA system is an integrated culture of organisms of various species belonging to different trophical levels in the same water body (but not necessarily at the same time), thereby guaranteeing a functional relation between the levels, i.e. an exchange of matter and/or energy among them. This is illustrated in this well-known conceptual diagram by Chopin et al. (2008) with three trophical levels. At the basis are the carnivores, usually fish that receive feed. Nutrients coming from fish excretions and feed rests will stimulate microalgae growth that serves as feed for bivalves (filter feeders), which will also feed on the feed rests directly. Finally, the nutrients from excretions from both fish and bivalves serve as a food source for seaweed cultures (primary producers).

Slide 4
Categories

As we have seen in the other presentations, different IMTA systems can be distinguished. Basically, two main categories exist: open systems and closed systems, that are nearly always (but not necessarily) land-based. In the open systems, different integrating cultures are placed in the same water body in the vicinity of each other. Typically, these include cage cultures (fish, other animals) and long lines for bivalves and seaweeds. Closed systems come in various types and sizes and range from semi-natural, for instance the earthen-pond type we will discuss in this course, or completely controlled and constructed, i.e. land-based tanks.

Slide 5
Comparison

Considering the management of these systems, one of the main differences between them is the degree of control that can be exercised on the exchange of energy and matter and the degree of contact with the environment. Open systems are in direct contact with the water, they form so to say a part of the environment. For closed systems, on the other hand, the contact is less direct and is regulated through the in- and outflow channels or pipes. This also indicates the amount of control on the exchange of energy and matter we will be able to exercise on these two systems. Control is of course minimal in open systems as this is basically driven by wind and tidal currents, whereas in closed systems the degree of control is much larger up to total. On the other hand, the energy required to run the system is generally considerably higher in closed systems, especially when pumping is involved.

Slide 6
Maps

As said, in this part of the course we will focus on closed, land-based systems. More specifically, we will be talking about the practice and possibilities of IMTA in earthen ponds in transformed salt marsh systems that are common of southwest Europe, both along the Atlantic and the Mediterranean coasts. An inventory of the most important systems along the south Atlantic coasts of Portugal and Spain was made in the framework of the project AQUA&AMBI (Support to wetland management in the southwest Iberia coastal zone: interactions between Aquaculture and Environment in the Euroregion Alentejo-Algarve-Andalusia) led by Integrate partner IPMA in which CTAQUA collaborated as well. As can be seen from the maps in this slide, the main areas where these systems can be found are in the Ria Formosa area in the Algarve,

Portugal, with some other sites more scattered in the west of the country. For Spain, important zones include the western part of the Huelva province bordering Portugal, and the area around the Bay of Cádiz. These zones are large wetland areas, harbouring a large biodiversity and of particular importance for migrating birds. Therefore, these zones are important stepping stones in the Natura 2000 network.

3 Short history of earthen ponds

Earthen ponds are actually transformed salt evaporation ponds. Salt production used to be an important economic activity in south of Spain and Portugal, however, around the 19 thirties for various reasons this activity started to decline and many ponds were left abandoned. This changed by the seventies of last century, when the growing population caused an increased demand of seafood that traditional fisheries could not meet. Slowly but steadily the former salt evaporation ponds were increasingly being transformed to be used for the cultivation of the fish, shrimps and other species that entered these ponds with the incoming seawater at high tide.

Slide 7
Companies

Initially, cultivation was limited to fattening of the individuals that had entered the systems spontaneously. However, by the mid to late eighties, technological advancement based on scientific studies led to the use of the ponds for the production of fry and fingerlings of the species sea bass (*Dicentrarchus labrax*) and gilt-head sea bream (*Sparus aurata*). In addition, the ponds were increasingly being used for the production of other fish species as well as bivalves as mussels and (Japanese) oysters. Currently, along the Andalusian Atlantic coast more than 7.000 ha of salt ponds is being used for aquaculture by more than 70 companies.

4 General functioning and uses of earthen ponds

Slide 8
Functioning

As said, originally these earthen pond systems were used as salt evaporation ponds. Nowadays this can still be seen from their general structure. In the following we will shortly describe the main structure of these ponds and the potential they offer for integrated aquaculture.

Water flows to the system by tidal forces through the entrance channel (caño de alimentación) and is collected in one or more large basins, called esteros. From the estero or esteros water is guided towards the retention areas (retenidas) and subsequently through a number of smaller tanks, thereby increasing the salt concentration, to finally end in the cristalization ponds (tajería). Water flow through all compartments is always driven by tidal force. Compartments are connected by small sluices, the compuertas, that allow for regulating water flow and preventing backflow at low tide when they are closed.

