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MULTIDISCIPLINARY APPROACH TO MANAGE THE STRIPED VENUS CLAM (CHAMELEA GALLINA) FISHERIES

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Summary

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Abstract

The use of hydraulic dredging for the catch of the striped venus clam *Chamelea gallina* (Linnaeus, 1758) has a long history in the Adriatic Sea. Starting from the early 70's the introduction of hydraulic dredges led to a rapid increase in landings (80,000-100,000 metric tons per year) followed by a progressive decline due to overexploitation and poor management. In the last 40 years the venus clam fisheries management faced several stages of development towards a more responsive fishery through the adoption of progressive technical restrictions. However, the adoption of management measures should be based on the best possible scientific evidence and sometimes the lack of knowledge can undermine the effectiveness of the measures taken; moreover, some information is very dated in time and recently new techniques have been adopted to study some biological aspects. Therefore, in the present PhD thesis some fundamental life history-traits of the species (e.g. age, growth, reproduction, size at maturity, fecundity, reburial ability and survivability) as well as the impact exerted by the dredge on the target and non-target species have been studied to identify reliable measures for a reasonable management of the clam fishery.

To estimate the age and growth of *C. gallina* populations in the mid-western Adriatic Sea, three ageing techniques were used: video-analysis of thin sections, video-analysis of acetate peel replicas (which involve shell sectioning) and observation of the surface growth rings. The two shell sectioning techniques were found not to be significantly different (χ^2 = 4.66, df = 3, p = 0.198), and were described by the same von Bertalanffy (VBF) growth curve parameters (L_∞ = 43.9, k = 0.26, t_0 = -0.84), whereas the surface growth rings approach proved unreliable and error-prone, as it underestimated the age and overestimated the growth rate (L_∞ = 26.4, k = 1.91, t_0 = - 0.11). The analyses demonstrated that shell growth is slower in the cold season. Furthermore, the shell growth rate decreases in older specimens.

The reproductive cycle of the species was investigated for a year-round by mean of microscopic, histological and video analysis techniques. The breeding season of the species was driven by rises in seawater temperature and chlorophyll-a concentration and its spawning period lasted from March to September. Size at sexual maturity was reached very early in the life cycle at about 11.2 mm within the first year of life. As regards partial fecundity – the number of mature oocytes potentially released by females with ripe gonads in a single release event – varied in relation to size. Nevertheless, the reduction on the Minimum Conservation Reference Size (MCRS) from 25 to 22 mm (Delegated Regulation (EU) 2020/2237) lead to a 40% reduction in the number of emitted eggs.

To test the reburial and survivability of undamaged clams that had undergone hydraulic dredging and mechanized sorting, two experiments, one in laboratory tanks and the other at sea, have been carried

out. In the tank experiments, the reburial times (time at which 50 and 90% of clams had reburied: T_{50} and T_{90}, respectively) of the whole sample was about 4 h (CI+ 4.4, CI- 3.6) and 8 h (CI+ 8.2, CI- 7.7), respectively. Clams survival rates was 94.8% and 96.2% respectively in the laboratory and at sea, without significant differences between the two experiments or among size classes. These findings demonstrate that undamaged *C. gallina* specimens returned to the sea have a very high survival probability and that they can contribute to mitigate the overexploitation of natural populations participating on the total reproductive output of the species.

The evaluation and quantification of shell damage on *C. gallina* populations in the mid-western Adriatic Sea showed that both in the non-sieved and sieved (*i.e.* commercial and discard) fractions the majority of individuals was intact and/or repaired: 86.3% and 79.4%, respectively. Shells with one or two valves chipped were mainly represented in relation to the remaining damage classes which on average cumulatively accounted always less than 9%. The dredge alone accounted for about 14% of shell damage, however the sieving process added an additional 6.8%, being 0.5 times higher than the previous one. The shell total length resulted significantly positively related to damage probability, however the magnitude of increase between treatments (non-sieved, commercial and discard) was higher for the discard fraction. Survival estimates assessed that only 8.3% of discarded clams died considering the whole size structure of this fraction.

The evaluation and quantification of damage induced by dredging on the discarded macro-benthic fauna living associated with *C. gallina* during the summer season highlighted that soft-shelled or soft-bodied species were the most affected by the harvesting process, whereas thick-shelled or thick-bodied species suffered minor damage. Overall, 61.0% of the individuals were undamaged, whereas 16.1%, 3.7% and 19.2% displayed slight, intermediate and severe damage, respectively. The two most damaged species were the sea urchin *Echinocardium cordatum* (>69%), and the bivalve *Mactra stultorum* (>35%). Mortality rate was 22.9% of all the discarded individuals with *E. cordatum* showing the highest mortality rate of 95.4%.

In conclusion, based on the information gained on the biological-traits of *C. gallina*, despite the reduction of the MCRS the potentially negative effect should not be detrimental and could be compensated by the restrictive measures provided for in the Italian Management Plans. However, on the eye of an ecosystem approach to fishery, mitigation measures should be tested and introduced to reduce the impact inflicted by dredging on the target species as well as on the macro-benthic communities.

Introduction

Fisheries context at national level

The striped venus clam *Chamelea gallina* (Linnaeus, 1758), known in Italy as “vongola” and locally as “cappola”, “lupino”, “cocciola” (Figure 1) is a bivalve of Veneridae family distributed throughout the Mediterranean, with high densities for a commercial use mainly in the western Adriatic, in the Marmara Sea (Turkey), in the southern Spain (Andalusia, Catalonia and mainly Gulf of Cádiz) and to a less extent in the Albanian (south-eastern Adriatic) and Moroccan coasts of the Alboran Sea. Italy, Turkey and Spain are the countries mainly involved in the venus clam fishery (Lucchetti et al., 2021).

In Italy the striped venus clam is particularly abundant along the central and northern Adriatic coast although noteworthy quantities are also caught in the south Adriatic and central-southern Tyrrhenian Sea (Lucchetti et al., 2021). *Chamelea gallina* is one of the most important edible bivalve molluscs and it is mainly found in the coastal fine well-sorted sand biocenosis described by Péres and Picard (1964) at depths between 2 and 12 m (Morello et al., 2006). Although the hydraulic dredger sector accounts for less than 6% of the Italian fleet, the quantities yearly landed (around 20 thousand tons) reach 15% of all fisheries production, and the Venus clam is the second most important species, second only to the anchovy (Lucchetti et al., 2021).

Originally the striped venus clam was harvested by hand, and its harvesting for food purposes has a long history in our country. However, as technological innovations developed the gears adopted were progressively more avant-grade, starting with a hand-operated fishing gear (traditional hand dredge) followed by a hydraulic dredge. The first hydraulic dredgers entered into service in the Adriatic Sea in the early 70s and in a few years exceeded the traditional dredges operated by hand because the catches and economic returns were much higher. In 1974 the hydraulic dredges numbered 383 (of which 240 were modified traditional dredges) along the entire Italian Adriatic coast, with annual landings overreaching 100 thousand tons in those years (Froglia, 1989). Ten years later dredges had increased in number to 607 in the same area. Dredges peaked at 778 in 1993 and then the fleet started decreasing. The increase of fishing effort (in terms of number of vessels) and catch efficiency, due to technical innovations, have led to intense exploitation, with a risk of over-exploitation (Carlucci et al., 2015).

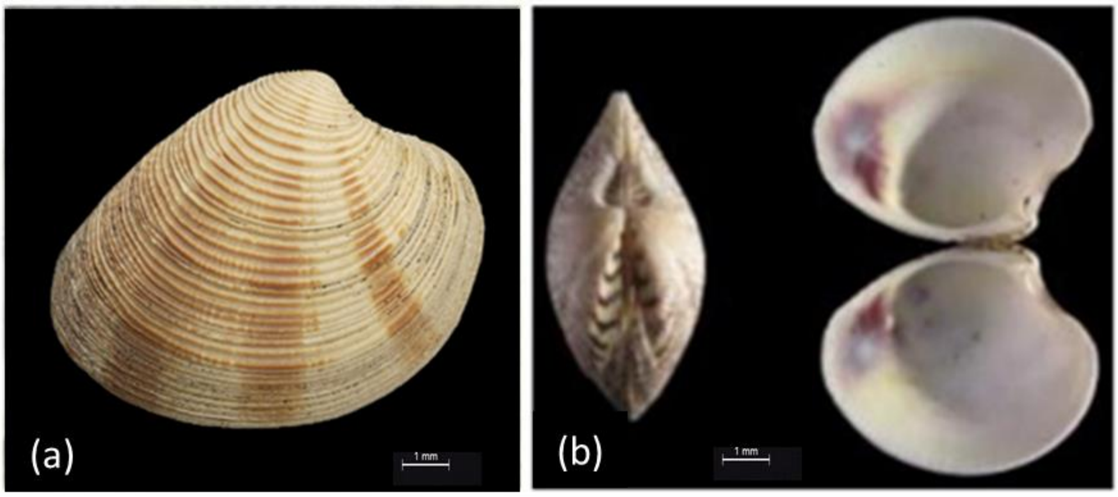

Figure 1. Photographs showing the target species C. gallina *a)* from the external and *b)* umbonal and internal side.

At present, clams are harvested with hydraulic dredges, which operate within a narrow coastal strip between the 0.3 and 2 nautical miles (NM), even if high densities for a commercial use are normally concentrated within 1 NM from the shore. The gear consists of a metal cage 2.5-3 m wide - made up of parallel metal rods 12 mm wide - mounted on two skid-sledge runners that facilitate the sliding motion on the seabed and prevent the cage from digging into the sediment. A sharp metal blade fitted on the lower part in front of the cage protrudes under the sledge runners a few cm (4-6 cm for the “vongolara” dredger), which favours the removal of the top part of the sediment. A hose connects the centrifugal water pump to nozzles placed at the dredge mouth and inside the cage; nozzles eject pressurized water towards the sea bottom to dislodge the marine organisms living in sediment and facilitate their catch. The vessel moves backwards during towing and, at the end of the tow, the cage is hauled on board at the bow and its content is tipped into a collecting box (Figure 2 and Figure 3). Selection is carried out either on the seabed by the dredge itself, either on board using a vibrating sieve, which consists of multiple grids. The dredge catch is processed on board and sorted mechanically into different commercial classes by the vibrating sieve, while discard (small clams) and other species without commercial interest, are directly thrown back into the sea.

Figure 2. Details of a hydraulic dredge.

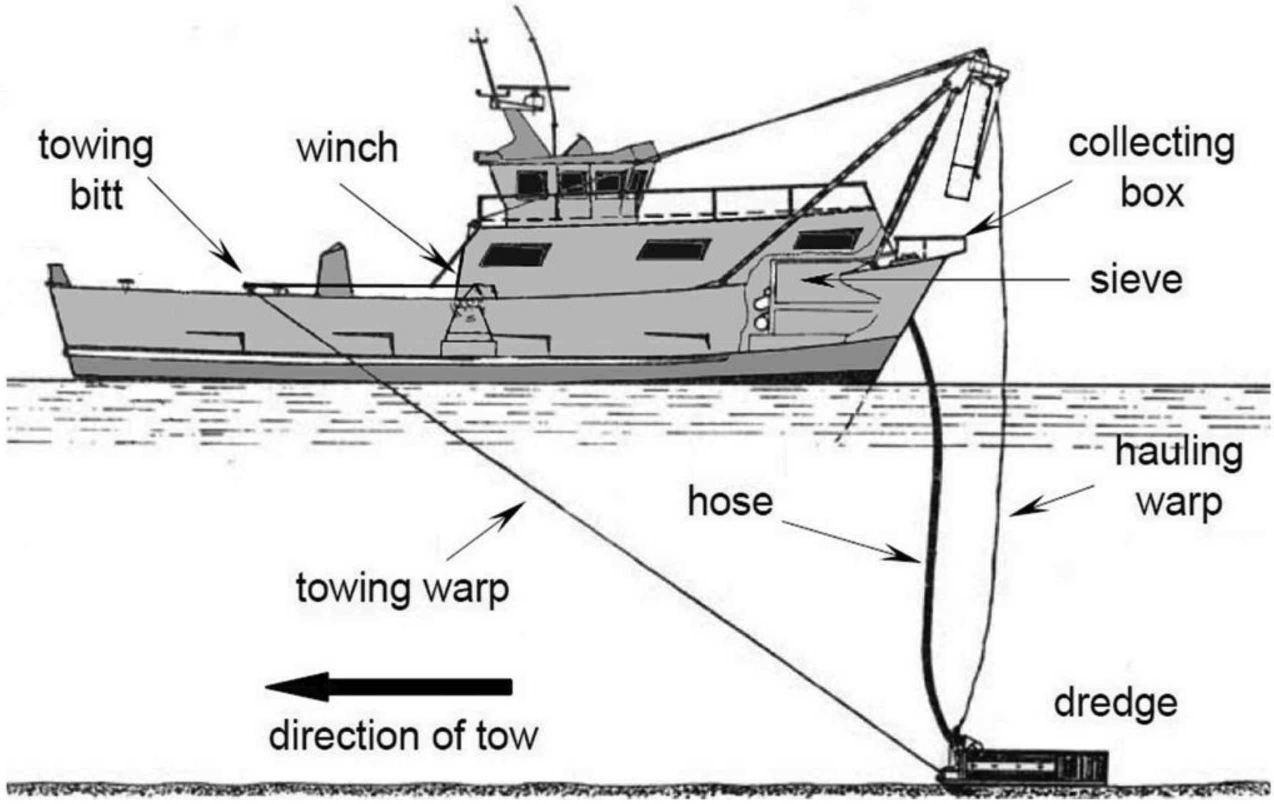

Figure 3. Representation of an hydraulic dredge showing its fishing method (Source: Lucchetti and Sala, 2012).

Fisheries employing hydraulic dredges (705 units in total) are carried out along about 1400 km of coastline over a total length of about 8000 km of Italian coasts. Updated data up to 2022 indicates that the total fleet of hydraulic dredges harvesting the striped venus clam is equal to 626 vessels, 38 of which mainly target the razor clam *Ensis minor* along the Tyrrhenian coasts, and only occasionally the venus clam. Therefore, 588 most productive vessels are concentrated along the Adriatic coasts, mainly in the Marche (37%) and Veneto (19%) regions. Overall production in 2018 and 2019 was around 19,000 tons per year and in 2020 and 2021 it exceeded 20,000 tons. Between 2018 and 2020 the striped venus clam landings accounted for about 10-15% by weight of the entire fishery national production and about 5-7.2% of total revenues (an average of about 50 million of euros per year) (Bargione et al., 2022). The fleet of hydraulic dredgers is currently uniform in terms of technology and dimensions (average LOA 15.5 m, average tonnage 9.98 GT, average engine power 150 kW). The total number of crew members on board is estimated at around 1500, which is equivalent to an average crew of two operators per vessel (Italian National Management Plan for hydraulic dredges, 2019).

Fisheries context in the Marche region

The most productive Italian region in the clam fishery sector is the Marche region whose shoreline, according to Regulation No. 6 of 19/10/2009, is currently divided into 4 fishing areas (called "A", "B", "C" and "D") in which 4 management Consortia for Mollusks Management (CO.GE.MO. Pesaro, CO.GE.VO. Ancona, CO.GE.VO. Civitanova Marche and CO.VO.PI. San Benedetto del Tronto) operate. Their territorial limits are shown in Figure 4.

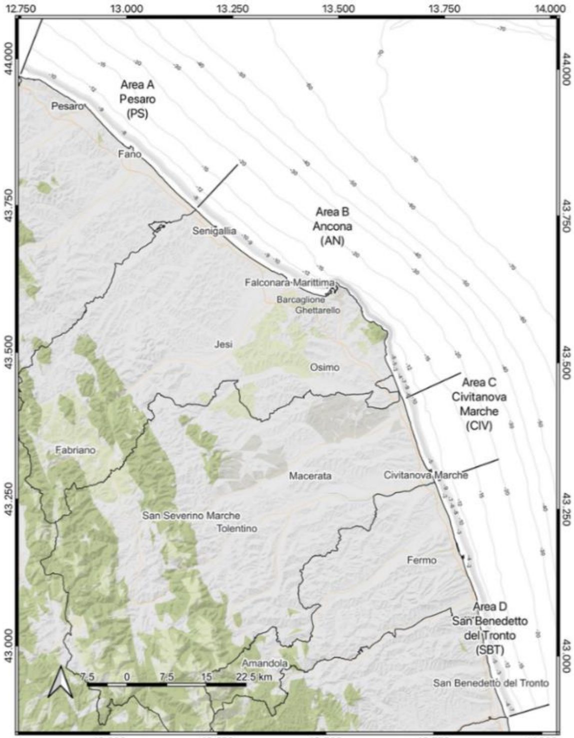

Figure 4. Map of the Marche region in the mid-western Adriatic Sea. From north to the south are showed the 4 Consortia for Mollusks Management present along the coast. (PS = CO.GE.MO. Pesaro, AN = CO.GE.VO. Ancona, CIV = CO.GE.VO. Civitanova Marche and SBT = CO.VO.PI. San Benedetto del Tronto).

The Marche region owns over the 30% of the total national fleet of hydraulic dredges followed by Veneto (about 23%), Abruzzo (about 15%) and Puglia (about 10%). Among the Consortia present in the Marche region, since 2010 the one of Ancona (CO.GE.VO. Ancona) is the one with the highest number of hydraulic dredges, followed in order by Pesaro, San Benedetto del Tronto and Civitanova Marche (Table 1), and their number of operating vessels has remained almost constant over the past 15 years.

Table 1: Number of hydraulic dredges divided by Consortia (Sources: IREPA and Consortia). AN = Ancona; CIV = Civitanova Marche; PS = Pesaro; SBT = San Benedetto del Tronto.

	2006	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**
AN	55	55	55	55	74	74	74	74	74	74	74	74	74	74	74	74
CIV	44	44	44	44	25	25	25	25	25	25	25	25	25	25	25	25
PS	64	64	64	64	65	65	65	65	65	65	65	65	65	65	65	65
SBT	56	56	56	57	57	57	57	57	57	57	57	57	57	57	57	57
TOT	219	219	219	220	221	221	221	221	221	221	221	221	221	221	221	221

Based on the data on landings annually furnished by each Consortium is possible to evaluate the pattern of the Catch Per Unit Effort (CPUE) expressed in terms of landings/vessel over time (Figure 5).

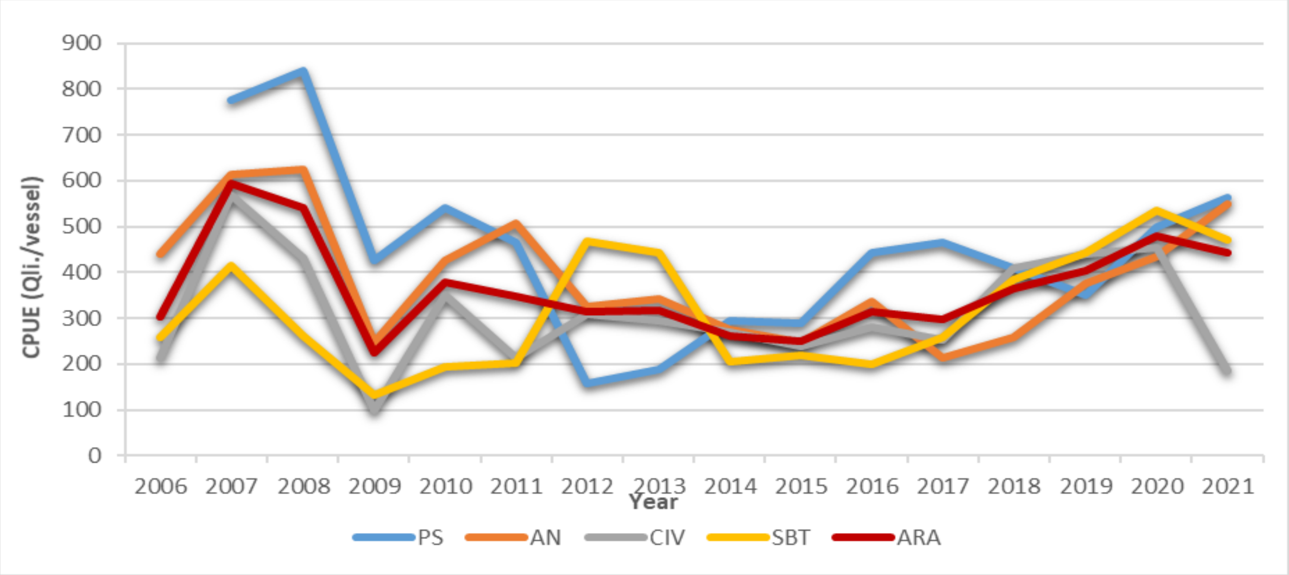

Figure 5. CPUE (Qli./vessel) for the Consortia of the Marche region over the period 2006-2020 (Sources: Consortia) AN = Ancona; CIV = Civitanova Marche; PS = Pesaro; SBT = San Benedetto del Tronto; ARA = Annual Regional Avarage.

