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1. Executive Summary

The main objectives of bivalve shellfish restoration are increasing fisheries production and/or improving health of coastal ecosystems. More effort has been devoted to reef-forming species, particularly oysters, but some projects have focused on infaunal species. The latter constitute the closest references for the common cockle. One of the first issues to consider when addressing restoration of an exhausted shellfish area is to identify the stress sources leading to depletion, in order to implement specific measures to eradicate or mitigate those sources. Then a project has to be design to create and keep efficient “spawner sanctuaries” in the area leading to successful recruitment waves, which will increase the “spawner collective”, up to reach demographic levels in accordance to the final objectives. This type of programmes should require various years to reach the objectives. Therefore, governance as well as social aspects of the programme have high relevance. This report provides a suite of succinct guidelines to restore exhausted cockle beds, focused on technical aspects.

The “spawner collective” can be constituted by deploying adult cockles, with ripe gonad or close to maturity, or spat/juvenile cockles that would need longer period since deployment to reach maturity. Adult cockles should be obtained from natural shellfish beds, while cockle spat can be also collected from natural shellfish beds or produced in hatchery/nursery facilities. A shortcut to deploy cockles more or less close to spawn would be supplying huge quantities of fertilised eggs or larvae to the area. If the area to be restored is seriously affected by a cockle disease, the “spawner collective” has to be produced with cockles resilient to that particular disease. Additionally, regarding genetic issues, the “spawner collective” should derive from cockles collected from areas as close as possible to the area to be restored, thus belonging to a population well-adapted to the geographical area and avoiding introducing discordant genetic elements that could impact neighbour areas. Furthermore, the introduction of pathogens that are absent in the area to be restored and in the neighbour areas must be avoided.

The “spawner sanctuaries” have to be built by deploying cockles in patches with high cockle density rather than evenly distributed in the area; the more patches the better. The younger the deployed cockles, the higher initial density should be used to assure enough

density when their gonad is ripe. The longer the period since deployment up to gonad ripeness, the higher risk of density decrease in the patches. Various factors can contribute to density decrease since the initial deployment, such as cockle death, predation and dispersal. Predator losses can be minimised by using different types of protective nets covering the plots with deployed cockles. It could also contribute to limit deployed cockle dispersal. Additionally or alternatively, pots or other trap devices to catch predators could be set in the area. Information on the hydrography of the area to be restored could help to decide the right location of the plots but, if it were lacking, a good choice would be places where cockles occurred before depletion. Specific monitoring has to be performed to evaluate the temporal evolution of the gonad condition of the deployed cockles in the immediate and consecutive reproductive seasons, as well as that of the number of live cockles, thus estimating the numbers of cockles actually spawning. This information will be highly valuable to introduce modifications with which improving the restoration process as it progresses year after year.

The larvae derived from the “spawner collective” could potentially settle inside the area to be restored or be exported outside. The availability of circulation models could help to predict areas where recruitment should be expected. Monitoring of recruitment in the area to be restored is crucial to evaluate whether the restoring progresses. The temporal evolution of demographic parameters (spatial distribution, cockle density, size distribution) should be estimated, which will be crucial feedback to redesign procedures as well as to validate or correct larval circulation models if available. Additionally, a specific genetic monitoring should be performed to discriminate between recruited cockles deriving from the deployed cockles (or the supplied hatchery-produced larvae) and recruits from any other origin, and to evaluate genetic diversity, because maintaining genetic variability and preventing from excessive inbreeding when hatchery produced cockles or larvae are deployed or released, is very important to assure sustainability of the population. Finally, monitoring the ecological changes, such as macrofaunal community structure and diversity and even evaluating any other cockle services could be worthy to demonstrate the benefits of restoration processes, going further than allowing sustainable fishery.

2. Introduction

Bivalve shellfish restoration has been traditionally focused on increasing short-term fisheries production. More recently, the health of coastal ecosystems has been considered as an objective and, frequently, as the priority of the restoration projects (Gann *et al.*, 2019). Overall, more effort has been devoted to epifaunal (living on the substrate) reef-forming species, particularly oysters (Lipcius & Burke, 2018; Pogoda *et al.*, 2019; Ridlon *et al.*, 2021), but there have been well-documented restoration projects focused on infaunal (those buried in the sediment) species (Rice *et al.*, 2000; Arnold *et al.*, 2002; Marsden & Adkins, 2010; LoBue & Bortman, 2011; Zhang *et al.* 2021). However, there are not well-documented information on restoration of cockle *Cerastoderma edule* beds, thus the programmes performed with other infaunal bivalve species constitute the closest references for the common cockle.