For aquaculture purposes, some adaptations have been made to this system. The main component in aquaculture is the estero. This basin or basins on average take up at least one third of the total area of the system. In general, they have a surface area of between 2 and 15 ha and are of 1 m average depth. This is the zone where many juvenile fish enter during spring tides and get trapped and are maintained for extensive (non-fed) aquaculture. When the fish reach commercial size they are harvested during a so-called despesque (defishing). Another zone of the former salt ponds that is used in aquaculture is the retention zone. Generally, this zone has been modified to form smaller channels or the larger earthen cultivation ponds that are used for semi-intensive and intensive (fed) aquaculture. Finally, to enable water circulation and renewal in the cultivation ponds, there is also an outflow channel (that can be the same as the inflow channel; inflow and outflow are regulated by opening and closing of the sluices).

So, in summary we have a system consisting of a series of interconnected ponds through which water is flowing driven by tidal force. As can be seen in the scheme, this offers various possibilities for integrated aquaculture. Cultures of different trophical levels can be maintained either together, or in separate compartments, connected by a semi-continuous flow of seawater. The manipulation of the sluices enables a certain amount of control over the system by controlling circulation rate. In the following we have used

these principles in the design of a demonstration pilot action of integrated multitrophic aquaculture in these former salt-ponds.

5 INTEGRATE pilot action CTAQUA

Slide 9
Pilot CTAQUA

The purpose of the pilot action of CTAQUA in the framework of the Integrate project is to design, operate and evaluate IMTA culture in earthen ponds in an existing and operational fish/oyster farm. The pilot experiment aims to demonstrate that cultivation of different marine species based on the principles of the IMTA-concept is viable in the earthen pond system. Furthermore, on a more scientific level, we aim to trace the nutrient flows in the system and to show how and where these are interconnected. The general goal of the pilot actions is to deliver IMTA technology, farming techniques and best practices. It is expected that these will be taken up at commercial level by collaborating farms and associated partners will channel them through to the broader aquaculture sector.

Slide 10
Location

The pilot action is carried out in the Salina de Belén de Poniente y de Levante near the city of Puerto Real in collaboration with the aquaculture company Estero Natural. This site, like many others in the area, is made of former salt evaporation ponds. Later, these were converted in fish cultivation ponds and the site has been in use for several years for the intensive cultivation of fish, mainly sea bass and sea bream. This activity ceased a few years ago, when intensive fish cultivation more and more took place indoor in recirculating aquaculture systems. In recent years, exploitation was taken over by Estero Natural, and activities are now mainly dedicated to oyster growing and extensive fish cultivation. Hydrologically it is connected to the inner zone of the Bay of Cádiz. Water exchange is driven by tides.

Slide 11
Species

The IMTA consists of three trophical levels: fed fish (gilt-headed sea bream, *Sparus aurata*, dorada in Spanish), filter feeders (Japanese oysters, *Magallena gigas*, formerly known as *Crassostrea gigas*) and primary producers (seaweeds *Ulva ohnoi* and *Gracilaria gracilis*). Foliose *Ulva* species are being commercialised under the name sea lettuce (lechuga de mar in Spanish), whereas *Gracilaria* is sold under its Japanese name ogonori. Sea bream and oysters have a large tradition of commercial exploitation and cultivation in these earthen ponds. Seaweeds, as in the most of Europe, have no traditional use but are increasingly being cultivated and exploited. Several seaweed exploitation activities have been developed in recent years by various companies in both Portugal and Spain. Their main use is currently in human food, but there is upcoming interest for the use of seaweeds in other sectors, such as in animal feed (incl. for aquaculture) and the use of their extracts in cosmetics and nutraceuticals.

6 Set-up and hydrology

Slide 12
Hydrology

The cultures have been set-up in two different ponds that are interconnected by a channel, which can be shut by a sluice (the “compuerta”). In downstream order, fish and oysters are grown together in the first pond and the seaweeds in the second pond. The co-cultivation of fish and oysters has been shown to be advantageous for both, as indicated by studies of Integrate partner IPMA among others. The continuous filtering of the water by the bivalves not only eliminates phytoplankton, it also greatly reduces potential fish pathogens as viruses and noxious bacteria from the water. On the other hand, large masses of seaweeds that photosynthesize during the day and respire in darkness cause strong diurnal dynamics in water oxygen levels. Especially on warm summer days this is problematic for the animals, hence this is why these are grown apart from each other.

To optimise the ponds for the IMTA, it is essential to make some small changes to the hydrology of the earthen pond system. In a standard salina, water intake and flow is reduced to a few days with a high tidal component, typically around spring tides. In many of the smaller and shallower channels, especially during warm summer months, salinity increases due to evaporation. These are less favourable conditions for algae

and oyster cultivation. Besides, reduced flow also means a reduced transport of nutrients and particulate matter from one culture to another. Hence, to improve IMTA efficiency, a more or less continuous flow and exchange with natural seawater is required. This is ensured by converting several “esteros” to collection basins (outlined in red in the figure). These are completely filled during days with high tidal component, typically around the spring tides, and their contents are gradually released through the other compartments.