Over the considered period CPUE pattern shows periodic fluctuations typical of an almost sessile species subject to intense exploitation or localized mass mortalities events which generally lead to the closure of the fishing activity until the resource recovers. This flows into a reduction of landings for the corresponding period as commonly observed in some Consortia of the northern Adriatic Sea (Barone et al., 2022). Furthermore, fluctuations in currents, temperature, salinity and other natural variables, such as the availability of space and nutrients, influence larval dispersal, recruitment and survival of the resource, thus shaping its abundance and distribution over space (Froglia 2000, Carlucci et al., 2015). The production peaks recorded in the various Consortia are generally temporally staggered, a clear sign that it is not only the environmental conditions that influence the state of the stocks. The fluctuations observed in CPUE can therefore be determined by a number of factors:

- environmental conditions (trophic conditions, physical parameters such as bottom seawater temperature and salinity, density, deaths, etc.);

- commercial considerations (the resource is harvested following the market demands);

- regulatory restrictions: starting from 2017 the maximum daily quota per vessel was reduced to 400 kg from the 600 kg previously envisaged (EU Regulation 2376/2016).

Moreover, CPUE generally seem to have increased from 2018 (except for CO.GE.MO. Pesaro), despite the introduction of the new Minimum Conservation Reference Size (MCRS) of 22 mm in 2017 (*see next sections*).

Management context

Hydraulic dredging was the first attempt in Italy of a fishery controlled through a system of licences, whose number had been set at the national level to keep the fleet and the fishing effort nearly unchanged. European, National and Regional management plans have led to a reduction of fishing capacity from 665 Adriatic vessels in 1998 to 588 vessels in 2002 (this number subsequently remained nearly unchanged). Therefore, at national level, the drop in the annual landings from around 100.000 t in the early 80s to the present (around 19,000 - 20,000 t in the last years) (Figure 6), can be explained by the reduced fishing effort, the variable market demand and the more restrictive management measures implemented over years, rather than reflecting the real status of the resource at sea (Italian National Management Plan for hydraulic dredges, 2019).

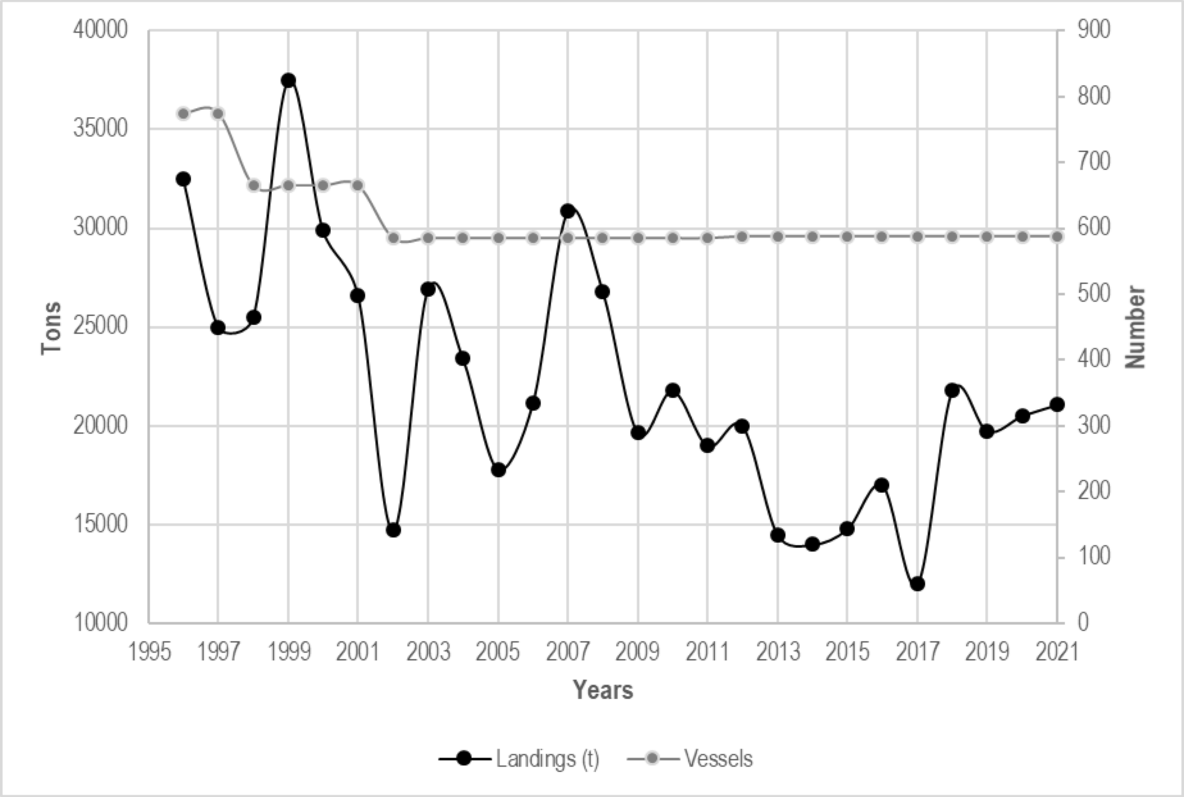

Figure 6. Total landings (tons) and number of vessels targeting the venus clams over the period 1996-2021.

Legal framework

The entry into force of Regulation (EC) 1967/2006, which implied a ban on hydraulic dredging within 0.3 miles of the coast, resulted in a significant reduction of the exploitable areas, compared to the previous legal framework (the previous legislation forbade the use of hydraulic dredges in those areas with a depth less than 3 m; M.D. 22/12/2000); the potential fishing grounds were affected differently by the new technical measures, depending on the nature of the coastal area concerned and on the spatial distribution of the species. The reduced exploited areas were significant: for example, for the most productive regions, Marche and Veneto, the fishing areas have been reduced of 35.3 and 56.3%, respectively. The reduction of the total fishing area at the national level was around 526.3 km^2 versus 1108 km^2 before the implementation of the Regulation, equal to a reduction of 52.5%.

At present, the main current legislations which regulate the clam fishing activity are:

- M.D. 22/12/2000 established the following characteristics for hydraulic dredges i) maximum cage width 3 m ii) maximum water pressure from the nozzles 1.8 bar iii) maximum gear weight 600 kg. The dredge on vessels targeting clams is also subject to the following limitations: the distance between the metal rods on the lower part of the cage must not be less than 12 mm. Instead of the rods, a metal grid with square mesh not less than 17 mm/side or rectangular mesh with sides of 12 and 25 mm respectively are allowed, or a perforated sheet of metal with holes with a diameter of not less than 21 mm and a full/ empty ratio of less than ½. Once hauled, the contents of the cage are turned out into the collection box then separated with sieves that have grids with the same characteristics as the cage described above;

- M.D. 22/12/2000 established two months fishing closures between April and October;
- Regulation (EC) 1967/2006 that bans the use of hydraulic dredges within 0.3 nautical miles of the coast;
- Regulation (EC) 1380/2013 establishes that for the species for which scientific evidence demonstrates high survival rates, among which the striped venus clam, the landing obligation is not applicable;
- The Ministerial Decree (M.D.) 27/12/2016 "National Discard Management Plan for clam stocks *(Chamelea gallina)*", which transposed the EU Regulation 2376/2016, setting the new Minimum Conservation Reference Size (MCRS) for *C. gallina* at 22 mm by way of derogation to the previous 25 (Annex III to Regulation (EC) 1967/2006) until 31/12/2019, and setting the new daily quota at 400 kg instead of 600 kg;
- Regulation (EU) 2019/1241 of the European Parliament and of the Council fixed that the maximum breadth of dredges shall be 3 m (the same measure was already set by the Regulation (EC) 1967/2006);
- The Commission Delegated Regulation (EU) 2020/3 of 28 August 2019 establishing a discard plan for Venus shells (Venus spp.) in certain Italian territorial waters, in the Article 2 has set that: a) the survivability exemption referred to in Article 15(b) of Regulation (EU) No 1380/2013 is applied, in Italian territorial waters of GFCM Geographical Sub-Areas 9, 10, 17 and 18, to catches of Venus shells (*Venus* spp.) below the minimum conservation reference size made with hydraulic dredges; b) all undersized clam shall be released immediately to the sea (until 31/12/2022); Article 3 has set the MCRS of venus clam at 22 mm (until 31/12/2020);
- The Commission Delegated Regulation (EU) 2020/2237 of 13 August 2020 has extended the MCRS of 22 mm until 31/12/2022.

Fisheries management and measures

Over a period of about 50 years, the management of *C. gallina* fisheries has undergone several evolutionary phases and only recently moved towards a more responsible fishing activity, following the footsteps of the most responsive fishery management systems on international scale. During the first twenty years the clam fishery management was based on a top-down system approach, where the authority (the Ministry) was the only agent designated in decision-making process. The management measures were only based on a fishing effort control and a few and weak technical restrictions: high daily quota and sporadic fishing closure, with no limitation to the number of fishing licenses. All the measures undertaken at that time were not built upon scientific studies and biological and economic analysis, but only on commercial and traditional rules (Carlucci et al., 2015). Despite an initial rapid increase in fleet size and economic revenues, such a centralized system without particularly restrictive management measures led in a few years to a socio-economic decline in the clam fishery sector, as well as a sharp decline in the stock (Spagnolo, 2007).

These conditions flowed into the transition from a micro-management to a co-management system represented by three independent agents, in line with modern international results–based management systems (RBMS): (i) an "Authority" defining specific and measurable and achievable objectives (outcome targets, OTs) for the utilization of fisheries resources, (ii) an Operator/resource user organizations taking responsibility for achieving these OTs and provide documentation that (iii) allows independent auditors (scientific support) evaluating the achievement of OTs (Nolde Nielsen et al., 2015; Santiago et al., 2015) (Figure 7). In light of this, in 1996 the Italian government (the "Authority" of the management system) launched a first clam management program, introducing a series of measures to contain fishing effort by delegating management responsibility directly to industries and fishermen (the "Operator"). The bivalves fishing management has been, therefore, entrusted to the Bivalve Molluscs Management Consortia, established pursuant to Ministerial Decree (M.D.) 44/1995 and 515/1998 and recognized by the Ministry of Agriculture and Forestry Polices. The operational procedures and the prerogatives of the Consortia were defined by the M.D. 22/12/2000 that amended the M.D. 21 July 1998.

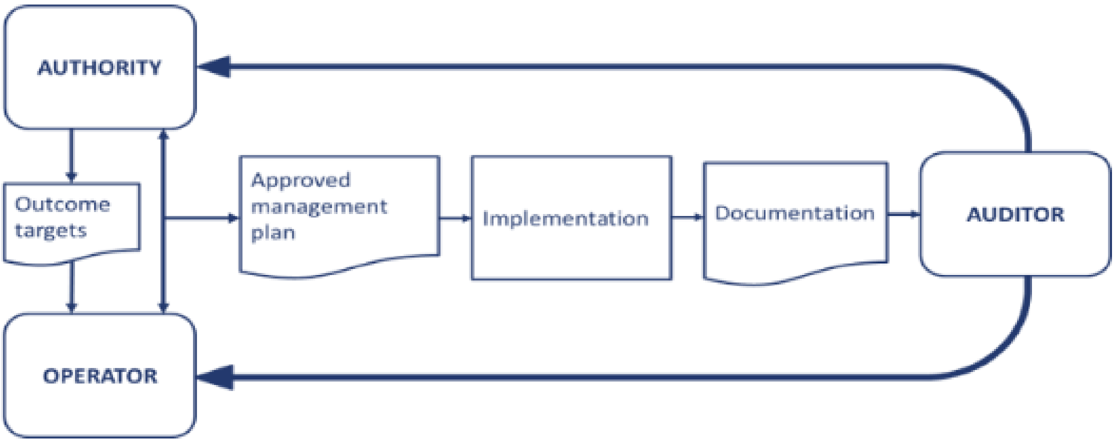

Figure 7. Schematic representation of the organization of the co-management or micro-management system (Source: Nolde Nielsen et al., 2015).

The framework and the general rules are decided at the central level by the Directorate General for Fisheries and Aquaculture, while the Consortia can regulate activities within the waters of jurisdiction adopting more restrictive measures within the boundaries set by national and EU legislation (see Figure 8). Therefore, bivalve molluscs management is actually based on territorial fishing rights, similar to those that exist in other Member States (TURF, Territorial Use Rights for Fishing). Territorial fishing rights, which provide for the full transfer of responsibility to the holders of these rights, is an appropriate and more easily applicable management system in cases where a few species with sedentary habits are targeted (in this case *C. gallina* is the only species with commercial interest). Each fishing district operates and has fishing rights exclusively in its own territory (Maritime District). Therefore, there is no competition between the parties who hold territorial rights and those who practice fishing outside the borders.

Figure 8. Map of Italy indicating all the Maritime Districts fishing for bivalve mollusks scattered along the Italian coasts (black dots), and representing the extension of their waters of jurisdiction (red bars).

The principle that inspired this management system, introduced in the 1990s by national policies was to ensure a balance between the fishing effort exerted in relation to the exploitable areas and the size of the stock. This management system, which is based on measures taken directly by the Consortia, was also adopted to increase the added value of the fished product (only what is required by the market is caught) without over-exploiting the resource.

The different Consortia, in relation with local specificities, have adopted different management measures to safeguard resources and maintain good social and economic conditions. At present, the Consortia have the power to suggest alternative technical measures to the Authority, taking as a reference the limits imposed by the general rules. The current management of fisheries has therefore turned into a bottom-up approach or rather a co-management. Moreover, the Consortia are responsible for the checks on the conformity of the gear used by the boats affiliated (dimension of the dredge, water pressure on the nozzles, space between the rods or diameter of holes of the vibrating sieve etc.) and they can also monitor the fishing areas exploited by the boats (through a GPS device installed in each vessel), to plan a shift of the fishing areas. Finally, the Consortia can carry out operations for moving or sowing the product from more productive areas to areas already exploited. At present, the most effective measures implemented are as follows:

- **Daily quota and fishing days**

The daily quota per vessel lowered over years from 2500 kg in 1986, 600 kg in 1989 to 400 kg in 2017 (M.D. 27/12/2016). At present, Consortia pay their attention to the market demand by collecting just the quantities requested by the market and harvesting even lower quantities than the maximum allowed whenever necessary. This allows to not saturate the market and keep higher prices. The maximum number of 4 fishing days per week foreseen by the law can be further reduced following the market request and the resource availability.

- **Technical and voluntary fishing closures**

Beyond the two compulsory months of fishing closure (set by the Authority) the majority of Consortia adopt additional periods of voluntary fishing closure, related to the market request, resource availability, the natural fluctuations of the stock and mass death events.

- **Fishing effort monitoring**

The vessels authorised to harvest clams adopted a system to monitor and record vessel position at sea as well as the fishing effort through GPS systems.

- **Monitoring of the resource at compartmental level**

The constant monitoring of the resource carried out autonomously by each Consortia with the support of a scientific body, is of key role to assess the spatial distribution and the abundance of both the commercial and undersized fraction. This monitoring is preparatory to adopt seeding, rotation and repopulation activities, which are the most important technical measures undertaken by most of the Consortia in relation to preserving the stock as well as fishing activities.

- **Commercial polices**

The avant-garde commercial policies introduced by the Consortia are of vital importance to make the fisheries profitable, even when the resource is not abundant. The opening of new commercial outlets, new destinations for export, promoting the product locally through events and fairs, are undoubtedly part of a strategy to be pursued as demonstrated by some Adriatic Consortia. In recent years, some Consortia have started to enhance the product caught through the eco-labelling and actually, some of them are certified MSC (Marine Stewardship Council).

- **Inter-Districts management**

The peculiar inter-districts management of two Consortia (Chioggia and Venice) of the Veneto region is the most forefront and interesting management method in the entire Italian national fishing industry. Indeed, all the members of both Consortia share further common management measures (beyond the ones foreseen by the National Management Plans) which resulted in *(i)* the establishment of an important Producer Organisation - the PO “Bivalvia Veneto” that drives the commercial management and hence also influence fishing activities and *(ii)* a fruitful cooperation through which companies are able to better overcome critical situations (e.g. massive mortality events, scarcity of resource) *(iii)* a rotation of the fishing grounds in a larger area, that allows better planning of spaces and better management of the resource.

Each Consortium mandatorily relies on the technical-scientific opinion of a research body of reference (the “Auditor”), chosen by the Consortium itself, for the constant monitoring of the resource and on the basis of which results adopts *ad hoc* management measures.

Annually a standardized survey to assess the clam biomass is carried out at national level, by all the Consortia with the scientific support of a Research Institute; this survey implies samplings on equidistant transects and perpendicular to the coast, with stations at different distance from the shoreline. The results gained are crucial to evaluate the status of the clam stocks in each maritime district and to identify possible further management measures to be applied. This monitoring activity is part of the program National Work Plan for Halieutics Data Collection (PNLRDA) under the EU

Data Collection Framework (DCF), Council Regulation (EC) 199/2008. By linking biological and social-economic aspects, the scientific bodies have defined average values for the density of clams, both as a threshold value below which fishing is not to be permitted (< 5 g/m^2), and an optimal value to be pursued to obtain optimal fishing revenue equal to ≥ 10 g/m^2 (all the values g/m^2 only refer to the commercial fraction) (Italian National Management Plan for hydraulic dredges, 2019).

Every year each Consortium draws up a report indicating the daily, monthly and annual landings of clams, the fishing effort exerted (total monthly fishing days), the months of closure of fishing carried out, both compulsory and optional, as well as a preventive plan that estimates in detail the actions to be taken for the following year. Every five years, based on the information gathered through: *a*) the constant monitoring of the resource at a local level, *b*) the standardized survey at a national level, *c*) the Annual Report drawn up by the Consortia, the Scientific body evaluates the measures taken by the Consortium expressing its favourable or contrary opinion on how the resource was managed in those years, possibly suggesting additional *ad hoc* management measures to be implemented. Based on the opinion of the Scientific Body, the Ministry evaluates the renewal of the management assignment of bivalve mollusc fishing by the Consortia.

What is still needed to be known

To guarantee the success of the management measures adopted (e.g. to define a minimum conservation reference size, closed areas, closed seasons etc.) is fundamental to constantly gain and provide new and updated information about the biological-traits of *C. gallina*. In particular, the aspects that require deeper investigations and considered in the present Ph.D. thesis work are the life-history traits of the species and the consequences experienced by clams and the mega-benthic fauna living associated with the target species due to the dredging activity.

Age and growth

Chamelea gallina optimal growth conditions depend on a series of often changing environmental parameters (e.g. temperature, salinity, dissolved oxygen, hydrology, nature of the sediment, trophism, inter and intraspecific competition; Barillari et al., 1979) which have to find a positive synergy with the biological recruitment peaks that occur along an extended reproductive season (e.g. Poggiani et al., 1973; Froglia, 1975; Valli and Zecchini-Pinesich, 1981; Casali, 1984; Ramón and Richardson, 1992; Gaspar et al., 2004; Rizzo et al., 2011). The environmental and anthropic pressures at which the natural exploited populations are subjected alter the growth parameters of the species, and it is therefore extremely important to know them in order to exploit the resource sustainably, especially if fishing management measures and a MCRS have been established.

In particular, the sound knowledge on the growth strategy and the growth parameters estimated for circumscribed populations represents a fundamental information to develop management plans on a local scale, especially in those areas where clams harvesting has an historical value and represents an economic sector of primary importance, as the case of the Italian striped venus clam fisheries in the Adriatic Sea (Finco and Padella, 2009).

Age and growth have extensively been investigated for bivalve molluscs in different geographical areas with a variety of methods, including mark and recapture (Jones et al., 1978; Ropes et al., 1984; Adjei-Boateng and Wilson, 2013), size-frequency distribution analysis (Froglia, 2000; Herrmann et al., 2009), shell surface growth rings (Fiori and Morsán, 2004; Adjei-Boateng and Wilson, 2013), thin sections (Christian et al., 2000; Dalgiç et al., 2010), growth lines in the internal umbo region (Peharda et al., 2002), acetate peel replicas (Ramón and Richardson, 1992; Gibson et al., 2001; Gaspar et al., 2004; Masu et al., 2008) and isotope analysis (Keller et al., 2002; Mancuso et al., 2019).