One of the first issues to consider when addressing restoration of an exhausted shellfish area is to identify the stress sources leading to depletion. The sources of stress affecting shellfish populations, alone or in combination, can include overfishing, degradation or destruction of habitat, degraded water quality (*i.e.*, anoxia, sedimentation, harmful algal blooms, pollution), diseases and predation. Obviously, specific measures have to be implemented to eradicate or mitigate those sources that contributed to depletion (Brumbaugh *et al.*, 2016). Then a project or programme has to be design to, basically, create and keep efficient “spawner sanctuaries” in the area leading to successful recruitment waves, which will increase the “spawner collective”, up to reach demographic levels in accordance to the final objectives, sustainable shellfishery and/or ecosystem health recovery. This type of programmes should require various years to reach the objectives, especially when the area to be restored is a large system, such as an estuary, a bay or a ria. Considering this length to keep operations in a large coastal area, with the need of legal restrictions (*e.g.* long closure periods) and disturbances of usual practices, governance as well as social aspects of the programme have high relevance (van Tatenhove *et al.*, 2020).

There are many stakeholders that care, for various reasons, about activities – including restoration – that affect the waterways near where they live, work or recreate. Engaging these

stakeholders is an important step in the development of a project as the right mix of partners can be a tremendous help in designing and implementing a successful restoration project and ensuring a sustainable result (Brumbaugh *et al.*, 2016). Different organizations or agencies possess different strengths, resources or capabilities, so building an effective coalition of partners is perhaps the best way to facilitate a project. Strengthening legal and regulatory policies that provide formal government support for the site would improve the likelihood of long-term success (Lo Bue & Udelhoven, 2013).

Shellfish restoration is still very much in its infancy and there is much room for further innovation and improvement (Brumbaugh *et al.*, 2016); this is particularly true for the case of the common cockle. With these issues in mind, next is a suite of succinct guidelines to restore exhausted cockle beds, focused on technical aspects.

3. Life stage and source of cockles to be deployed

The usual way to recover infaunal mollusc populations in an exhausted area is to create concentrated patches of spawners that will supply recruits in the area. It could be achieved through three main approaches, according to the life stage to be deployed, either adults, spat or fertilised eggs/larvae (Arnold, 2001; Arnold *et al.*, 2002; Stewart & Greese, 2002; Marsden & Adkins, 2010). The most immediate option to get massive spawning in place would be deploying adults in the spawning season (i.e. with ripe gonad or close to it), while spat/juvenile cockles would need longer period since deployment to reach maturity. The longer the period to reach maturity the higher the percentage of individual losses; thus, the younger the life stage used, the higher the number of individuals that should be deployed. A shortcut to deploy cockles more or less close to spawn would be supplying huge quantities of fertilised eggs or larvae to the area, thus circumventing the risk of poor gamete release or fertilisation failures and the expensive and labour-intensive process of producing spat in hatchery/nursery facilities.

Adult cockles, with ripe gonad or close to maturity, should be obtained from natural shellfish beds; cockle spat can be also collected from natural shellfish beds or produced in

hatchery/nursery facilities, while fertilised eggs and larvae have to be produced in a hatchery. Therefore, the availability of “healthy” shellfish beds that can be used as source (donor) of cockles (adults or spat) to be deployed in the restoring area without threatening the sustainability of the donor beds and, alternatively, the availability of hatchery/nursery facilities in which producing cockle fertilized eggs, larvae or spat would condition the choice.

Historically, the relaying (collecting individuals from a bed and deploying them in other area) approach has been the most frequently used for restoration of infaunal mollusc populations, mostly due to the economical and/or technical difficulties for the hatchery production of most species (Rice *et al.*, 2000). A successful reference of the adult relaying approach is the restoration of the hard clam *Mercenaria mercenaria* population in Gran South Bay, Long Island, NY, USA (LoBue & Bortman, 2011; LoBue & Udelhoven, 2013). Regarding the impact on the donor bed, collection procedures and quantities have to be adjusted to avoid threatening its sustainability but, in case recruitment is very abundant, with high cockle spat density, the impact of thinning out would be negligible or even beneficial for the donor bed (Dijkema *et al.*, 1987). Nevertheless, using a donor bed could be avoided (and thus any impact) if hatchery/nursery facilities provided enough quantity of cockle spat. There are procedures to produce cockle spat in hatchery/nursery facilities (Pronker *et al.*, 2013; Fernández Otero *et al.*, 2021), some of them developed within project COCKLES (Joaquim *et al.*, 2021), which could be effectively used in cockle bed restoration programmes (Villalba *et al.*, 2021).