Other important points to be taken into account is that especially the seaweed cultures require a bit larger minimum depth than maintained usually in these systems (depending on the cultivation system). This can be obtained by manipulating the sluices through the placement of (wooden) boards. In this case, a minimal water depth of 80 cm was established in the seaweed pond, which is sufficient to maintain both longlines with seaweeds as the floating cage system we deployed later on and which we will explain in more detail below.

7 Main nutrient flow and interactions

Slide 13
Nutrient flow

Slide 13 shows a schematic representation of the main flows, sinks and sources of nutrients and matter. Some of these are controlled by us, whereas others, in particular the microbiological processes that will appear on the next mouse click, are inherent to the system. First of all, there is the basic flow that we might call the intended nutrient flow in the IMTA. There are two distinguished nutrient inputs: on one side there is the feed given to the fish and on the other side there are the nutrients that are already present in the incoming seawater (background levels). These nutrients (in both input streams) are available in various forms: particulate, dissolved inorganic (phosphates, nitrate, nitrite and ammonium), and dissolved organic nitrogen (N) and phosphorous (P). The feed is largely consumed by the fish, although some rests will remain and decompose, releasing organic and inorganic nutrients.

Consumed feed is partly (approximately 20 – 25%) converted in new fish biomass; the rest is excreted. Dissolved nutrients coming from the excretions, feed and background levels stimulate phytoplankton growth. Phytoplankton, together with non-consumed feed, is filtered from the water by the oysters and in turn converted in to oyster biomass. Oyster excretions will also be remineralised to dissolved inorganic N and P. Nutrients are transported with the water flow to the algae pond, where the dissolved inorganic and part of the organic nutrients will be incorporated and converted in to new seaweed biomass.

Tightly linked to this basic flow is the flow caused by the chemical and (micro)biological processes inherent to these systems that will be visible after the mouse click. Crucial in the coupling of the flows is the remineralisation of organic material (uneaten feed, fish and oyster excretions, other particulate material). This process largely depends on temperature, rate of material deposition, etc. No attempts are made here to describe these processes in detail, nor to describe the other (microbiological) processes involved, as this falls outside the scope of this course. However, it is important to point out that the decomposition and remineralisation routes of nitrogen and phosphorous have some important differences. Phosphorous ions have important geochemical properties that may cause the sediment to act as a (temporal) sink for phosphorous. P regeneration is therefore controlled by both chemical and biological processes. Nitrogen, on the other hand, is more controlled by microbiological processes. Furthermore, denitrification, the conversion of dissolved inorganic nitrogen in to N_2 -gas that subsequently will disappear in the air is a highly significant nitrogen sink in coastal marine environments and can easily reach between 30 and 50% or higher of the total nitrogen input.

8 Research methods

Slide 14
Methods fish

After some initial tests with the different species, first trials for the pilot were started in July 2018. Juvenile dorada fish were bought from a local supplier and released in the system. Start density and biomass were

1.500 individuals and 60 kg respectively. This density responds to the so-called “improved extensive” category in the culture permit systems of the Andalusian regional government; i.e. a density higher than natural occurrence where feeding of the fish is required. Other categories include extensive (low density of fish that got trapped in the system with the tidal flow (feeding not required), semi-intensive and intensive. The latter two categories require monitoring of dissolved oxygen in the water and regular gassing with oxygen to prevent massive fish die-offs due to hypoxia or even (temporal) anoxia. Ponds are protected by nettings on top to keep piscivorous birds as the cormorant out. Fish density and biomass will be assessed twice per year following a defishing of the estero. Fish are hand-fed; the weekly amount of feed administered is registered and the feed is analysed for its nutrient content. The amount of N and P input to the system in the form of feed is hence calculated as the amount of feed times its N and P content respectively.

Slide 15
Methods oysters

As said above, oysters are grown with the fish. As is common in commercial oyster cultivation, oyster seeds are grown in mesh bags. Common practice is to leave the bags in crates on the sediment surface. Alternatively, bags can be attached to floaters that are in turn attached to long lines, so the oyster bags are floating some 10 cm below the water surface, a method developed by the Portuguese IMTA partner IPMA and also adopted in this pilot experiment. As the oysters grow, density (related to bag) has to be prevented from becoming too large by doubling the amount of bags and reseeded. In total, 15 kg of oysters was sown in July, reaching commercial size and a total weight of 51 in December. A new and larger batch was seeded in February 2019. Oysters are sampled monthly to determine survival, size and weight and a small sample of animals (15) is sacrificed to determine the so-called condition index; i.e. the amount of flesh with regard to the total weight including the shell.