Most of these methods involve some disadvantages. Mark and recapture experiments require lengthy procedures to obtain the data (especially in slow-growing species) and involve marking a large number of specimens. Size-frequency distribution analysis is suitable only for fairly young clams since the slower growth of older specimens makes the statistical modes undistinguishable; in addition, it is unsuitable for species with a relatively long annual recruitment period, as previously described for the striped venus clam; and/or highly variable growth within age classes (Gaspar et al., 2004). For *C. gallina* Polenta (1993) also described a broad growth variability. In the Adriatic Sea, its spawning season, which peaks in late spring-early summer, spans approximately from early spring to early autumn (Cordisco et al., 2003a; Rizzo et al., 2010; Scopa et al., 2014). The shell surface growth ring approach (Figure 9a) often yields conflicting results, due to the difficulty of distinguishing true annual rings from false ones, which are generally caused by disturbances such as changes in environmental parameters (e.g. salinity, oxygen concentration, temperature, food availability, pollution, predation), endogenous factors (e.g. reproduction, disease) and even dredger-related stress (Carlucci et al., 2015). In addition, in older specimens the most recent rings are deposited close to one another and near the ventral margin, hampering their distinction, especially if the margin is eroded.

Techniques that overcome these problems, such as acetate peel replicas, thin sections (Figure 9b and Figure 9c) and isotope analyses, have been developed to assess the age and growth of *C. gallina* as well as for several other bivalve species (e.g. Jones et al., 1990; Moura et al., 2009; Versteegh et al., 2010; Ezgeta-Balić et al., 2011; Hernández-Otero et al., 2014), but they are more expensive and time-consuming. By investigating the growth lines in the shell sections, albeit disturbance lines are visible even along the shell section, it is easier to distinguish between true and false annual rings.

In bivalves the annual growth rings consist of a wide opaque bands (light zones) laid down during the period of fast growth and a narrow translucent bands (dark zones) laid down during the slow growth period. The period of deposition of the two kinds of growth bands is species-specific, and for the striped venus clam the former is laid down in summer while the latter in winter (Arneri et al., 1995). However, in the youngest specimens, growth is generally faster during the first years of life, where the majority of the energetic budget is invested in somatic growth rather than reproduction (Bayne and Bayne, 1976).

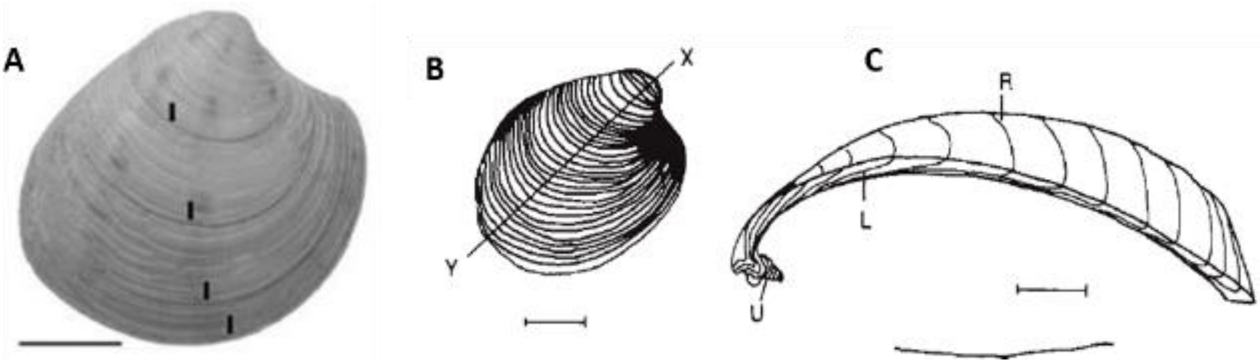

Figure 9. On the left (A), the picture shows surface growth rings (dashes) visible along the shell surface of *C. gallina*. Scale bar 1 cm indicated by the black bar (Source: Gaspar et al., 2004). In the middle (B), it is shown how to make a radial section of the shell along the X-Y axes. On the right (C), is represented a shell section where is possible to observe the internal growth rings along the internal shell section (L) as well as in the internal umbo region (U), while surface growth rings (R) are visible on the shell surface (modified by Gosling, 2008).

To date, most of the works carried out aimed at investigating the growth dynamics of bivalves, including the striped venus clam, by adopting at the same time more than one of the methods described above, in order to compare the results obtained, as well as being able to compare recent works with those of the past which adopted only one of the aforementioned methods.

In general, each technique shows a certain inter-individual growth variability. The extended reproductive period of the species explains much of this variability, being growth extremely influenced by sea water temperature, so that the moment at which birth occurs within the reproductive period will influences the size at age. Moreover, the changing growth parameters in relation to environmental and anthropic pressures over time and space stress out the importance to constantly gain updated information on them for a correct management of the resource.

Reproductive cycle

Bivalves generally have an annual reproductive cycle characterized by multiple spawning events of different intensity (peaks) within the same reproductive season (Park and Choi, 2004). Both in females and males, gametogenesis starts with the proliferation of gonial cells (oogonia and spermatogonia) by mitotic divisions. Oogonia and spermatogonia differentiate into primary oocytes and spermatocytes, respectively. At this stage the processes of meiosis start. The first meiotic division produces secondary oocytes and spermatocytes. In males, during spermatogenesis the second meiotic division occurs soon after the first one, producing four haploid spermatids. These mature into spermatozoa during the process of spermiogenesis. In females, during oogenesis the first meiotic division is arrested at the prophase I when the paired chromosomes are dispersed in the enlarge nucleus (named in oocytes the germinal vesicle). This stage is followed by oocyte growth during vitellogenesis during which reserve materials, such as lipids and glycogen, are synthetized and accumulated. After reaching full size through growth during vitellogenesis, the primary oocytes undergo two meiotic divisions, and the germinal vesicle breakdown occurs through induction by fertilization (Llodra, 2002). Ooocytes can undergo to lysis phenomena especially towards the end of the reproductive cycle (Erkan, 2009).

Chamelea gallina is a dioecious species although some authors (e.g. Corni et al., 1985b) reported some cases of rudimentary as well as functional hermaphroditism. The sex ratio of the species was found to be almost equal to 1:1 in the studied populations (Valli and Zecchini-Pinesich, 1981; Froglia, 1989). The reproduction is external and the life-cycle of the species is characterized by two different stages: the planktonic larval stage (veliger) in the water column, and the adult benthic stage living buried in the sediment (Cordisco et al., 2003a).

The reproductive activity of the striped venus clam is highly controlled by environmental factors such as temperature and food availability, affecting the starting and the long-lasting of the reproductive season over time and space (Cordisco et al., 2003a). *Chamelea gallina* reproduction in the Adriatic is generally reported to span from March to September (e.g.Valli and Zecchini-Pinesich, 1981; Cordisco et al., 2003; Scopa et al., 2014) even if occasional late autumn reproductive events have also been detected (Cordisco et al., 2003, 2005; Rizzo et al., 2011).

Gametogenesis is strongly dependent on the bioenergetics budget of the species and the optimal allocation of energy between somatic growth and synthesis of reproductive material (Giangrande et al., 1995). The allocation of energy from growth to reproduction characterize older specimens, while in the youngest growth is rapid during the early years and lower is the investment in reproduction (Honkoop et al., 1998; Johnson and Smee, 2012). Fecundity in bivalves, expressed as the number of emitted eggs within the same reproductive season, increases with shell size (Park and Choi, 2004; Mzighani, 2005; Delgado et al., 2013). However, a great difficulty exists in studying the reproductive output of bivalves due to the widespread gonad tissue inside the visceral mass (Chavez-Villalba et al., 2003), except in the Pectinidae family where the gonad is a discrete organ. For this reason, so far on bivalves mainly qualitative reproduction studies using standard histological techniques have been conducted (e.g. Gaspar and Monteiro, 1998; Moura et al., 2009; Joaquim et al., 2014), rather than the quantitative ones. However, the semi-quantitative or quantitative methods applied were both direct or indirect. For example, on live specimens the reproductive investment was assessed by inducing spawning through thermal shock or chemical injection to count the number of emitted eggs (Fong et al., 1996; Utting and Millican, 1997; Hamel et al., 2001), or weighting the organisms prior to and after spawning (Pouvreau et al., 2000). On dead specimens instead, only an indirect estimation can be made such as through strip spawned methods (Hendriks et al., 2003, 2005), volumetric reconstruction methods (Galinou-Mitsoudi and Sinis, 1994; Mzighani, 2005; Moles and Layzer, 2008), histological (Joaquim et al., 2011) and immunological methods (Kang et al., 2003).

Another key parameter to be assess in bivalves, especially for the commercially exploited ones, is the size at first maturity (TL_{50}) which can help to evaluate the spawning fraction of the papulation and its related potential fecundity (Delgado et al., 2013). In the Adriatic different authors reported controversial size at maturity (L_{50}) ranging from 12 to 18 mm TL which is commonly reached within the first year of life (e.g. Poggiani et al., 1973; Corni et al., 1985a; Romanelli et al., 2009). Moreover, this value can change over time and space in relation to different local environmental conditions such as sea water temperature and food availability, as well as to anthropogenic, genetic and physiological factors (Da Costa et al., 2013). For this reason, it is important to constantly update these kind of basic biological information of the commercially exploited species *C. gallina*.

Survivability

Data on the effects of fishing on clam survivability are limited (Froglia, 2000; Marin et al., 2003; Morello et al., 2005b; Moschino et al., 2008). Clams harvested with hydraulic dredges are hauled up from the seabed, dumped into a collecting box on board and conveyed to a mechanized sieve for sorting. Since the smaller specimens that pass through the sieve are returned to the sea through a waste exhaust pipe, discarded clams undergo considerable physical stress (Morello et al., 2005a). Even though discards are believed to mitigate the overexploitation of natural populations, the mechanical stress to which they are subject has the potential to reduce their survivability (Moschino et al., 2003, 2008).

At present many studies on different marine species tried to focus on vitality and survival of discarded species (e.g. Hyvärinen et al., 2008; Humborstad et al., 2009; Hochhalter and Reed, 2011). Captive observation is therefore a common technique where discarded animals are transferred into containment facilities (e.g. tanks or underwater cages), after experiencing representative fishing conditions (i.e. capture, handling and release) *in situ* (Enever et al., 2009; Revill et al., 2013; Depestele et al., 2014) or after simulation. However, the species are not actually discarded, but are retained in captivity for a period of time to monitor their vitality and survival. This approach facilitates to monitor the studied species, and allows both dead and surviving animals to be collected to assess for injuries, physiological status and vitality.

Some constraints related to survival estimates are due to the maintenance of wild unacclimatized animals in captivity which can induce stress (Snyder, 1975; Portz et al., 2006) which in turn can lead to captivity-related mortality besides to the treatment effect. Moreover, when transferring the organisms into containment facilities, additional handling can induce further stress underestimating the true value of survivability. On the other hand, many of these techniques isolate the captive population from their natural predators, so they will not account for any predation effects on discard survival (e.g. Raby et al., 2013).

When captive observations are carried out in the field, captivity may also exclude stressors that would otherwise be experienced by discarded animals and so it is possible that the specimens may survive better in the containment facilities than if released. However, in general the additional stressors associated with being contained are considered to have a larger effect on the specimens (Portz et al., 2006), or rather that the method is more likely to induce mortality than to increase survival.

When survival assessment is carried out in the laboratory the specimens are more likely to die immediately after being placed in the tanks or around the end of experiments due to containment (i.e. highly stressed individuals attempting to survive in laboratory conditions eventually die), although in some cases specimens kept in captivity for longer periods can become acclimatized to captivity and potentially behave differently than wild specimens (ICES, 2015). So, it is essential to keep the investigated specimens under as close to natural conditions as possible.

The great difficulty represented by recreating the natural marine environment (e.g. water quality, water movement, food availability, substratum) for the striped venus clam in laboratory conditions explains why so far the majority of the studies investigating the species survival have been conducted in terms of the natural ability of bivalves to survive periods of aerial exposure (e.g. Brooks et al., 1991; Eertman et al., 1993; Moschino and Marin, 2006). Only a long term study was carried out placing clams into controlled laboratory conditions for 7 days to assess their survivability, though without recreating the substrate where clams live (Anjos et al., 2018). The quality of a live sandy sediment deteriorates over time becoming anoxic preventing clam survival, therefore trying to mimic the real habitat of *C. gallina* is not a small challenge. Whereas, when testing the striped venus clam reburial ability in a short period of time (4 h) into laboratory tanks the live sandy sediment was placed (Morello et al., 2006). However, considering that bivalves as *C. gallina* live burrowed in the sediment, it would be of great importance to be able to mimic their natural environment in the laboratory to monitor survivability over days, minimizing stress just by checking the presence/absence of dead clams on the sediment surface, a condition not as easily feasible in the field.

Feasibility is a limiting factor when planning to carry out survivability experiments. As least as far it concerns assessing survivability on bivalve molluscs, while in the field digging the sandy bottom to place anchored cages covered with sediment may limit the level of replication, in the laboratory the costs associated with building up an aquarium system could negatively affect the feasibility of the experiment although allow a higher number of replicates. These kind of studies for *C. gallina* are still lacking, although a great urgency exists to assess the suitability of the management measures undertaken so far.

Impact on the target species

Hydraulic dredges introduction raised concern over the years as this fishing technique leads to the death of target and non-target species, alter habitats and disturb seabed sediments (Hall-Spencer and Moore, 2000; Tuck et al., 2000; Gaspar et al., 2003b; Constantino et al., 2009; Lucchetti and Sala, 2012). Operating on the seabed inevitably causes a physical disturbance to the bottom destabilizing and modifying the conditions of the sediment resulting in a decrease in habitat complexity, with consequences on benthic communities (Kaiser et al., 2000; Carbines et al., 2004; Gilkinson et al., 2005; Vergnon and Blanchard, 2006; McLaverty et al., 2020). Most recent efforts to understand the impact of dredges have focused on deep water fisheries and less is known about the impact of dredges in the costal environment (Soon and Ransangan, 2019). In particular, in costal environments studies have been mainly performed to assess the impact of the dredge on by-catch and macro-benthic communities (Gaspar et al., 2002, 2003a, 2003b; Hauton et al., 2003; Urra et al., 2017; Anjos et al., 2018; Baeta et al., 2021a, 2021b). Studies related to the impact exerted by hydraulic dredging on the target species are few (e.g. Marin et al., 2003; Moschino et al., 2003; Schejter and Bremec, 2007; Vasconcelos et al., 2011; Soon and Ransangan, 2019; Urra et al., 2021) and even lesser the ones that have addressed mitigation measures or gear modifications to reduce the damage inflicted by the gear and increase the post-fishing survival probability on the target species as well as on the by-catch species (Gaspar et al., 2001, 2002, 2003a; Leitão et al., 2009). Shell damage caused on bivalve clams by fishing has been widely recognized, representing a threat on discarded individuals as well as on dislodged ones left on the dredge passage (e.g. Robinson and Richardson, 1998; Gaspar et al., 2003a, 2003b; Hauton et al., 2003; Moschino et al., 2003; Baeta et al., 2021a; Urra et al., 2021). Evaluation of scarring on shells surface has also been adopted as a method to evaluate fishing intensity in dredged areas compared to pristine ones, especially where fishing effort data were lacking (Witbaard and Klein, 1994; Klein et al., 1995; Ramsay et al., 2000; Schejter and Bremec, 2007). The shell damage suffered by the target species can take place i) in the sediment due to compaction of the sand, ii) inside the dredge due to the contact and abrasion between bivalves, debris, and the metallic grid, iii) when tipped from the dredge to the collecting box, or iv) due to the mechanized sieve during the sorting process (Gaspar et al., 2001). Nevertheless, not all the individuals with shell damage die as repaired shells and abnormal calcifications are commonly observed in bivalve mollusks (Lomovasky et al., 2005; Schejter and Bremec, 2007), indicating the ability of the organism to repair the damaged shell (Day et al., 2000; Alexander and Dietl, 2001; Harper et al., 2009).

Multiple factors may influence the fraction of damaged dredged clams such as shell thickness, burrowing depth, shell total length (TL), tow duration, the characteristics of the gear (e.g. tooth length and spacing, inclination of the blade), the increase in engine power and fishing intensity (Gaspar et al., 1994; Gaspar and Monteiro, 1998; Ramsay et al., 2000; Vasconcelos et al., 2011). Nevertheless, the damage induced during fishing operation may cause, in addition to direct fishing mortality, also indirect fishing mortality on discarded and dislodged clams, exerting economic loss. This threat has been ignored for a long time, however for managing purposes it is of key importance to understand the effect of dredging on the discarded fraction and the ability of clams to survive once rejected to the sea.

Impact on the macro-benthic communities

Bycatch is defined as the capture of non-target organisms accidently caught belonging to the discards, viz catches rejected at sea because they are unmarketable species, highly damaged species or individuals below the Minimum Conservation Reference Size (MCRS) (Kelleher, 2005; Tsagarakis et al., 2014). Discards amount is highly variable depending on the métier and sometimes can represent a large fraction of the total catch (Veale et al., 2001; Kelleher, 2005). Concern has raised over years on the impact exerted by the different fishing activities which generate discards (Pranovi et al., 2001; Urra et al., 2017). So far, most of the researches has mainly focused on the impact caused by deep-sea trawling fisheries on bycatch species (Bergmann and Moore, 2001a; Bergmann et al., 2001; Thrush and Dayton, 2002) as well as on commercial ones (Bergmann and Moore, 2001b). On the contrary, albeit attention is now increasing in several parts of the world, only few studies assessed the impact caused by bivalve dredging on shallow costal fishing grounds on the target species (Moschino et al., 2003; Vasconcelos et al., 2011; Soon and Ransangan, 2019) and on the macro-benthic communities (Gaspar et al., 2002, 2003a, 2003b; Urra et al., 2017; Anjos et al., 2018; Baeta et al., 2021a, 2021b). Dredging has traditionally been considered among those fishing activities with a greater impact on costal benthic ecosystems (Collie et al., 2000). However, impact also depends on many other factors such as the time scale (i.e. short and long term) (Piersma et al., 2001), the technical features of dredges (e.g. mesh size, tooth length, water jects, etc.), the fishing effort, the local conditions (e.g. depth, type of sediment, benthic community composition, other stress factors) (Collie et al., 2000) and seasonality (Urra et al., 2017; Baeta et al., 2021a). On the long term, removing species and individuals from their habitat through the generation of discards during common fishing practices – viz changing the species relative abundance and size, together with the population structure of prey and/or predators – can lead to structural and functional disturbances in the ecosystem (Pauly et al., 2002; Thrush and Dayton, 2002). For example, the accidental catch of non-target species can modify the diversity, biomass and productivity of the associated biota (Jennings and Kaiser, 1998), disrupt trophic interactions (e.g. removal of prey and/or predators; Pauly et al., 1998) with subsequent modifications to food webs (Gaspar et al., 2001), change the structure of the benthic communities in the short and long term (Hall-Spencer and Moore, 2000; Jenkins et al., 2001; Ragnarsson et al., 2015), alter species foraging behavior (FAO, 2003), reduce the ratio of large-to-small bodied species (Bianchi and Morri, 2000) and affect fishing yields in other fisheries (Clark and Hare, 1998). For this reason, it is of key importance to have a sound knowledge on the effects derived from fishing at ecosystem level, in order to adopt suitable management plans and actions with the aim to achieve a responsible and sustainable fishing activity.

However, it is known that dredging affect the benthic communities either directly or indirectly (Pranovi et al., 2001; Gaspar et al., 2002). Indeed, discarded or dislodged organisms left on the dredge path may be killed outright, suffer different damage levels which make them susceptible to predation or the ones with minor damage should be able to recover and survive (Mensink et al., 2000; Gaspar et al., 2003b). The injuries suffered by benthic organisms, which may result into death, can occur during the towing of the dredge on the seabed as specimens hit against the bars of the gear or because of abrasion inside the dredge, or during the sieving and discarding processes (Veale et al., 2001). Therefore, it is crucial to analyse the composition of discard in order to propose new strategies to minimize their impact (Urra et al., 2017).