Information on cases using the larval approach is very scarce. Success was elusive in attempts performed with larvae of the hard clam *M. mercenaria* (Arnold, 2008), which contrasts with the good results obtained when larval release was implemented to restore severely depleted populations of an epifaunal mollusc, the bay scallop *Argopecten irradians*, in estuaries of West Florida (USA, Leverone *et al.*, 2010). Anyway, the above-mentioned approaches should not be considered mutually exclusive.

In the case that the area to be restored is seriously affected by a cockle disease, such as exhausted cockle beds due to marteiliosis, cockles to be deployed must be resilient (resistant or tolerant) to that particular disease, marteiliosis-resistant cockles if that is the disease.

Therefore, cockles to be deployed in the exhausted area have to be collected from another area (if any) where resilience to that particular disease has been proved or they have to be produced within a selective breeding programme to increase resilience to that disease. In this context, guidelines to produce a marker-assisted selective breeding programme to produce Marteiliopsis-resistant cockles have been provided as an output of the project COCKLES (Villalba & Martínez, 2021).

Another important consideration is related to the genetic characteristics of the cockles to be deployed. Cockles to be deployed, or those to be used as broodstock in hatchery facilities to produce larvae or spat with which address restoration, should be collected from areas as close as possible to the area to be restored, in order they belong to a population well-adapted to the geographical area and to avoid introducing discordant genetic elements that could impact neighbour areas by the future migration of larvae from the restored area.

A serious risk that must be avoided, if the relaying approach is chosen, is the introduction of pathogens that are absent in the area to be restored and in the neighbour areas. This risk would be higher if cockles to be relayed were collected from far distant areas.

4. Deployment of cockles

Cockles have to be deployed in areas considered as “spawner sanctuaries”. The quantity of larvae resulting from deployed “spawners” is influenced by the number of spawners but also by its close proximity to assure that released spermatozoa meet oocytes maximising fertilisation. Therefore, cockles should be deployed in patches with high cockle density rather than evenly distributed in the area; the more patches the better. Ideally, densities close to 100 ready-to-spawn cockles per m² should be used in plots of at least 100 m² scattered in the area. As mentioned above, the younger the deployed cockles, the higher initial density should be used to assure enough density when their gonad is ripe. The longer the period since deployment up to gonad ripeness, the higher risk of density decrease in the patches.

Various factors can contribute to density decrease since the initial deployment, such as cockle death (by multiple possible causes), predation and dispersal (horizontal translation out of the patches). Post-settlement dispersal of infaunal molluscs is frequently overlooked. A study by Hunt *et al.* (2020) showed that New Zealand cockles *Austrovenus stutchburyi* were able to disperse at least 50 cm within 1 tidal cycle (12 h). Dispersal was size dependent, with adult cockles dispersing less than juveniles. Post-settlement dispersal varied both spatially and temporally. Dispersal was greatest at the most wave exposed site, and during a storm event. Even horizontal active locomotion has to be considered, as it has been described for multiple infaunal bivalves, through movements known as leaping (Ansell, 1969), crawling and walking (Tettelbach *et al.*, 2017). Multiple predators, both invertebrates (snails, crabs, sea stars, ...) and vertebrates (finfishes and birds) can contribute to increase losses of deployed cockles, seriously compromising the success of restoration (van der Heide *et al.*, 2014; Wilcox & Jeffs 2019). Predator losses can be minimised by using different types of protective nets as predator deterrent measures (Cigarría & Fernández, 2000; Beal & Kraus, 2002; Tan & Beal, 2015), which could cover the plots with deployed cockles. It could also contribute to limit deployed cockle dispersal. Preliminary trials could inform whether or not the use of netting is needed (Cummings *et al.*, 2007). Additionally or alternatively, pots or other trap devices to catch predators could be set in the area.

An important decision is the location of the plots where deploying cockles. Information on the hydrography of the area to be restored could help to decide but, if it were lacking, a good choice would be places where cockles occurred before depletion. Preparation of the plot substrate before deploying cockles could be required if the sediment looked degraded.

Once the cockles have been deployed in the plots and, thus, the “spawner sanctuaries” have been settled, the cockles will release gametes in quite synchronic waves during the spawning season. Specific monitoring has to be performed to evaluate the temporal evolution of the gonad condition of the deployed cockles in the immediate and consecutive reproductive seasons, as well as that of the number of live cockles, thus estimating the numbers of cockles actually spawning (Joaquim *et al.*, 2007; Doall *et al.*, 2008). This information will be highly

valuable to introduce modifications with which improving the restoration process as it progresses year after year.