Slide 16
Methods seaweeds

Finally, the third culture, seaweeds, were deployed in the adjacent and interconnected pond. As said above, two species were selected that commonly occurs in these environments, the chlorophyte *Ulva ohnoi* and *Gracilaria gracilis*. Seaweed cultivation is still in its infancy in Europe and several methods are being tested for suitability depending on the environment. These include spore production and seeding on specially produced textiles, seeding of vegetative material on long lines, and seeding of vegetative material in cages. Vegetative seeding in floating cages gave the best results. The system consists of mesh cages, kept suspended by buoys and submerged to approximately 70 cm, some 10 – 20 cm above the sediment at minimum water level. Algae are seeded at a density of 1 kg per cage. To prevent selfshadowing due to high densities and subsequent reduction of the light levels reaching the alg, frequent harvesting to original density is required; i.e. monthly in spring and autumn and every two weeks in summer. Every harvest, samples are taken as well for analysis of nutrient (carbon, nitrogen and phosphorous) contents.

Slide 17
Methods water

Nutrient flows between the compartments in the system and the background input in the incoming seawater are quantified by taking water samples of the incoming water in the fish pond, the outgoing water of the fish pond to the algae and the outflowing water of the algae pond. Samples are analysed for nutrients twice per month with neap and spring tide. This way, based on the amount of water flowing through the system, calculated using the tidal components and the concentrations of the nutrients in the water, measures of the minimum and maximum nutrient exchange through the system are obtained.

Slide 18
Methods mass balance

Combining all data together, we can then construct seasonal or annual nutrient mass balances for N and P. These mass balances describe the major known nutrient sources and sinks in the system. On the left side are the main nutrient inputs consisting of the input through feed and inflowing water. On the right will go the outputs: nutrients converted in biomass (fish, oysters, and algae) and the nutrients flowing back to the ocean. This way, we obtain insight in the efficiency of utilization of the nutrients of the feed and their possible faith in the system. We can expect inputs to be considerably larger than outputs. The missing amounts are accounted for by processes mentioned earlier, such as deposition of organic matter, denitrification, immobilisation of phosphate, etc.

Slide 19
Methods isotopes

The mass balance in itself is no proof that nutrients actually pass from fish feed to oysters. In order to be able to really trace the flow of nutrients through the system a different method is needed. Such a method is the analysis of stable nitrogen and carbon isotopes (^{15}N and ^{13}C respectively). Stable isotope studies are

increasingly used in ecological studies to reveal nutrient pathways in ecosystems and to define main nutrient sources and sinks. The idea behind is that fish feed has a different isotope content (isotope signature) than natural marine sources, as is illustrated for nitrogen in the figure. This difference, although diluted, will still be visible in the other compartments of the system. Hence, by analysing the isotope signature of the different compartments of a system the actual flow of nutrients through the system can be shown. During the pilot, two sampling campaigns, in spring and in summer, are foreseen. Natural abundance of stable C and N isotopes will be analysed in fish feed and oyster, algae and in water samples from the IMTA and in oyster, algae and water samples from a reference site outside the IMTA.

9 Potential for amplification and conclusions

In summary, during this part of the course, we have introduced to a definition of land-based IMTA and provided an overview of the presence and possibilities of these systems along the Iberian Atlantic Coasts. As we have shown, with some minor modifications, these former salt evaporation ponds can be transformed and prepared for IMTA.

Slide 20
Conclusions

The basic system deployed by CTAQUA in the pilot study consists of three cultures that all are able to generate economical revenues: fish, oysters and seaweeds. However, due to the open character of these earthen pond systems, other cultures that also represent an economical value can easily be integrated especially if they possess complementary ecological functionality. Examples include, but are not limited to, omnivorous and detritivorous fish species such as mullets (mugilidae), omnivorous (detritivorous) and herbivorous invertebrates such as shrimps, sea urchins, holothurians and abalones, and other suspension and/or filter feeding bivalves. Some of these examples are also presented in the contributions of the other Integrate partners.

In conclusion, we have shown the many possibilities the earthen pond systems present for the development of integrated aquaculture. Given that many of these systems along the Spanish and Portuguese coasts are currently under-used and under-exploited and considering the large surface area they occupy, it seems that development of IMTA could be a great way of enhancing a sustainable and diverse type of aquaculture in this region. The Integrate project intends to support this development by transferring the knowledge gained during the project to the local aquaculture sector. Several mechanisms are foreseen in order to do this, among which are the publication of good-practice guides and the organization of "living labs"; undertake technical visits to the IMTA facilities in the project to demonstrate the current developments to a wider group of professionals, end-users and representatives of public bodies.

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