Objective of the thesis

At present the venus clam fishery is highly regulated by national and international laws and the variety of *ad hoc* management measures applied by Consortia aim at making the managing system increasingly sustainable both at social and ecological level. To guarantee the conservation of the resource, the ecosystem and the fishing activities over time, it is of key importance to have a sound knowledge of basic biological information about the species and the effects exerted by the dredge on the target and non-target species followed by their interaction with the fishing gear. In this thesis I deepened and investigated some old and new fundamental life history-traits of the species (e.g. age, growth, reproduction, size at maturity, fecundity) and the consequences experienced by clams (i.e. reburial, survival potential and damage) as well as by the macro-benthic fauna (i.e. damage and mortality rate) after fishing operations. Many of these aspects have been already investigated in the past such as age, growth, reproductive cycle and size at maturity, however these traits are influenced by changing environmental conditions and anthropogenic pressure, and require continuous updating over time. On the eye to provide supporting information for a responsive managing of the striped venus clam fisheries in Italy, new and updated information about the biological traits of *C. gallina* and the interaction gear-species were investigated. Indeed, dated or lacking primary information might compromise the current management and conservation of the stock.

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Chapter 1

Age and growth of striped venus clam *Chamelea gallina* (Linnaeus, 1758) in the mid-western Adriatic Sea: a comparison of three laboratory techniques

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Age and Growth of Striped Venus Clam *Chamelea gallina* (Linnaeus, 1758) in the Mid-Western Adriatic Sea: A Comparison of Three Laboratory Techniques

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Abstract

Age and growth studies provide critical data for clam fishery management. Three aging techniques, thin sections and acetate peel replicas – which involve shell sectioning – and surface growth rings were used to estimate the age and growth of *Chamelea gallina* populations in the mid-western Adriatic Sea. Their results were compared to identify the most reliable and least time-consuming approach. There were no significant differences between the two shell sectioning techniques ($\chi^2 = 4.66$, df = 3, $p = 0.198$), which were described by the same von Bertalanffy (VBF) growth curve parameters ($L_\infty = 43.9$, $k = 0.26$, $t_0 = -0.84$), whereas significantly different L_∞ and k values were found between the two shell sectioning techniques and surface growth rings (L_∞: $\chi^2 = 13.62$, df = 1, $p < 0.001$; k: $\chi^2 = 9.18$, df = 1, $p < 0.002$; these statistics refer to the comparison between acetate peels and surface growth rings). The latter approach proved unreliable and error-prone, as it underestimated age and overestimated the growth rate ($L_\infty = 26.4$, $k = 1.91$, $t_0 = -0.11$). Although the thin sections and acetate peel techniques both provide reliable age and growth estimates, the former approach was less time-consuming. Our analyses demonstrated that shell growth is slower in the cold season and in older specimens and that it has slowed down over the past few decades.

Keywords: *striped venus clam, age, growth, thin sections, acetate peels, surface growth rings*

Introduction

Chamelea gallina (Linnaeus, 1758) is an infaunal filter-feeder clam of the Veneridae family (Bivalvia: Lamellibranchiata: Veneridae) that inhabits the fine well-sorted sand biocenosis described by Péres and Picard (1964). It is widespread in the Mediterranean and Black Seas and along the eastern Atlantic coast. The striped venus clam tolerates limited salinity and temperature variations and requires sandy and muddy–sandy sediments (Moschino and Marin, 2006). In Italy it inhabits a narrow coastal strip at depths ranging from 0 to 12 m up to 1–2 nautical miles (NM) off the coast (Morello et al., 2006). It is particularly abundant in the western central and northern Adriatic Sea, where the massive Po River outflow and the currents flowing along the Italian coast provide abundant nutrients, particles and organic matter (Orban et al., 2007).

Chamelea gallina is a major edible bivalve species throughout the Mediterranean, especially in Italy, Spain, Turkey, and Morocco. In Italy, the fishery consists of 626 hydraulic dredgers, 588 of which operate along the Italian Adriatic coasts, employing ca. 1500 workers and totaling in the last years (2018-2021) annual landings of about 19,000 - 20, 000 metric tons per year and mean revenues even reaching over €50 million (Bargione et al., 2022). *Chamelea gallina* is managed by detailed national and international regulations. In Italian territorial waters its Minimum Conservation Reference Size (MCRS) has been reduced from 25 mm total length (TL) [Council Regulation (CE) No. 1967/2006, 2006 of the European Community (EC)] to 22 mm TL [Delegated Regulation (UE) No. 2016/2376, 2016 of the European Union (EU) and Commission Delegated Regulation (EU) 2020/3, 2020].

Studies of bivalve population dynamics require a thorough knowledge of their growth rate and age and are essential to develop effective fishery management measures (Mancuso et al., 2019). Age and growth have extensively been investigated for bivalve mollusks in different geographical areas with a variety of methods, including mark and recapture (Jones et al., 1978; Ropes et al., 1984; Adjei-Boateng and Wilson, 2013), size-frequency distribution analysis (Froglia, 2000; Herrmann et al., 2009), shell surface growth rings (Fiori and Morsán, 2004; Adjei-Boateng and Wilson, 2013), thin sections (Christian et al., 2000; Dalgiç et al., 2010), acetate peel replicas (Ramón and Richardson, 1992; Gibson et al., 2001; Gaspar et al., 2004; Masu et al., 2008) and isotope analysis (Keller et al., 2002; Mancuso et al., 2019).

Most of these methods involve some disadvantages. Mark and recapture experiments require lengthy procedures to obtain the data (especially in slow-growing species) and involve marking a large number of specimens. Size-frequency distribution analysis is suitable only for fairly young clams since the slower growth of older specimens makes the statistical modes undistinguishable; in addition, it is unsuitable for species with a relatively long annual recruitment period and/or highly variable growth within age classes (Gaspar et al., 2004). In *C. gallina* gonad development and gamete emission are closely related to water temperature, as reported for various venerids living in temperate areas (Tirado and Salas, 1998; Rizzo et al., 2011). Polenta (1993) also described a broad growth variability for this species. In the Adriatic Sea, its spawning season, which peaks in late spring-early summer, spans approximately early spring to early autumn (Cordisco et al., 2003; Rizzo et al., 2010; Scopa et al., 2014). The shell surface growth ring approach often yields conflicting results, due to the difficulty of distinguishing true annual rings from false ones, which are generally caused by disturbances such as changes in environmental parameters (e.g., salinity, oxygen concentration, temperature, food availability, pollution, predation), endogenous factors (e.g., reproduction, disease) and even dredger-related stress (Carlucci et al., 2015). In addition, in older specimens the most recent rings are deposited close to one another and near the ventral margin, hampering their distinction, especially if the margin is eroded.

Techniques that overcome these problems, such as acetate peel replicas, thin sections and isotope analyses, have been developed to assess the age and growth of *C. gallina* as well as for several other bivalve species (e.g., Jones et al., 1990; Moura et al., 2009; Versteegh et al., 2010; Ezgeta-Balic et al., 2011; Hernández-Otero et al., 2014), but they are more expensive and time-consuming.

Given the sedentary habits of bivalves and the influence exerted by environmental and endogenous conditions on growth in different geographical areas (Gaspar et al., 2004), the assessment of age population structure for management purposes must be performed on the local scale. This study examines the age structure and growth rate of *C. gallina* populations harvested in the mid-western Adriatic Sea by three techniques – thin sections, acetate peel replicas and shell surface growth rings – to identify the most accurate and least time-consuming method. The results are discussed in the light of current regulations and in particular compared with those obtained by similar studies conducted in the same area.

Materials and Methods

Sampling

Chamelea gallina individuals were obtained from November 2018 to October 2019 from commercial hydraulic dredges conducting normal fishing operations in the Ancona and San Benedetto del Tronto Maritime Districts (mid-western Adriatic Sea; Figure 1). The dredges exploited fishing grounds characterized by sandy sediment located more than 0.3 NM from the coast at depths ranging from 5 to 12 m. An unsieved sample of about 30 specimens comprising all the available size classes, including individuals under the MCRS, was obtained monthly from the dredge collecting box. Only clams with undamaged valves were studied. For each specimen, the TL (maximum distance along the anterior-posterior axis) and height (H; maximum distance along the dorsoventral axis) of the shell were measured to the nearest 0.1 mm using a digital vernier caliper. The valves were then opened with a cutter to remove the flesh, air-dried and numbered for further processing.

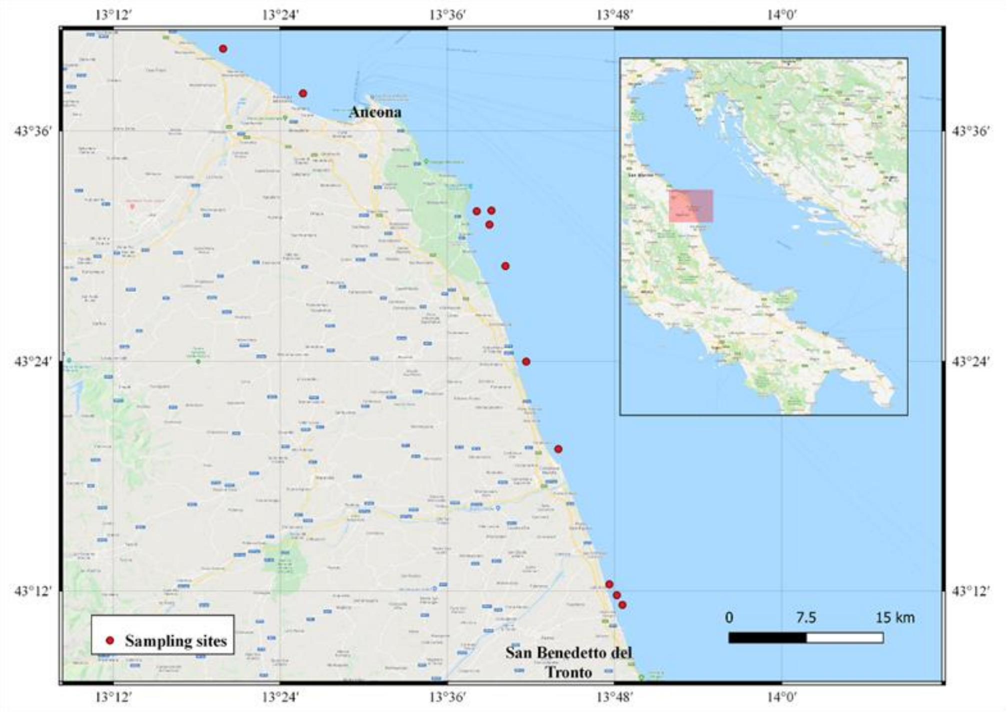

Figure 1. Map of the sampling area.

Thin Sections

The right valves of 353 specimens were placed in a silicon mold and embedded in polyester resin as described by Rhoads and Lutz (1980). Once hardened, the blocks were cut radially into 1mm-thick sections, from the umbo to the ventral margin along the axis of maximum growth, using a high-speed saw equipped with a diamond blade. The surfaces of each section were ground flat using successive finger grits (800, 1000, 1200 µm) and wetpolished with a polishing compound to obtain the required texture and thickness. In each thin section, the number of annual growth rings was determined by counting the alternating opaque (carbonate matrix) and translucent (carbonate-organic matrix) increments visible on the shell cross-section (Arneri et al., 1995) using a dissecting microscope under reflected light at low magnification (6.4x). Alternatively, growth rings were counted in the region of the umbo when not easily detectable along the shell section. However, assuming that the growth rings are laid down yearly, the age of each clam was estimated by counting all the translucent zones after marking them with a black marker pen along the shell section. The distance between the umbo and the ventral margin of each growth ring was measured with a vernier caliper. For edge analysis, the distance between the last annual growth ring and the ventral margin of the shell was measured using a video analysis system connected to the dissecting microscope, to determine how the shell margin extent (opaque zone) varied over the months.

Acetate Peels

The other halves of the resin blocks, which were processed to obtain the thin sections, were used to prepare the acetate peel replicas. For this time-consuming analysis we only prepared a subsample, which yielded a total number of 118 readable slides, encompassing all the available size classes but not all the months. After grounding flat and polishing their surfaces as described above, the half blocks were immersed for 2 min in 1% HCl solution, which dissolves the carbonate parts but preserves the organic part of the matrix, resulting in a relief on the sectioned shell surface. Acetate peel replicas were prepared as described by Rhoads and Pannella (1970) and Richardson et al. (1979). The pattern emerging after treatment in HCl solution was transferred to a 0.1 mm thick sheet of cellulose acetate, previously immersed in an ethyl-acetate solution and then laid on the shell surface block. Once dried, the acetate peels were placed on a microscope slide and photographed under a light microscope connected to a video analysis system, to identify the annual growth rings. Each annual ring was marked directly on the peel using a black marker pen. The distance between the umbo and each ring was measured with a digital vernier caliper.

Surface Growth Rings

All the left valves of the 353 specimens were examined for the annual growth rings, which appeared as smooth clefts on the shell surface and as strong pigmented lines across the anterior-posterior axis. The distance between the umbo and each ring was measured using a digital vernier caliper along the dorsoventral axis.

Data Analysis

Since the measurements taken with all three techniques were relative to shell height (H, maximum distance between the dorsal-ventral margin), the height-at-age data were converted to shell TL using an equation resulting from the height-length relationship, as follows:

$$H = 0.815\ TL + 1.5645\ (r^2 = 0.98;\ N = 353)$$

The mean length-at-age was calculated and compared among techniques. The annual increments were calculated by subtracting the mean TL of the younger age class from the mean TL of the older class immediately above it.

The age of each individual was calculated at half a year, based on the capture date and on the assumption that the translucent bands form in winter, considering as conventional birthday the 1st of July. Accordingly, half a year was added to all the specimens collected from 1st January to 30th June and to all those aged 0+. The age readings were performed by the same reader 2 weeks apart. Their accuracy was estimated by calculating and comparing the average percent error (APE) and average coefficient of variation (ACV) of each technique. To describe the growth patterns, the following von Bertalanffy Growth Function (VBGF) was fitted to the length-at-age data:

$$E\,[L]\,t = L_{\infty}1 - e^{-k(t-t_0)}$$

where E[L]t is the mean length-at-age t and L_{∞} (theoretical maximum length), k (growth coefficient) and t_0 (theoretical age at length zero) are the parameters to be estimated.

The LogLikelihood ratio test was applied to increasingly less complex nested models by setting and varying the parameters of the two VBGFs (Ogle, 2016), to compare the VBGF parameters of pairs of techniques. The first two models to be tested were the ones which showed different and equal values of the von Bertalanffy (VBF) growth curve parameters (L_{∞}, k, t_0). Bias plots were built according to Campana et al. (1995) and Muir et al. (2008) to compare the age estimates obtained by the two shell sectioning methods. All analyses were performed using the free software R (R Core Team, 2013) and the FSA package (Ogle et al., 2020).

Results

Thin Sections and Acetate Peels

The specimens analyzed using the thin sections technique ranged in size from 12.4 to 37.7 mm. The annual growth rings, consisting of wide opaque bands (light zones) laid down in summer (fast growth period) and of narrow translucent bands (dark zones) laid down in winter (slow growth period) were easily detected (Figure 2a). Since the bands are deposited parallel to the ventral margin of the shell, they could also be seen in the region of the umbo (Figure 2b), which helped the counts when the sections were not clearly readable. The method provided age estimates ranging from 0.5 to 6.5 years (Figure 3a). Indices of age precision within readings performed by the same reader were very low (APE = 1.22%; ACV = 4.34%), reflecting good method consistency and reproducibility. The mean length-atage and the standard error (SE) are reported in Table 1. In particular, 1-year-old specimens reached 14.57 ± 0.11 mm TL and 2-year-old specimens 21.0 ± 0.13 mm TL. The older individuals showed increasingly narrow annual growth rings (Table 2). Moreover, edge analysis showed that the largest margin increment was laid down in summer and the smallest was deposited in autumn-winter

(Figure 4). This was confirmed by the margin extent of a specimen caught in June 2018, which measured 2.4 mm (Figure 2a).

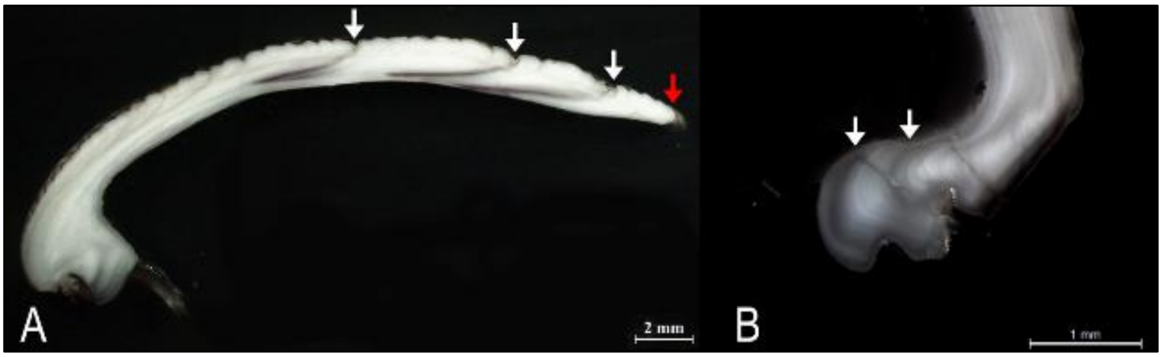

Figure 2. (A) Thin section of a *Chamelea gallina* shell from a specimen harvested in June 2018. White arrows: annual growth rings; red arrow: marginal increment. (B) Additional individual in which annual growth rings were observed in the umbo region (white arrows) when growth rings along the sections were not clearly readable.

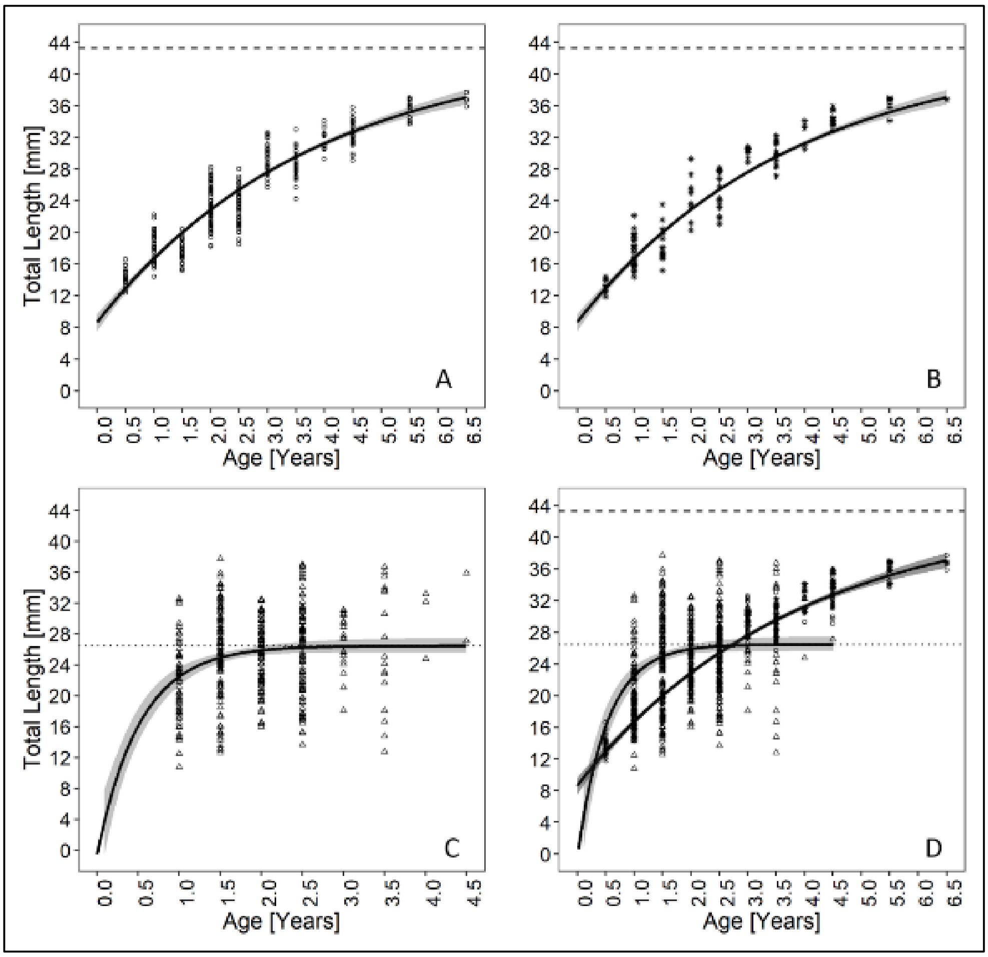

FIGURE 3. *Chamele gallina* growth curves determined using (A) thin sections, (B) acetate peel replicas, (C) surface growth rings and (D) by fitting all the von Bertalanffy (VBF) growth curves together. The shaded areas around the growth curves are the 95% CIs. Different dot types represent the observed individuals, being respectively 353 for both thin sections and surface growth rings techniques and 118 for the acetate peels.