Cockles are able to recondition (restore) their gonads after spawning; in other words, their gonads go through successive waves of gametogenesis, ripeness, spawning, reabsorbing and resting along cockle life. Even in southern latitudes, such as in the Iberian Peninsula, cockles are able of restoring the gonad immediately after spawning without a resting stage trough a long annual spawning period (even from May to September). Nevertheless, cockle fecundity tends to decrease with age. According to this, live cockles can stay in the plots releasing gametes various spawning seasons but, if available, young cockles should be deployed in the plots every year to readjust the density with highly fecund spawners. The deployed cockles will modify the sediment favouring burrowing of new recruits or the new ones to be deployed (Donadi *et al.*, 2014).

If larval release were the selected approach, a way to limit larval dispersal from the area where settlement is wished would be setting up floating enclosures (floating impermeable barriers forming a corral); the enclosures would be taken away some days after releasing the larvae inside them (Leverone *et al.*, 2010).

5. Recruitment

An undetermined percentage of the larvae derived from the spawning of the deployed cockles (or directly supplied from hatchery facilities, if that occurred) will settle and will become new recruits, where will they do it? The pelagic period of cockle larvae before their settlement in the natural environment may last from 15 to 30 days or longer, which involves potential dispersal for long distances, mainly depending on water circulation. According to this, the larvae could potentially settle inside the area to be restored or be exported outside (Lundquist *et al.*, 2009). The availability of circulation models in the area to be restored could help to predict areas where recruitment should be expected (Lundquist *et al.*, 2009; Bidegain *et al.*, 2013; Goodwin *et al.*, 2019). The recruits, once they reach maturity, will contribute as new spawners.

Monitoring of recruitment in the area to be restored is crucial to evaluate whether the restoring progresses. The temporal evolution of typical demographic parameters, such as spatial distribution, cockle density, size distribution, should be estimated. This knowledge will be crucial feedback to redesign procedures (revision of plot location and size, cockle density and age at deployment, anti-predator actions...) as well as to validate or correct larval circulation models if available (Zhang *et al.*, 2019), because all the process has to be maintained and improved year after year to take advantage of every reproductive season, until the cockle population area reaches a level allowing sustainable exploitation. Meanwhile, any shellfish exploitation and any other activities interfering the restoration process should be banned.

6. Genetic discrimination of recruits

A specific genetic monitoring to discriminate between recruited cockles deriving from the deployed cockles (or the supplied hatchery-produced larvae) and recruits from any other origin should be performed. Larvae deriving from cockles outside the area being restored could enter the area and settle there. Additionally, cockles existing in the area that were not deployed within the restoration process could also contribute to new recruitments. Therefore, is important to discriminate the origin of recruits to accurately evaluate the contribution of deployed cockles to new recruitment (Wilbur *et al.*, 2004; Díaz Puente *et al.*, 2016), which is particularly important if deployed cockles have a specific character, such as disease resistance, that is important to maintain during the restoration process. Furthermore, maintenance of genetic variability and prevention from excessive inbreeding when hatchery produced cockles or larvae are deployed or released, is very important to assure sustainability of the population (Gaffney, 2006; Hornick & Plough, 2019, 2021). The genetic monitoring will allow evaluate genetic diversity and assess if there are some genetic structure between years (Zhang *et al.*, 2021). Again, this knowledge will feedback the process, disclosing deviations to correct by procedure modifications. Deep knowledge of the cockle genome (Pardo *et al.*, 2021) and the genetic population structure of cockles along the European Atlantic coast (Vera *et al.*, 2021,

2022) has been generated within the project COCKLES, which will allow to design accurate genetic monitoring procedures for the purpose of this section.

7. Evaluation of ecological benefits and other services

The common cockle is a key engineer species (Dairain *et al.*, 2020; Maire *et al.* 2021) and ecosystem changes are expected to be a consequence of the restoration of exhausted cockle beds. Monitoring the ecological changes, such as macrofaunal community structure and diversity (Hewitt & Cummings, 2013; Shantharam *et al.*, 2019; Blanchet *et al.*, 2021), along and following the restoration process and, even evaluating any other cockle services (Carss *et al.* 2020; 2021) could be worthy to demonstrate the benefits of restoration processes, going further than allowing sustainable fishery.

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