Table 1. Maximum, minimum and mean length-at-age and respective SE of *Chamelea gallina* specimens from the mid-western Adriatic Sea calculated with the three ageing techniques.

	Age (years)	Mean TL (mm)	SE (mm)	TL min (mm)	TL max (mm)
Thin sections	1	14.57	0.11	9.12	21.39
	2	21	0.13	14.64	27.16
	3	26.33	0.16	20.41	30.96
	4	30.55	0.26	25.81	34.52
	5	34.57	0.28	31.82	36.85
	6	36.39	0.51	35.25	37.71
Acetate peels	1	15.25	0.18	10.84	19.67
	2	21.69	0.25	15.99	26.79
	3	27.45	0.28	22.86	32.68
	4	31.7	0.43	27.16	35.01
	5	34.19	0.63	29	36.48
	6	36.54	0.91	35.13	37.73
Surface growth rings	1	17.61	0.26	3.96	30.94
	2	21.96	0.32	8.26	33.54
	3	24.16	0.7	10.71	36.48
	4	27.26	1.15	24.58	30.59

Table 2. Mean annual growth rate calculated with the three ageing techniques in the different age classes.

	Mean size (mm)		
Age class (years)	Thin sections	Acetate peels	Surface growth rings
0-1	14.5	15.2	17.6
1-2	6.43	6.45	4.34
2-3	5.33	5.75	2.2
3-4	4.22	4.25	3.1
4-5	4.02	2.49	_
5-6	1.82	0.94	_

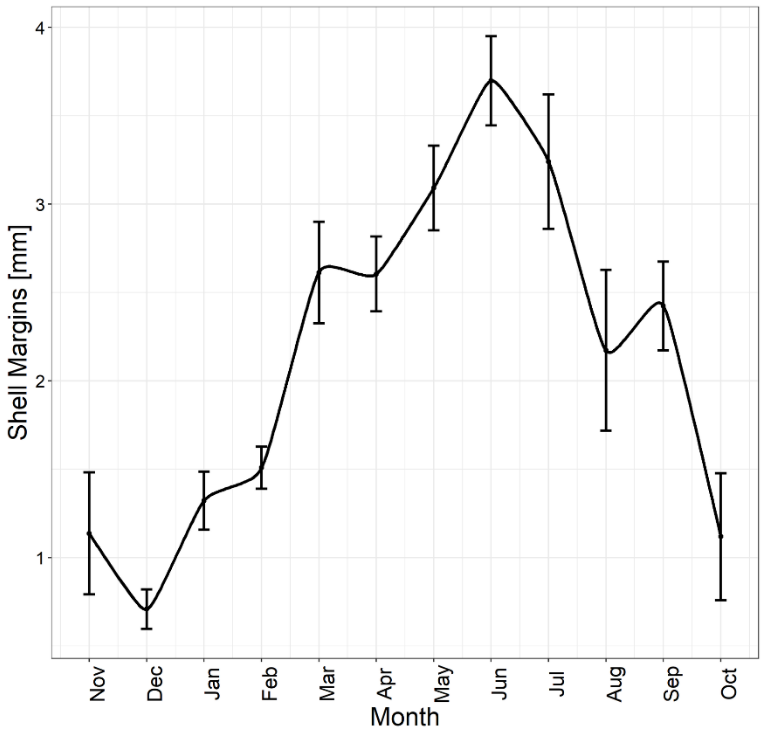

Figure 4. Edge analysis. Different mean shell margin extents across months and associated SE (black bars) of 353 specimens analysed by mean of the thin section technique throughout the year, highlighting the maximum margin extent occurring during the summer season.

The acetate peel replica technique allowed identifying seasonal growth bands deposited parallel to the ventral margin on the outer (prismatic) shell layer. Annual growth rings were identified as alternating clusters of wider and narrower bands. Under the light microscope the growth bands were visible as more (Figure 5a) or less distinct (Figure 5b) dark lines. The annual growth rings were formed by clusters of wide and narrow bands, which often ended with a cleft (Figure 5c,d). Occasionally, a ring showed two clefts (Figure 5e). This regular pattern allowed distinguishing real rings from false ones, which though characterized by a cleft, appeared as a sudden interruption of the regular pattern and did not show a regular narrowing of the band, but a mixture of different growth increments (Figure 5f).

The age of the specimens analyzed, which measured 11.9 to 37.7 mm, was estimated to range from 0.5 to 6.5 years (Figure 3b). The values of the indices of age precision were very low (APE = 1.18%; ACV = 2.92%), reflecting good method consistency and reproducibility. The mean length-at-age and SE were comparable to those obtained with the thin section method (Table 1). In particular, 1-year-old clams measured 15.25 ± 0.18 mm TL and 2-year-old specimens 21.69 ± 2.10 mm. The annual growth rate was faster in the first year of life (ca. 14– 15 mm) and slower in the following years, as also noted in the thin sections (Table 2). The absence of significant differences between the two shell sectioning approaches on the LogLikelihood ratio test ($\chi^2 = 4.66$, df = 3, $p = 0.198$) indicated that both were described by the same VBF growth curve parameters. Their VBF growth curve parameters (L_∞ = 43.9, k = 0.26, t_0 = −0.84) and their 95% confidence intervals (CIs) are compared with those of the shell surface growth rings in Table 1. Moreover, the ACV and APE between techniques were low, respectively 4.61 and 3.26%. Age bias plots showed that the mean age points lie almost perfectly on the agreement line, without differences in the age estimates yielded by the two techniques (Figure 6).

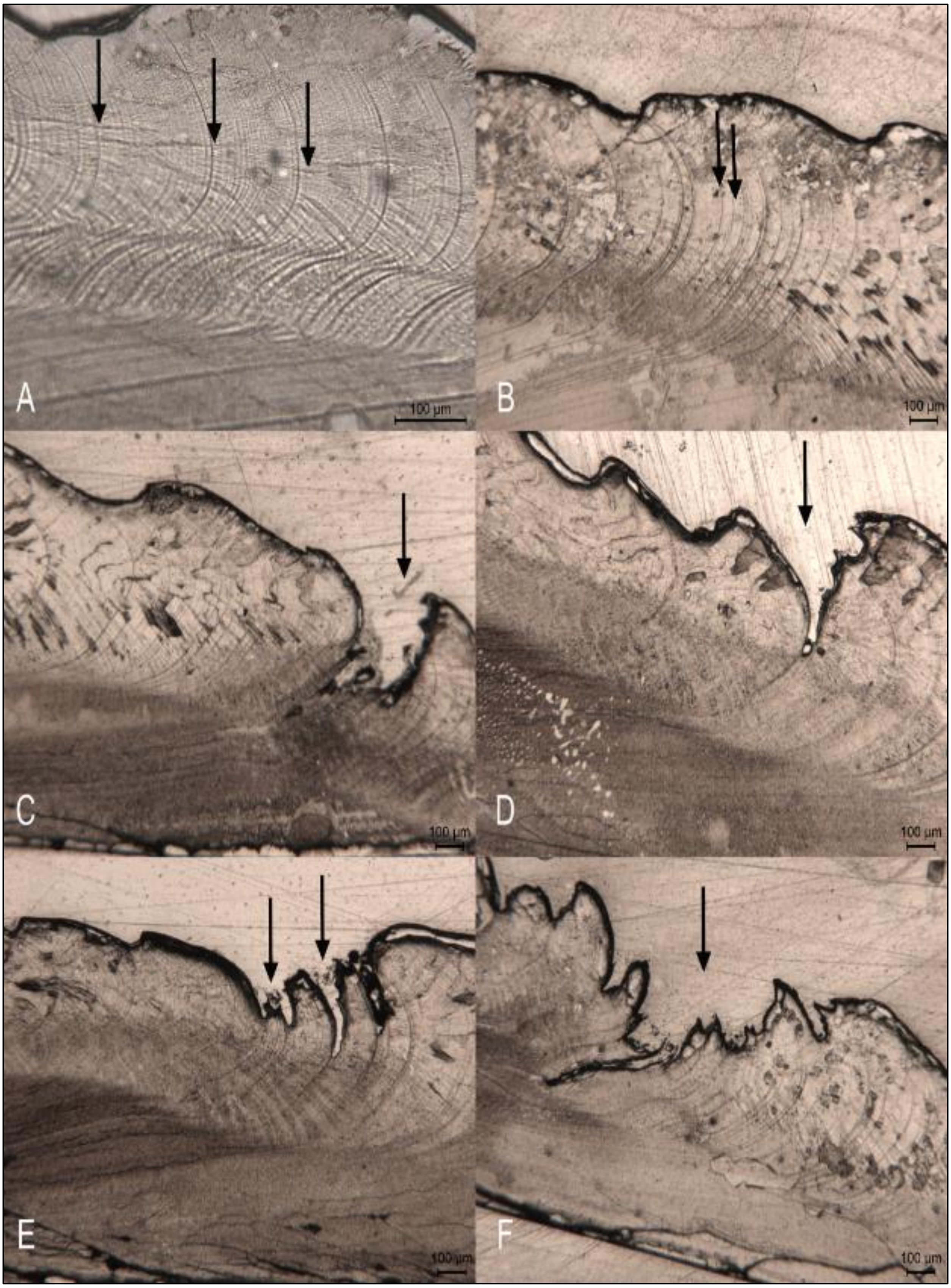

Figure 5. Photomicrographs of acetate peel replicas of *Chamelea gallina* shell sections. (A) Widely spaced and marked bands deposited during the fast growth period (arrows). (B) Narrower and less defined bands deposited during the slow growth period (arrows). (C, D) Annual growth ring with an associated cleft (arrow). (E) Annual growth ring with two occasional associated clefts (arrows). (F) A stress ring (arrow).

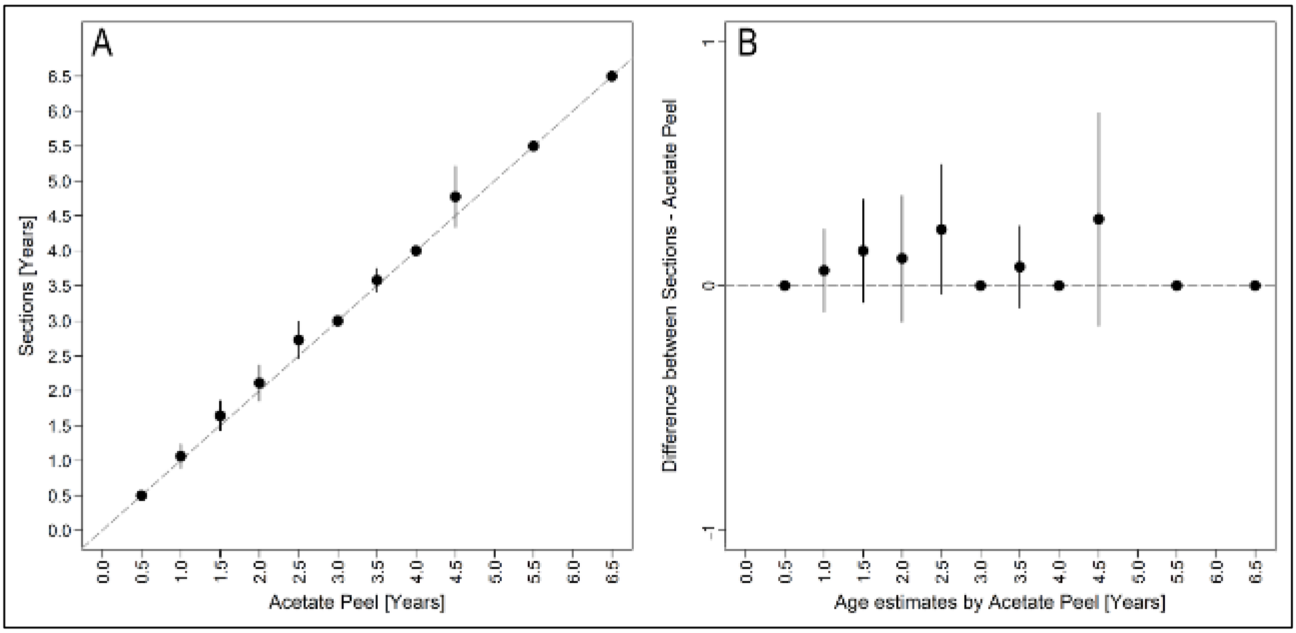

Figure 6. Bias plot comparing (A) differences in age readings between the two shell sectioning techniques as described by Campana et al. (1995), and (B) the differences between thin sections and acetate peels readings versus the acetate peels age estimates as described by Muir et al. (2008).

Surface Growth Rings

All the specimens used to asses age with the thin sections (length range, 12.4 – 37.7 mm) were also analyzed by the surface growth ring technique (Figure 7). The age estimates provided by this method ranged from 1.0 to 4.5 years (Figure 3c), thus missing the smallest and the largest age classes compared to the other two techniques (Figure 3d). Moreover, according to the VBF growth curve most specimens were aged 1.0–2.5 years, irrespective of their size, despite the relative low values of the precision indices (APE = 5.55%; ACV = 7.85%) reflected good estimates of the age. The VBF growth curve parameters ($L_\infty = 26.4$, $k = 1.91$, $t_0 = -0.11$) and their 95% CIs (Table 3) differed widely from those of the other two techniques. Indeed, the LogLikelihood ratio test highlighted significantly different L_∞ and k (L_∞: $\chi^2 = 13.62$, df = 1, $p < 0.001$; k: $\chi^2 = 9.18$, df = 1, $p < 0.002$) between acetate peels/thin sections and surface growth rings, whereas the t_0 values did not show any significant differences ($\chi^2 = 0.93$, df = 1, $p = 0.335$). The very high value of the instantaneous growth rate ($k = 1.95$) indicated a very fast growth, while the other methods yielded values ranging from 0.24 to 0.36, indicating a slow growth. The asymptotic length (26.3 mm) assumed by the model was also much lower than the maximum shell length of 37.7 mm recorded at sea. The mean length-at-age underestimated age size classes 3 and 4 compared with the other two techniques (Table 2), even without considering the absence of age classes 5 and 6. The minimum TL measured in 1-year-old specimens was an unrealistic 3.96 mm, indicating that false rings may occur throughout the shell height. Unlike the shell sectioning techniques, this method highlighted an alternate annual growth rate pattern (Table 2). Due to the very different age estimates, the age bias plot of the surface growth rings and one of the two shell sectioning methods is not reported.

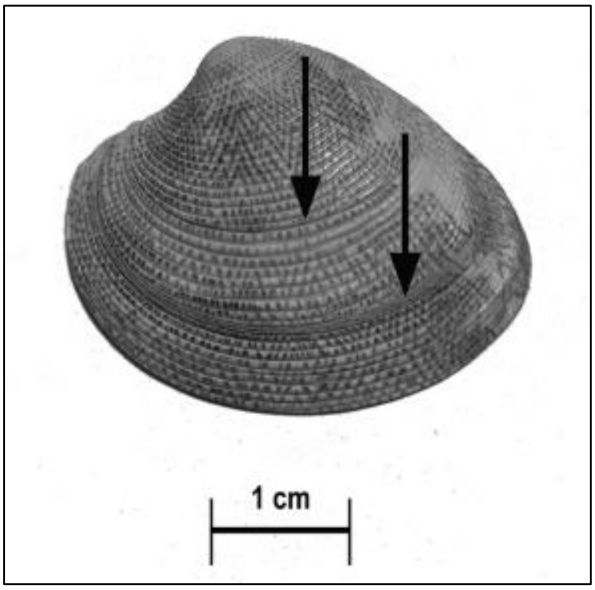

Figure 7. Surface growth rings (arrows) on the shell of *Chamelea gallina* aging 2 years old following this approach. The specimen measured 37.7 mm total length and was aged 6 years according to both the thin section and the acetate peel replica techniques.

Table 3. Mean VBF growth curves parameters and respective CIs assessed by the model for *Chamelea gallina* from the mid-western Adriatic Sea using the three ageing techniques. Thin sections and acetate peels shared similar growth parameters.

		Mean	CI -	CI+
Thin sections and acetate peels	L_∞	43.27	40.39	47.12
	k	0.26	0.21	0.31
	t_0	-0.84	-1.05	-0.66
Surface growth rings	L_∞	26.46	25.64	27.48
	k	1.91	1.49	2.47
	t_0	0.01	-0.11	0.09

Table 4. Length at age, k, L_∞, t_0 and maximum estimated age values obtained with different ageing techniques in C. gallina specimens harvested inside and outside the Adriatic Sea (W = western, E = eastern, N = northern, S = southern).

Location	**Method**	**L1**	**L2**	**L3**	**L4**	**L5**	**L6**	**L7**	**L8**	**L9**	**L10**	**Age max**	**L max**	**L_∞**	**k**	**t_0**	**Reference**
Mid-W Adriatic	Thin sections	14.57	21	26.33	30.55	34.57	36.39					6	37.7	43.27	0.26	-0.84	Present study
Mid-W Adriatic	Acetate peels	15.25	21.69	27.45	31.7	34.19	35.13					6	37.7	43.27	0.26	-0.84	Present study
Mid-W Adriatic	Surface rings	17.61	21.96	24.16	27.26							4	37.7	26.46	1.91	0.01	Present study
Mid-W Adriatic (Ancona)	Length-frequency	17	25									2	50				Froglia (1975,1989, 2000)
Mid-W Adriatic (Ancona)	Thin sections	17	25	30	34	38	39	42	45			8	49	49.63	0.25	-0.79	Polenta (1993)
Mid-W Adriatic (Ancona)	Back-calculation	16	25	31	35	37	38.5	39.5				7	49	40.83	0.48	-0.06	Polenta (1993)
Mid Adriatic	Thin sections											8		41.6	0.48	-0.01	Arneri et al. (1995)
E Adriatic	Thin sections											6	46	39.5	0.52	-0.13	Arneri et al. (1997)
Mid Adriatic (Fano-Pesaro)	Surface rings	15	24	30	33	35						5	46				Poggiani et al. (1973)
S Adriatic (Bari)	Surface rings	15	23	31	35	38	42					6					Marano et al. (1982)
N Adriatic (Gulf of Trieste)	Isotopes											4					Keller et al. (2002)
W Adriatic	Surface rings											3		41.36	0.54		Mancuso et al. (2019)
W Adriatic	Thin sections											3		42.02	0.5		Mancuso et al. (2019)
W Adriatic	Isotopes											3					Mancuso et al. (2019)
E Adriatic (Gulf of Manfredonia)	Surface rings														0.79		Vaccarella et al. (1996)
S Portugal	Surface rings	19.2	28.8	32.7	36	37.5						5	40	37.55	0.71	0.01	Gaspar et al. (2004)
S Portugal	Acetate peels	17.3	25.3	30.7	33.6							4	40	38.95	0.47	0.24	Gaspar et al. (2004)
S Portugal	Length-frequency	18.2	24.7	29.5	33	35.5						4	40	42.15	0.32	0.76	Gaspar et al. (2004)
W Mediterranean (Valencia, Spain)	Acetate peels	20	25	28	31							4		36.12	0.35		Ramón & Richardson (1992)
W Mediterranean (Valencia, Spain)	Length-frequency													40.05	0.4		Ramón (1993)
W Mediterranean (Ebro, Spain)	Surface rings	18	24	28	31							4		36.12	0.35		Vives & Suau (1962)
N Marmara Sea, Turkey	Surface rings	16.15	22.44	26.53	29.2	30.93						5	34.5	34.17	0.43		Deval & Oray (1998)
N Marmara Sea, Turkey	Acetate peels	15.3	20.8	24.7	27.3	29.2	30.5	31.4				6	34.3	33.46	0.37		Deval (2001)
N Black Sea	Thin sections											9	31	27.5	0.61	-0.14	Boltacheva & Mazlumyan (2003)
Black Sea	Thin sections	6.7	10.4	13	14.8	16	17.2	18.7	19.9	20.9	22.2	10	28.7	26	0.16	-1.96	Dalgiç et al. (2010)

Discussion

Age and growth studies provide crucial clam fishery management data. Although these parameters are generally considered together, each provides unique and useful information on specimens and populations. A variety of aging methods have been applied to assess *C. gallina* growth and population structure (Table 4). In this work, three techniques (thin sections, acetate peel replicas and surface growth rings) were compared to identify the most reliable and least time-consuming approach. Acetate peel replicas had never been employed to estimate the growth of *C. gallina* harvested in the Adriatic, despite its successful use in other geographical areas (e.g., Ramón and Richardson, 1992; Deval, 2001; Gaspar et al., 2004). According to our findings, the two shell sectioning techniques were equally reliable, whereas the shell surface growth ring approach underestimated age. Indeed, the shell surface growth ring approach has yielded contrasting results in various bivalve species, either underestimating (Gaspar et al., 2004; HernándezOtero et al., 2014) or overestimating age (Gaspar et al., 1995; Peharda et al., 2002), despite the occasional success (Mancuso et al., 2019). In our study, underestimation was due to the large amount of shells presenting a smooth surface with no clefts, independently from the size, which led to a mismatch with the length-at-age data derived from the internal readings. The L_8 value of 26.46 mm estimated by the model was lower than the maximum specimen length (37.7 mm) measured at sea; this result, combined with age underestimation (which in turn led to overestimation of the k value), made the approach unreliable. Moreover, the wholly unrealistic age attributed to the smallest specimens, aged 1, 2, and 3 years (measuring respectively 3.96, 8.26, and 10.71 mm TL) demonstrated that false rings may occur throughout the shell surface, whereas the age of 1 year, attributed to a specimen measuring 30.94 mm TL, was the result of the absence of visible annual growth rings, a feature that was shared by the majority of our samples. The method was therefore unreliable and error-prone, due to inherent difficulties related to the absence/misinterpretation of rings on the shell surface. Misinterpretation problems can partly be overcome by investigating the growth lines in the shell sections, albeit disturbance lines are visible even along the shell section, it is easier to distinguish between true and false annual rings. The growth bands on *C. gallina* thin sections has been validated in the western Adriatic Sea by Arneri et al. (1995), who determined that the translucent (dark zones) bands are laid down once a year, approximately between October and February, whereas the opaque bands (light zones) are deposited from March to September. Indeed, our edge analysis results, indicating that the largest margin increments were recorded in summer and the lowest in autumn-winter, were also confirmed by the observation of a specimen caught in June 2018, where the shell margin extent was 2.4 mm.

The two shell sectioning techniques demonstrated that the growth rate decreases as specimens become older, highlighting a very fast growth in the first year of life (of 14–15 mm TL). In the second year the growth rate had more than halved already. Several factors, including spawning, food availability, type of substratum, depth, light, temperature, salinity and population density may affect shell growth rate (Gaspar et al., 2004; Dalgiç et al., 2010). A recent study by Mancuso et al. (2019) did not highlight any difference in the growth parameters of *C. gallina* over a wider latitudinal range along the Italian Adriatic coasts, even including our area of investigation. This is why we did not consider to investigate differences in growth parameters over sites. Growth is the result of linear extension along the umbonal-ventral axis per unit of time, and slows down with increasing age or size (Lorrain et al., 2004), as also confirmed by isotope analysis (Keller et al., 2002; Mancuso et al., 2019).

A comparison of our length-at-age results with data from works conducted in the same area (Froglia 1975, 1989, 2000; Polenta et al., 1993; Arneri et al., 1995) highlighted a reduction in the maximum shell length ever recorded at sea on fishing grounds (see Table 4). The differences in length-at-age data reported outside the Adriatic might be explained by different ecological conditions (Polenta, 1993; Gaspar et al., 2004; Dalgiç et al., 2010). In the Adriatic the estimated length at 1 year was very similar with all techniques, whereas differences emerged from the second year. In the past, 2-year-old clams have been reported to have a mean length of about 25 mm TL (the previous MCRS), whereas in this study it was just under 22 mm (the current MCRS), reflecting a reduction in shell growth rate over time that has already been reported by Biondi and Del Piero (2012) in the Gulf of Trieste. Froglia (1987), by mean of the length frequency distribution approach, described a limited proportion of 3-year-old clams (TL > 35 mm) in the areas examined in the present study, making us thinking that the growth rate was faster at the time. We can speculate that the fishing pressure may have induced a reduction in shell growth in the Adriatic, as in the Black Sea the growth rate has declined in areas subject to high fishing pressure compared to non-dredged areas, where clams grew faster (Dalgiç et al., 2010).

However, fishing pressure cannot be computed as the only cause of the potential shell size reduction. Surveys conducted in the Adriatic by the National Research Council - Institute for Biological Resources and Marine Biotechnologies (CNR – IRBIM) of Ancona (Italy) in 2017, 2018, and 2019, when the MCRS was already 22 mm TL, found tens of thousands specimens under the MCRS per 100 m^2 (DGPEMAC, 2019), reflecting not only a strong recruitment of the species supporting the commercial fractions of the stock for the following years, but also a very high density of specimens per unit area. In areas characterized by such density the strong competition for food limits growth and in extreme cases leads to mass mortality events (Liu et al., 2006). Fluctuations in salinity and rising chlorophyll concentration negatively affect *C. gallina* growth in the western Adriatic Sea (Mancuso et al., 2019), as well as temperatures below 10°C and above 27°C slow or inhibit shell linear extension rates (Ramón and Richardson, 1992; Moschino and Marin, 2006;

Romanelli et al., 2009). Even a study carried out at the coastal station of the LTER (Long Term Ecosystem Research) Senigallia-Susak transect, ca. 40 km to the north of our study area, showed significantly increased phytoplankton abundance and biomass and inorganic nutrient concentrations in the period 2007–2016 compared with 1988– 2002 due to increased Po River flows, which were observed since 2007; moreover, abnormally abundant rainfall combined with greater freshwater inputs in 2007–2016, especially in winter, explained the decreasing salinity trend recorded in the same years (Totti et al., 2019). Water acidification also affects clam shell growth (Fabry et al., 2008) and reduces shell thickness (Bressan et al., 2014). Even though increasing water acidification has been documented in north Adriatic dense deep waters (Luchetta et al., 2010), the change is too limited to reduce the saturation state of carbonates to an extent that would significantly affect clam calcification processes (Totti et al., 2019).

Therefore, shell growth decline may be the result of the synergistic action of multiple factors. Studies should also be carried out in non-dredged areas, to understand and quantify the role of fishing activity. Detailed studies of the population age structure and growth of *C. gallina* are essential, since uncertainties in age estimation undermine the effectiveness of management actions. A responsible management plan for the striped venus clam fishery should take into account the biological aspects of the species and the effects of the gear on the populations, and provide guidelines to ensure the persistence and conservation of the species over time. A management plan capable to return larger individuals as in the past should not to be intended as an appropriate measure, not possible condition due to the intrinsic characteristics of the gear, but a suitable plan is the one able to allow the exploitation of the resource not exceeding the maximum sustainable yield. The present study found that clams of 22 mm TL were, on average, individuals of two years old while in the past decades the achievement of the second year was on average reported at about 25 mm TL. Therefore, to allow the maintenance in Italian territorial waters of the present MCRS set at 22 mm TL [Delegated Regulation (UE) No. 2016/2376, 2016; Commission Delegated Regulation (EU) 2020/3, 2020] by way of derogation to the previous 25 mm TL [Council Regulation (CE) No. 1967/2006, 2006] further studies including investigations into the reproductive potential of *C. gallina* at different sizes should be carried out based on the knowledge that the species reaches the size of maturity during the first year of life (Cordisco et al., 2005).

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics Statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Author Contributions

GB wrote the manuscript with the contribution of CV, MV, and AL. GB, MV, and AP collected the samples. GB, FD, and AP conducted the different lab activities. CV performed the statistical analysis. AL was the scientific responsible of the study. All the authors contributed to the article and approved the submitted version.

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Chapter 2

Chamelea gallina reproductive biology and Minimum Conservation Reference Size: implications for fishery management in the Adriatic Sea

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RESEARCH ARTICLE Open Access

Chamelea gallina reproductive biology and Minimum Conservation Reference Size: implications for fishery management in the Adriatic Sea

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Abstract

Background: The striped venus clam *Chamelea gallina* is an economically important species in Adriatic Sea fisheries. The use of hydraulic dredging for its catch has a long history in Italy and its management faced several stages of development in the last 40 years. A great effort has been made in the past two decades to move from poorly or weakly managed fisheries to a well-structured co-management system to improve the sustainability of this fishery. However, a prerequisite for appropriate resource management is a sound knowledge of the biology and reproductive strategy of the species.

Results: We investigated three major biological features– the gametogenic cycle, size at sexual maturity and partial fecundity – by microscopic, histological and video analysis techniques. We demonstrated that its breeding season is driven by rises in seawater temperature and chlorophyll-a concentration and that its spawning period lasted from March to September. Size at sexual maturity was reached very early in the life cycle. As regards partial fecundity – the number of mature oocytes potentially released by females with ripe gonads in a single release event – varied in relation to size. Nevertheless, the reduction on the Minimum Conservation Reference Size (MCRS) from 25 to 22 mm (Delegated Regulation (EU) 2020/2237) lead to a 40% reduction in the number of emitted eggs.

Conclusions: We suggest that the ability of Adriatic clam stocks to withstand the strong fishing pressure of the past 40 years and the present one is due to their high reproductive potential and multiple spawning events combined with the effect of management measures (closed areas/seasons, quota, MCRS) and technical constraints on the gear and the sieve on board. Moreover, since the reduced MCRS for Venus shells is still larger than the size at maturity, it will probably not be detrimental to the reproductive capacity of the stock.

Keywords: *Chamelea gallina*, Reproductive cycle, Sexual maturity, Partial fecundity, Minimum Conservation Reference Size (MCRS), Fishery management

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Introduction

The striped venus clam *Chamelea gallina* (Linnaeus, 1758) is an economically important species in the Mediterranean Sea, where it thrives at depths of 2 – 12 m [2, 3] in the coastal biocenosis of well-sorted fine sands described by Pérès and Picard [4], although it can even reach greater depths up to 20 m [4, 5]. In Italy, the fleet targeting *C. gallina* consists of 635 active hydraulic dredges, 601 of which are concentrated along the Adriatic coasts [6]. Hydraulic dredges harvesting *C. gallina* operate in a narrow strip between 0.3 and 2 nautical miles (NM) off the coast (depth range 3–15 m), and along 1400 km of the 8000 km of the Italian coastline. In the early 1970's, the transition from hand-operated to hydraulic dredges resulted in an immediate and steep yield increase of up to 80,000–100,000 tons/year that was followed by a progressive decline due to overexploitation and poor management [7]. In the past two decades, considerable effort has been made to move to a well-structured co-management system, to improve the sustainability of this fishery [8]. In recent years (2018-2019), annual production has been around 19,000 tons (~ €50 million), accounting for 11% of fishery production in Italy in weight and for 6% in revenues [6]. However, the declining landings do not accurately reflect the status of the resource at sea, which is influenced by factors such as natural population fluctuations; the variable catch quota, which is set on the basis of market demand; and the wide range of restrictions that have been adopted over time to promote the sustainability and responsiveness of the fishery. The *C. gallina* fishery is managed through technical measures that:

a) set dredge dimensions (maximum width, 3 m; maximum weight, 600 kg; Ministerial Decree 22/12/2000 [9]);
b) regulate the fishing effort through closed areas (dredging is banned within 0.3 NM of the coast; Regulation (EC) 1967/2006 [10]);
c) ban fishing activities in some periods (dredging is forbidden for 2 months between April and October [6]);
d) limit the catch of juveniles through constraints on the technical features of the dredge and the mechanical sieve on board (Ministerial Decree 22/12/2000) [9];
e) establish the Minimum Conservation Reference Size (MCRS), which is currently 22 mm (Delegated Regulation (EC) 2016/2376, Regulation (EC) 2020/3, and Delegated Regulation (EC) 2020/2237 [11–13]) by way of derogation from the previous 25 mm (Annex III to Regulation (EC) 1967/2006 [10]).

A prerequisite for appropriate resource management is a sound knowledge of its biology and reproductive strategy. The reproductive cycle of *C. gallina* in the Adriatic has been reported to span from March to September [14–16]. However, there is disagreement on its size at first sexual maturity

(TL_{50}), which has been described to range from 11 to 18 mm [4, 17, 18]. A thorough knowledge of this parameter is crucial to evaluate the spawning fraction and fecundity of the population that has not been harvested, which contributes to its reproductive output [19].

Chamelea gallina reproduction has been the subject of several qualitative studies in the Adriatic Sea and elsewhere [20–24]. On the other hand, there is only one quantitative study investigating the potential number of emitted eggs per females in a single spawning event in relation to shell size [19]. The disproportion is due to the diffusion of gonad tissue in the visceral mass, which hampers the study of reproductive output and investment in all bivalves [25] except Pectinidae, whose gonad is a discrete organ. Quantitative reproduction data, like gonad biomass and fecundity, are critical to understand the life history of marine bivalves and to manage them successfully, i.e. to define the MCRS [26–28].

Various semi-quantitative and quantitative methods have been applied to estimate bivalve fecundity also in relation to their reproductive strategy and ovary structure, even though quantitative investigations are still much fewer than qualitative studies [29]. For example, the reproductive investment has been assessed in live specimens by inducing spawning through thermal shock or chemical injection, to count the number of eggs released [30–32] or by weighing them before and after spawning [33]. Dead specimens can be analysed indirectly by strip spawning [34, 35], volumetric reconstruction [36–38] and histological [39] and immunological methods [40]. However, all of them underestimate the reproductive output, since incomplete spawning is not infrequent and spawning events of different intensity may occur several times during the reproductive season [41]. This is the case of *C. gallina*, a multiple partial spawner with intra-individual asynchronous ovary development [42]. Bivalve fecundity is closely related to size and age [7, 38], although it can also be influenced by phylogeny and environmental conditions [25, 43–45].

Altogether, the information on the reproductive biology of *C. gallina* in the Adriatic Sea is dated and limited, which has the potential to undermine stock management and conservation efforts. The aim of this study is to provide new and updated information on the reproductive cycle of *C. gallina*: *i)* by investigating the gametogenic cycle using histological techniques and evaluating its relationships with temperature and chlorophyll-a (Chl-a); *ii)* by estimating T L_{50} by microscopic observation; and *iii)* by assessing partial fecundity (PF) by means of histological and image analysis approaches. Since in Italian territorial waters the MCRS has temporarily been reduced from 25 mm to 22 mm total length (TL) we also describe how the reduction affects clam fecundity. The study findings provide insights for fishery management, such as the MCRS and the time of the year when fishing should be closed.

Methods

Sample and data collection

Clam samples were collected monthly, from November 2018 to October 2019, during commercial fishing operations conducted on sandy bottoms (depth, 5 to 12 m) in the Ancona Maritime District (central Adriatic Sea, Figure 1). From 2 to 3 individuals per size class (width, 2 mm) were measured to the nearest 0.1 mm with a Vernier calliper along the anterior-posterior shell axis. The number of specimens each month analysed depended on the size classes available in the sample (maximum size range 18 - 36 mm TL, overall mean size (± Standard Deviation, SD) 25.4 ± 3.8 mm TL). Testing for differences in the gametogenic cycle in relation to shell size was not considered, however a gonad fragment from each specimen was placed in Dietrich solution [46] for subsequent histological analysis.

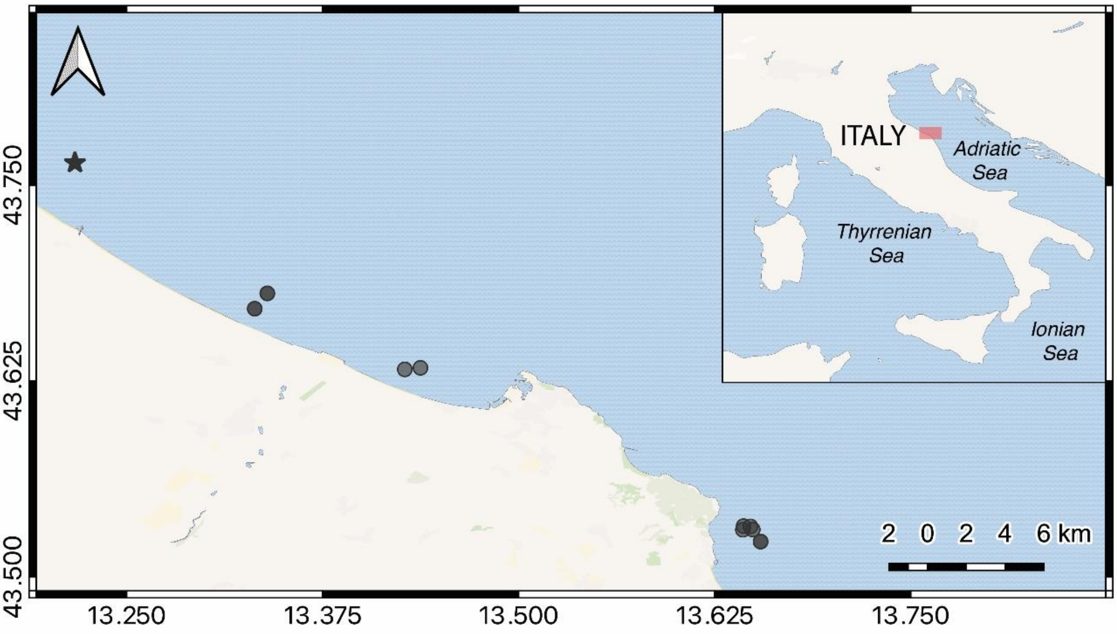

Figure 1. Map of the sampling area generated through the QGIS software version 3.20 "Odense" (www.qgis.org). Dots indicate the sampling positions. The star marks the Tele-Senigallia research pylon, where the bottom seawater data were recorded.

Bottom seawater temperature (BST) data were obtained from the Tele–Senigallia pylon, a research tower located 1.3 NM off Senigallia, which is close to the sampling area and has been collecting oceanographic data since 1988 [47]. Temperature data were recorded every 10 minutes on a daily scale at 12.5 m depth. The daily Chl-a values were freely downloaded from the EU Copernicus Marine Service Information website [48] by tracing a polygon overlapping with the sampling area.

Mean monthly values were than calculated for both parameters to observe how in parallel gonadal maturity stages changed over months.

Histology

The gametogenic cycle of females and males was investigated using a standard histological protocol. Gonads were dehydrated through increasing ethanol concentrations and embedded in paraplast. Serial 6-μm-thick transverse sections were cut with a microtome, mounted on slides, stained with Harris haematoxylin and eosin [49] and finally examined under a light microscope at 5–40 × magnification. Maturity stages were assigned according to the 6-stage scale proposed by Joaquim et al. [24] for both sexes, with a slight modification of the last stage, as follows: F1/M1, inactive; F2/M2, early active; F3/M3, late active; F4A/M4A, ripe; F4B/M4B, partially spawned; and F5/M5 regressing (i.e. gonads in resorption with residual unreleased gametes inside the tissue) rather than "spent" as in the original scale (i.e. totally reabsorbed gonad). When multiple stages coexisted in an individual, the predominant stage was assigned

Size at sexual maturity

TL_{50} was determined in specimens obtained from additional samples collected on alternate weeks in the central part of the reproductive season (May to August, 8 additional samples and in total 492 specimens analysed). From 3 to 4 individuals per size class (width, 1 mm) were measured and analysed and the total number of individuals per each sample always varied depending on the size classes available in the sample (maximum size range 4 - 36 mm TL, overall mean size 18.7 ± 9.5 mm TL). An *ad hoc* dichotomous maturity scale (1, not sexually mature; 2, sexually mature) was applied to classify specimens based on microscopic features. Gonad material was smeared on slides and examined under a light microscope linked to a video analysis system (Las Image Analysis, Leica). Females were classified as immature if only previtellogenic (immature) oocytes ($d_{max} \leq 60$ μm) were detected, and as mature when vitellogenic (mature) oocytes (maximum diameter, $d_{max} > 60$ μm) began to develop. This threshold (≤ or > 60 μm) was set based on what described by Corni et al. [18, 21] in the Adriatic where *C. gallina* previtellogenic oocytes had a different shape (more irregular) and dimension ($d_{max} \leq 60$ μm) compared to vitellogenic ones. Males were classified as mature/immature based on the presence/absence of spermatozoa with well elongated branched tails. Whenever not possible to assess the sex of small individuals they were classified as indeterminate and excluded from the calculation of TL_{50}. TL_{50} was assessed in both sexes by fitting a logistic model to the proportion of mature specimens per size class:

$$y = \frac{1}{(1 + \ exp^{-(a+bcx)})}$$

where y is the relative frequency of mature individuals; x the size of individuals, exp is the basis of the Neperian logarithms, a and b are the regression constant [50], using the R package sizeMat [51].

Partial fecundity

A total number of 26 females (20 in stage 4A and 6 in stage 4B) ranging from 19 to 33 mm TL, collected during the reproductive season and relative to the additional sampling, were examined to investigate the relationship of gonad volume (G_v) with TL, the number of oocytes contained in gonadal tissue and the percent G_v occupied by oocytes. PF was assessed in stage 4A females by histological and video analysis methods, to provide an estimate of the number of gametes released in a single release event in relation to TL. Histological analysis and image post-processing took at least 8 h per individual.

All specimens were measured and opened. All the organs (shell, mantle, siphons, gills) except the visceral mass and the foot were removed before storage in Dietrich solution for subsequent histological analysis. A procedure similar to the one described by Delgado et al. [19] was employed for G_v calculation. In brief, the entire visceral mass was cut into sections; a 6-µm-thick section every 100 µm was stained with Harris haematoxylin and eosin and viewed under a stereomicroscope connected to a video analysis system (Leica Application Suite V4.12) using reflected light at low magnification (0.76 ×). The area of the gonad (G_a) was measured in each section using Image J software, which allowed calculating G_v. In addition, 6 randomly chosen fields per gonad were digitized under a light microscope at 10 × magnification and used to assess the d_{min} (minimum diameter) and d_{max} of oocytes, which were sectioned through the nucleus. Oocyte volume was then calculated assuming cells to be spheroid ($O_v = 4/3 \times \pi \times d_{min} \times d_{max}^2$). The total oocyte number of each clam was estimated by standardizing the observations from each field to the entire gonad volume. PF was estimated by summing the number of mature oocytes in each 4A female. The relationship between PF and TL was explored by regression analysis.

Statistical analysis

The percent G_v occupied by all types of oocytes was used to test for statistical differences between maturity stages. The use of percentages allowed to control for differences in G_v between individuals. One-way analysis of variance (ANOVA) was applied to establish whether 4A and 4B females showed a significantly different mean percent G_v occupation. Two-way ANOVA was applied to investigate possible differences in mean percent G_v occupation between maturity stages and between oocyte development stages (mature and immature). Before result interpretation, the data were explored to check the assumption of normality, homoscedasticity and independence. All assumptions were met.

After the tests, the Tukey HSD test was performed to explore differences among the levels of significant terms.

Analysis of covariance (ANCOVA) was used to test for statistical differences in oocyte number accounting for differences in TL between individuals. Analogously, a two-way experimental design was used to test for the effect of Maturity stage (2 levels: 4A and 4B) and Oocyte development stage (2 levels: mature and immature) controlling for the covariate, TL. Prior to statistical analysis, the data were explored to check the assumption of normality, homoscedasticity, independence, linearity of regression and homogeneity of slopes. Abundance and length data were log-transformed to meet the assumptions. Finally, a simple main effects test was conducted to explore the interaction between the levels of each term.

All statistical analyses and visualizations were produced in R (v 4.0.3; R Core Team [52]).

Results

Environmental parameters

The mean monthly BST and Chl-a values exhibited a seasonal trend (Figure 2). BST was 16.6 ± 1.4 °C in November, dropping to 10.7 ± 0.5 °C until March with a minimum at 8.8 ± 1.1 °C in January. It gradually rose from January, and in June it approached the values recorded in November (16.4 ± 1.0 °C). From July to October, BST ranged from 20.6 ± 2.2 to 25.8 ± 1.5 °C, peaking in August. Chl-a fell steeply from 7.8 ± 4.3 mg/m^3 in November to ~2.2 ± 0.8 mg/m^3 in December-April, it increased in May-July with a peak in June (4.6 ± 1.9 mg/m^3) and fell again from August to October (1.6 ± 0.4 mg/m^3).

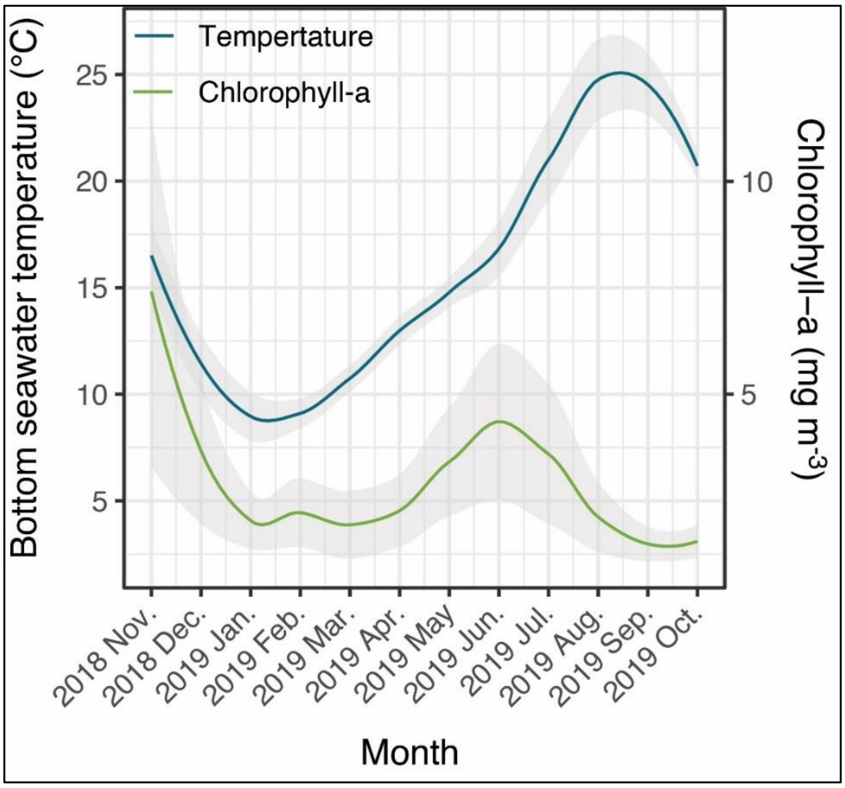

Figure 2. Mean monthly bottom seawater temperature and chlorophyll-a concentrations recorded between November 2018 and October 2019. Grey area: standard deviation.

Histology

Altogether 213 females, 205 males and 64 indeterminate individuals were subjected to histological analysis. The progress of maturity stages over the months is reported in Figure 3. During the 12-month sampling, the 64 indeterminate individuals were recorded only in November, December and October. At this stage sexes were not distinguishable, therefore the inactive stage is hereafter reported as F1/M1. The inactive stage was characterized by abundant connective tissue occupying the whole visceral mass without follicles/acini or gametes; only indeterminate cells were present and the sexes were indistinguishable (Figure 4a and Figure 5a).

In November gametogenesis had already resumed, as 56.5% and 76.7% of females and males were respectively in stage F2 and M2 (early gametogenesis). The gonads of F2 females showed fully developed follicles and oogonia (diameter, 5-6 µm) together with some small previtellogenic oocytes in early stage of development (diameter, 15-20 µm) around the follicle wall; only vesicular cells were seen in the lumen (Figure 4b). This stage was predominant in November and December (~60%), whereas in January-March an almost equal proportion (~50%) of females were in stages F2 and F3. In April, 72% of females were in stage F3 (late active stage) and 28% were still in stage F2. Stage F3 oocytes were considerably larger and most were in the previtellogenic and pedunculated stages.

Pedunculated oocytes protruded into the lumen of the follicle through their stalk, whereas a small number of vitellogenic oocytes were seen free in the lumen (Figure 4c). In May, 95% of females had ripe gonads (F4A stage) with pedunculated and vitellogenic oocytes filling the lumen (Figure 4d). In March, a small fraction of females (14%) were already in the ripe stage. Evidence of partial release (F4B, partially spawned stage) was detected since May (5%); this stage became more represented in June and July, when females with full or partially empty gonads were in almost equal proportion (~50%). The partially empty follicles indicated that a first release event had already occurred. In F4B females new and residual pedunculated and vitellogenic oocytes were detected in the lumen together with oocytes in an earlier stage of development attached to the wall, indicating the resumption of gametogenesis (Figure 4e). In August, 67% of females had regressing gonads (stage F5) with residual oocytes in the collapsed lumen and connective tissue and indeterminate cells surrounding the gonad area (Figure 4f). In September, we collected a single female (stage F5). In October, the gonads had completely regressed and females could no longer be identified, since all individuals were in the inactive stage (M1/F1).

In November, males were in the early gametogenesis stage (M2) with fully formed acini, spermatogonia surrounding the walls and vesicular cells filling the lumen (Figure 5b). Between December and February, males were almost exclusively (94-100%) in late active gametogenesis (stage M3), with germ cells of decreasing size – spermatocytes and spermatids – arranged centripetally and projecting into the lumen (Figure 5c). From March to July, most males (67-100%) had ripe gonads (stage M4A), with the lumen of the acini filled with spermatids and spermatozoa (Figure 5d). Partial release events (stage M4B) spanned from June to September, peaking in August (84.5%). In M4B males, new spermatids and spermatozoa occupied a portion of the partially emptied acini, although cells in earlier development stages were also detected along the acinar walls (Figure 5e). Gonad regression (stage M5) began in August, the majority of males in this stage being observed in September (73.9%); the acini were collapsed, with connective tissue and indeterminate cells beginning to surround the gonad area; residual spermatozoa were present except where a total release event had occurred (Figure 5f). In October, a residual of 0.7% of males were still in regression, whereas all the other individuals (97.3%) had inactive gonads.

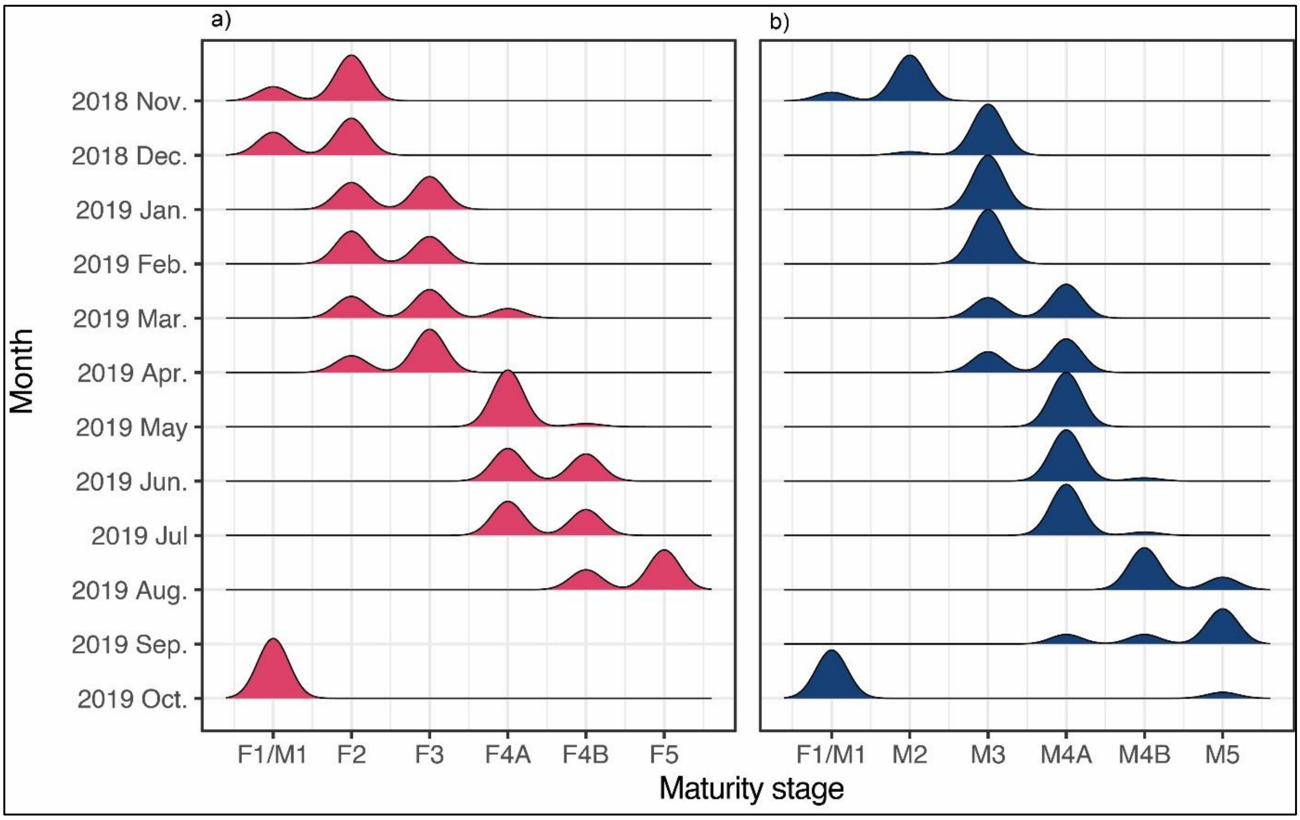

Figure 3. Ridge plots showing the progress of maturity stages over the months in (a) females and (b) males. The curves illustrate the percentage of individuals in each maturity stage. The sum of the areas defined by the curves corresponds to the total monthly observations.

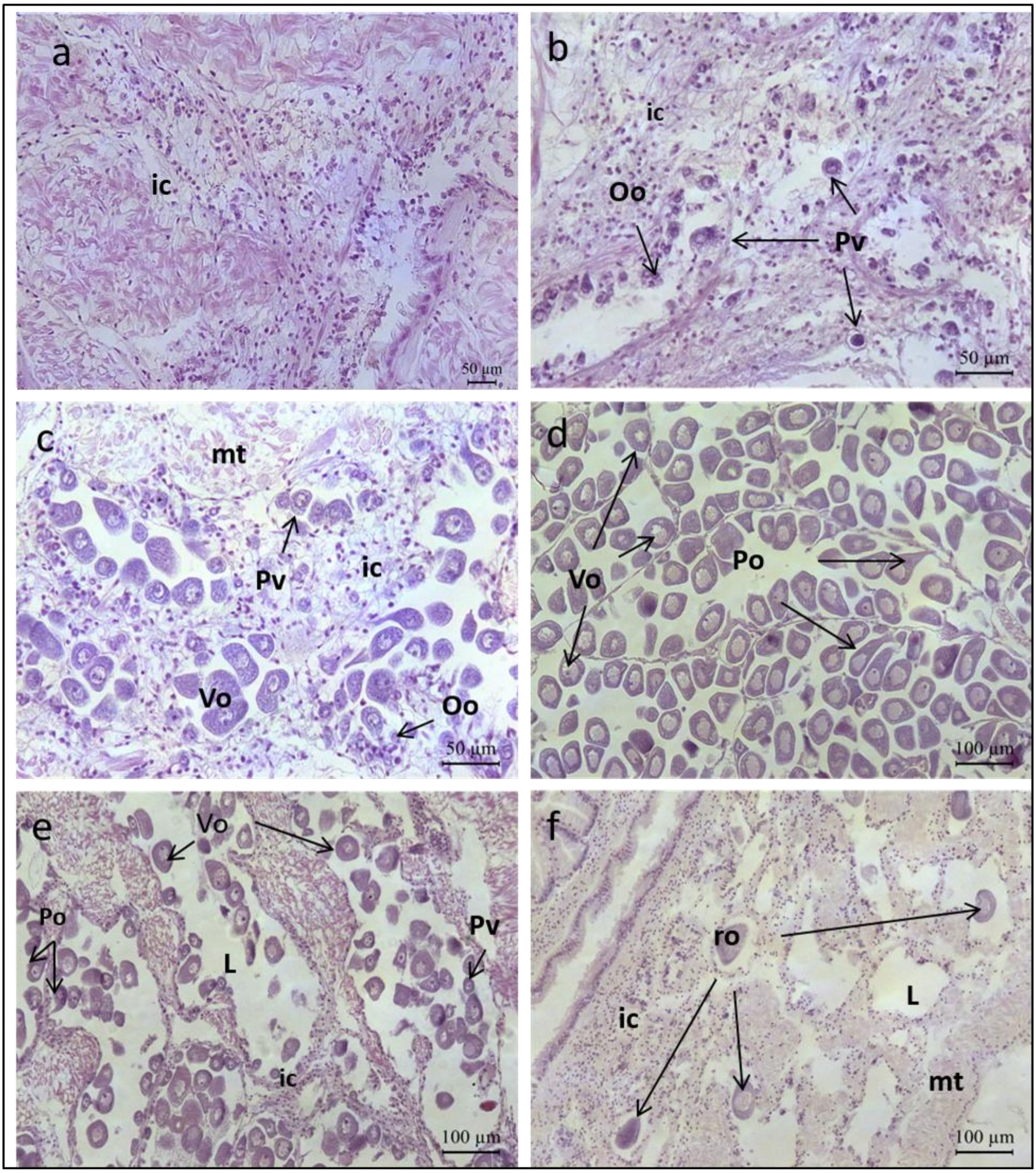

Figure 4. Histomorphological maturity stages in *C. gallina* females: (a) inactive stage, M1/F1; (b) early active stage, F2; (c) late active stage, F3; (d) ripe stage, F4A; (e) partial emission stage, F4B; (f) regressing stage, F5. Abbreviations: ic: immature cells; Oo: oogonia; Pv: previtellogenic oocyte; Po: pedunculated oocyte; Vo: vitellogenic oocyte; L: lumen; ro: residual oocyte, mt: muscle tissue.

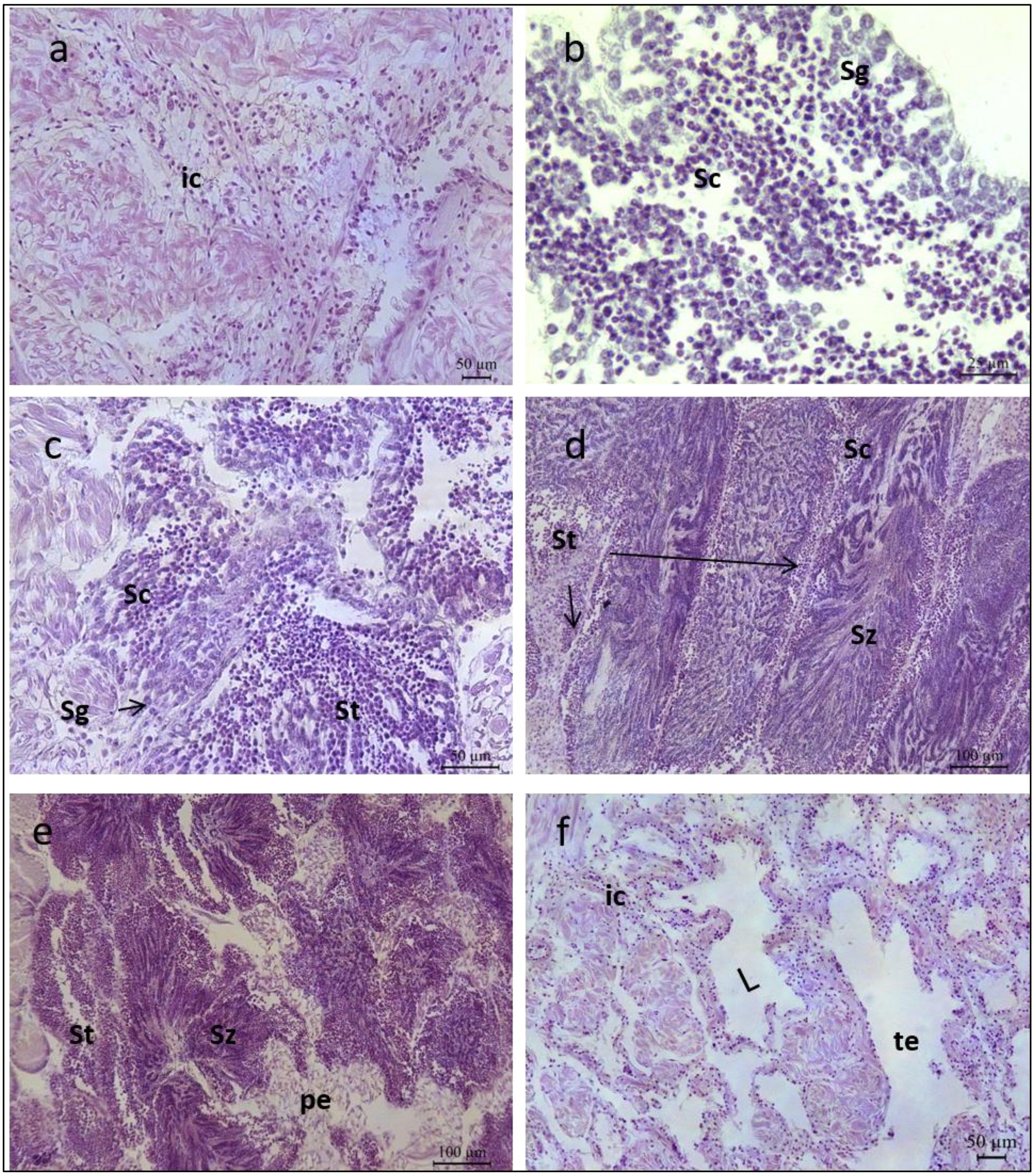

Figure 5. Histomorphological maturity stages in *C. gallina* males: (a) inactive stage, M1/F1; (b) early active stage, M2; (c) late active stage, M3; (d) ripe stage, M4A; (e) partial emission stage, M4B; (f) regressing stage, M5. Abbreviations. ic: immature cells; Sg: spermatogonia, Sc: spermatocytes; St: spermatids; Sz: spermatozoa; pe: partial emission; te: total emission; L: lumen.

Size at sexual maturity

A total of 504 additional individuals (227 females, 243 males and 34 indeterminate), collected during the *ad hoc* sampling carried out in the middle of the reproductive season, were analyzed to assess TL_{50} in both sexes. The smallest females and males with well-developed gametes measured 9.6 mm and 9.9 mm TL, respectively; TL_{50} was ~11.0 for females and 11.5 mm for males, whereas the TL_{50} of the entire sample was ~11.2 mm (Figure 6). Above 15 mm TL, all males and females were sexually mature.

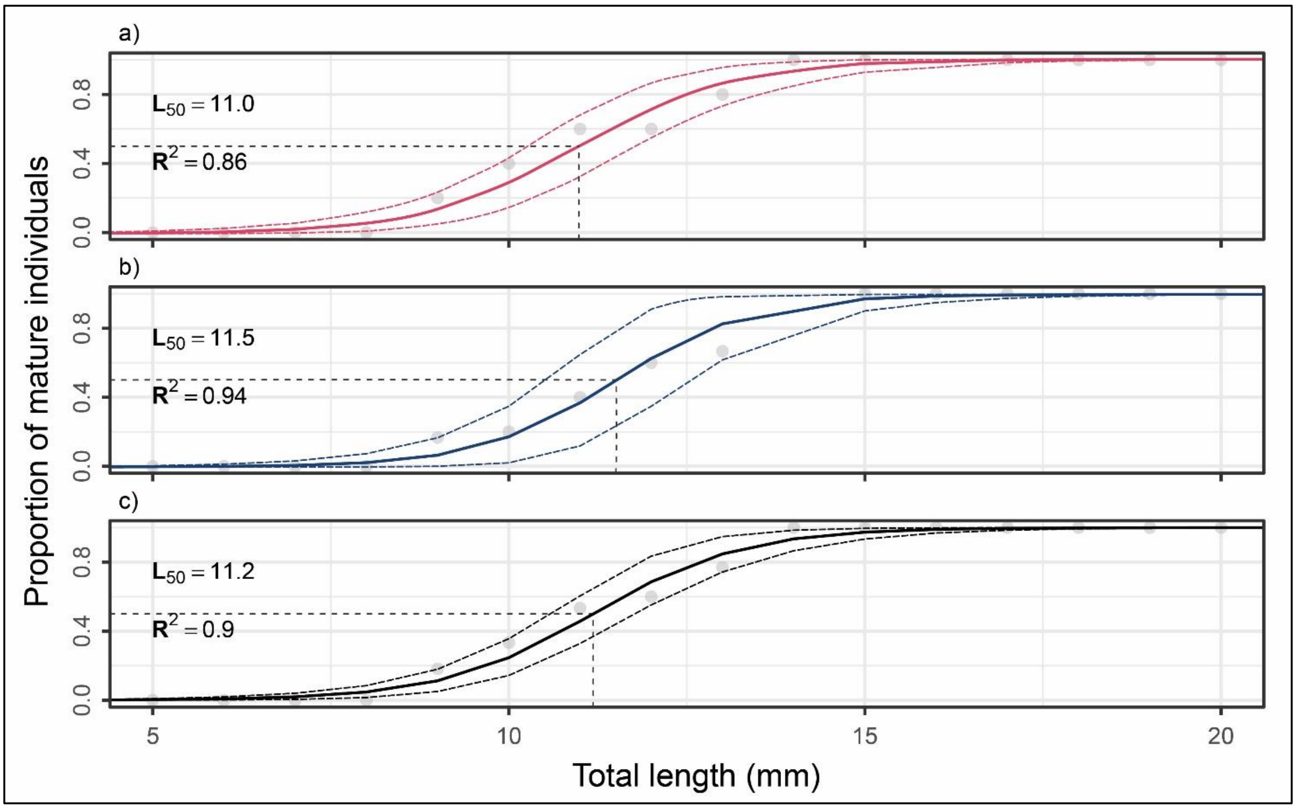

Fig.6 Size at sexual maturity assessed in *C. gallina* (a) females, (b) males and (c) pooled sexes.

Partial fecundity

The gonad volume ranged from 25.2 to 280 mm^3 and was significantly and positively related to size ($G_v = 17.2 \times TL - 304.5$; adj. $R^2 = 0.97$; $F_{1,24}=772.5$; $p < 0.001$). The percent G_v occupation by all types of oocytes was significantly different between maturity stages (ANOVA, $F_{1,24} = 64.4$; $p < 0.001$) and was 39.6% and 21.8% in stage 4A and stage 4B, respectively. Two-way ANOVA, testing for the effect of Maturity stage and Oocyte development stage on the percent G_v occupation, highlighted significant differences between the two parameters and their interaction. Although the interaction was statistically significant (Table 1), the G_v occupied by mature and immature oocytes (19.1% and 20.5%, respectively) in 4A females was not significantly different, whereas in 4B females immature oocytes occupied almost twice the volume compared with mature oocytes (14.0% and 7.8%, respectively; Table 1).

Table 1. Results of two-way Analysis of Variance (ANOVA) and the Tukey HSD test for the effects of Maturity stage (4A and 4B), Oocyte development stage (Mature / Immature), and the interactions of the two terms.

ANOVA				
	Df	**Sum Sq**	**F value**	**Pr(>F)**
Maturity stage	1	976.2	76.342	< 0.001*
Oocyte stage	1	136	10.637	< 0.01*
Maturity stage * Oocyte stage	1	72.2	5.645	< 0.05*
Residuals	48	613.8		

Tukey HSD test				
	diff	**lwr**	**upr**	**p adj**
4B - 4A	-8.906	-10.955	-6.857	< 0.001*
Mature – Immature	-3.235	-5.230	-1.241	< 0.01*
4B Immature - 4A Immature	-6.484	-10.321	-2.648	< 0.001*
4A Mature - 4A Immature	-1.372	-4.736	1.993	0.7
4B Mature - 4A Immature	-12.699	-16.536	-8.863	< 0.001*
4A Mature- 4B Immature	5.112	1.276	8.949	< 0.01*
4B Mature - 4B Immature	-6.215	-10.471	-1.959	< 0.01*
4B Mature- 4A Mature	-11.328	-15.164	-7.491	< 0.001*

The size frequency distribution of oocyte d_{max} between the two maturity stages showed that the mode was 53 mm in stage 4A and 41 mm in stage 4B (Figure 7). In mature females, the largest d_{max} values of mature occytes were 154.89 µm and 139.21 µm in stages 4A and 4B, respectively, and the smallest d_{max} values in immature oocytes were 5.85 µm and 9.54 µm, respectively. The mean diameter of mature and immature oocytes was respectively 70.3 µm, 41.5 µm in stage 4A and 70.1 µm and 38.6 µm in stage 4B.

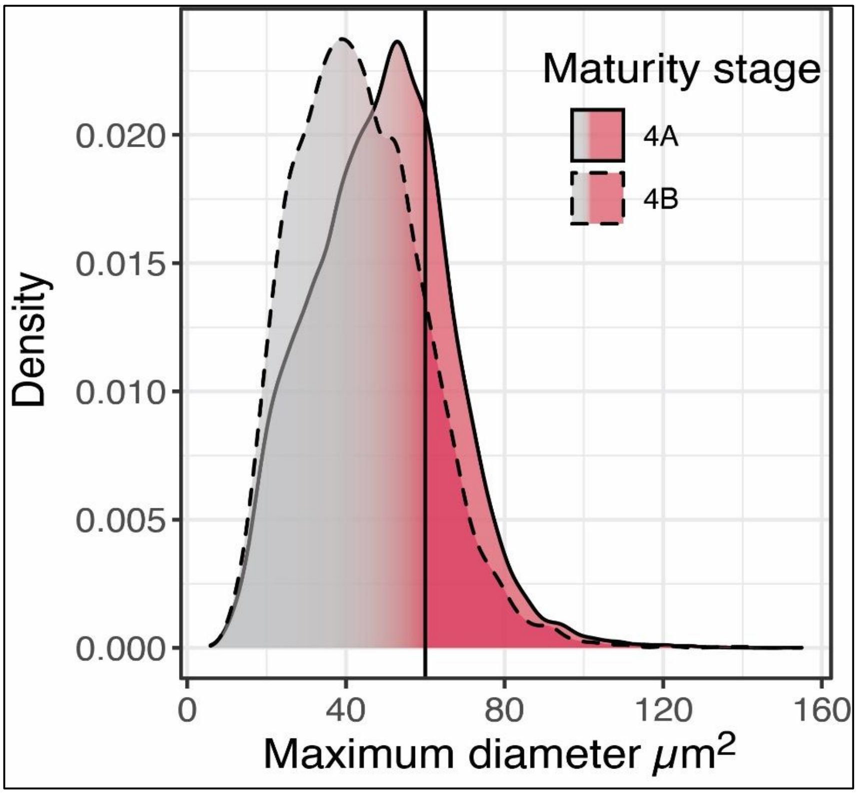

Figure 7. Size frequency distribution of maximum oocyte diameter in 4A and 4B females. The vertical line divides oocytes into those larger and smaller than 60 μm in maximum diameter.

There was a strong positive and significant linear relationship between the number of any type of oocytes and TL, irrespective of maturity stage (Figure 8). Two-way ANCOVA indicated that, while controlling for TL, there was a significant difference in the total number of oocytes between 4A and 4B females (ANCOVA, $F_{1,47} = 121.638$; $p < 0.001$). Similarly, there was a significant two-way interaction between Maturity stages and Oocyte development stages in the number of oocytes while controlling for TL (ANCOVA, $F_{2,47} = 186.131$; $p < 0.001$). A simple main effects test for Maturity stage and Oocyte development stage demonstrated that mature oocytes were more numerous in 4A than 4B females ($F_{1,23} = 30.8$; $p < 0.001$), whereas the difference between 4A and 4B females in terms of number of immature oocytes was not significant ($F_{1,23} = 0.185$; $p < 0.671$).

In 4A mature females size ranged from 19.2 to 29.9 mm TL with a PF from 3.6×10^4 to 3.7×10^5 oocytes/female depending on size (average, 1.6×10^5 oocytes/female). The linear regression analysis ($PF = 3.01\times10^4$ TL -5.21×10^5; adj. $R^2 = 0.85$; $F_{1,14} = 83.8$; $p < 0.001$) suggested that 4A females can release ~1.4×10^5 (95% confidence interval, CI ± 2.3×10^4) oocytes/female at size 22 mm TL (present MCRS) and 2.3×10^5 (95% CI ± 2.7×10^4) oocytes/female at size 25 mm TL (ex-MCRS).

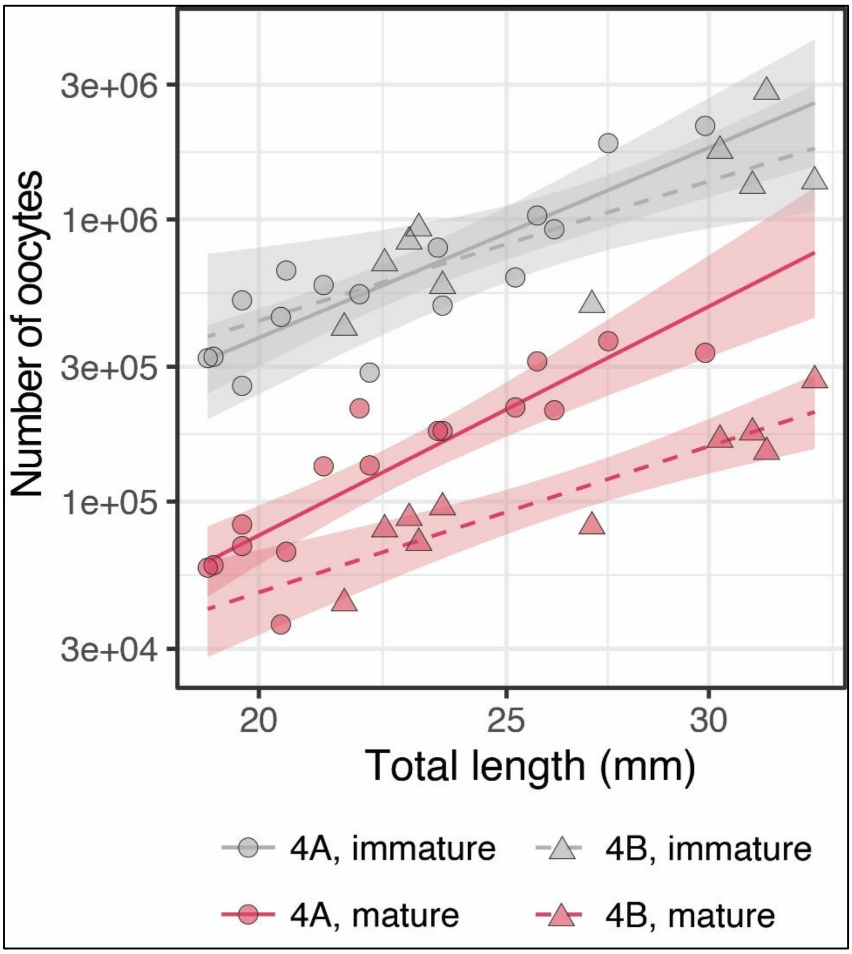

Figure 8. Scatterplots illustrating the relationship between oocyte number and total length. The log-scale emphasizes the differences between and within maturity stages (4A and 4B) in terms of oocyte maturity (mature / immature).

Discussion

Reproductive biology

This study describes the year-round reproductive cycle of the commercially valuable species, *C. gallina*, in the western Adriatic Sea. The gonad development of *C. gallina* exhibits a cyclical annual pattern influenced by BST and Chl-a. In November, when we began sampling, gametogenic activity was already detectable and an important fraction of females and males were in the early active stage (F2/M2). In November, the high Chl-a concentration and the high BST, which was similar to the one recorded in June (~16 °C), when the clams were spawning, may have acted as a trigger. Indeed, temperature abnormalities (> 14-18 °C) have been suggested account for the advanced stage of maturity and reproduction seen in clams in autumn and winter [15, 53, 54]. In temperate climates the most common bivalve gametogenesis pattern is initiated by the seawater temperature reaching a certain threshold [55].

High energy stores in late autumn – related to the high Chl-a concentration detected in November – combined with high BST values, probably drive gonad development to the next stage (F3/M3). In December, Chl-a and BST both dropped; in January and February – the two coldest months, with BST under 10 °C – gonad maturation stopped. The percentage of females and males in the different stages of maturity remained almost unchanged. Our observations agree with studies indicating that clam growth [56] and gonad development [57] slow down when BST is less than 10 °C. In March, when BST exceeded 10 °C and Chl-a began to increase, gametogenic activity resumed and ripe gonads were first detected, especially in males. Most spawning events, highlighted by evidence of partial release and gonad recovery, occurred from May to August as both BST and Chl-a rose.

In August, when BST peaked, some clams began to show gonad regression (F5/M5). This stage was predominant in September and was followed by the inactive stage (F1/M1) in October. Similarly, a study of *Ensis arcuatus* in north-western Spain highlighted that the last spawning event before gonad regression was associated with an increase in surface temperature [58]. Several studies have demonstrated that water temperature and food availability significantly influence the reproductive cycle of *C. gallina* [19, 22, 24] and other bivalves [58–60]. This is especially true in temperate regions, where increasing temperature and food supply accelerate gonad development in numerous clam species [61–63]. Indeed, the striped venus clam shows an opportunistic reproductive strategy, since gonad development and sexual maturation are closely associated with nutrient accumulation, i.e. food availability [29, 64]. Whereas we detected two Chl-a peaks, one in early autumn and the other in late-spring/early-summer, in other temperate areas Chl-a peaks in late autumn [24] or in late summer and winter [19], despite similar seasonal seawater temperature patterns. However, bivalve reproductive activity is controlled not only by environmental factors, but also by their interaction with endogenous processes [60, 64, 65].

In recent years, the reproductive cycle of *C. gallina* has widely been investigated, especially along the Spanish, Portuguese and Turkish coasts, whereas the majority of studies in the Adriatic are fairly dated (*Additional file 1*). An extended spawning period has been described by most studies in all areas [14, 23, 24, 66], although a shorter period has also been reported [22, 54, 57]. In the Adriatic Sea, the reproductive cycle of *C. gallina* commonly spans from March to September, with some additional reproductive events in early autumn, whereas studies conducted at different temperate latitudes have described reproductive events only from late spring to late summer (*Additional file 1*). The reproductive plasticity of *C. gallina* can be explained by changes in local environmental and trophic conditions over time and by geographical location [53, 64]. In temperate areas eggs are released in favourable conditions for the development of planktotrophic larvae, when phytoplankton and Chl-a concentrations are abundant and when the water temperature ranges from 18 to 27 °C [15].

In the present study, evidence of partial gamete release and developing gametes in the same acinus/follicle in 4B individuals, heralded further spawning events as long as environmental conditions would be favourable for reproduction within the same reproductive season, otherwise gametes are reabsorbed at the end of it. These findings confirm that the striped venus clam is a multiple partial spawner [19–21, 42], even though single spawning events have been described by other authors [17, 24, 67]. In our study, all specimens were gonochoric, albeit cases of hermaphroditism have been reported [7, 21]. Gonad development was synchronous in females and males, as reported in several studies [24, 57, 66], a strategy that maximizes reproductive success. Another well-established feature of *C. gallina* is interindividual asynchrony, whereby specimens in different maturity stages coexist in the same period (*Additional file 1*). In contrast, intraindividual asynchrony – where different maturity stages coexist in the same individual – has rarely been described before the present study [19–21].

Both sexes of the striped venus clam reach sexual maturity at about 11.2 mm TL, in the first year of life; indeed, the first year specimens grow to about 15 mm TL [68]. Clams longer than 15 mm TL were all found sexually mature. Our findings are consistent with previous studies reporting a similar or even smaller size at sexual maturity [18, 53, 66, 69–71]. Yet, a TL_{50} of 9 to 18 mm is commonly described in the Adriatic Sea and elsewhere (*Additional file 1*). Such different values, reported even in the same area, may be attributed to the intrinsic reproductive variability of the species in relation to local environmental conditions such as seawater temperature, food availability and to anthropogenic, genetic and physiological factors [64], as well as to the different methods adopted to asses maturity.

Although the estimation of potential annual fecundity is critical to understand bivalve production and population dynamics, it is little explored [72]. *Chamelea gallina* is characterized by indeterminate fecundity, or better by a potential annual fecundity that is not known before the onset of spawning, since unyolked oocytes continue to mature and be spawned throughout the reproductive season [73]. Even though we were able to estimate the PF related to a single egg release event, in multiple partial spawning bivalves the number of spawning events occurring in the same reproductive season and the intensity of each reproductive peak are unknown [41], and are different in different years [74]. Only another study by Delgado et al. [19], conducted in the Gulf of Càdiz (south-western Spain), has assessed the fecundity of *C. gallina*. The results of the two investigations are quite similar; in particular, Delgado and co-workers analysed females in a size interval (20-30 mm TL) similar to ours, they found similar estimates of gonad volume (range, 37.25-205.95 mm^3) and reported that the percent G_v occupied by all types of oocytes and by mature oocytes was respectively 37.71% and 18.38% in 4A females and 31.30% and 14.23% in 4B females. Nevertheless, their estimated PF (range, 7.6×10^4 – 7.9×10^5 oocytes/female) is higher than ours, despite a similar order of magnitude of oocyte number in relation to TL. The difference may lie in the method used to calculate PF: we only considered oocytes sectioned through the nucleus, which involves that the actual number of oocytes in the gonad may have been underestimated, whereas Delgado et al. [19] did not report it. Before egg release, the oocytes can reach a diameter of 110-120 µm [18], which is comparable with the d_{max} values we found in 4A and 4B mature oocytes. We found that fecundity is related to size, as noted by other authors [38, 44, 75], since in younger individuals growth is fast and the investment in reproduction limited, whereas in older bivalves energy is switched from growth to reproduction [44, 45].

A variety of studies have tried to estimate fecundity in various bivalve species, despite the problem of gonad tissue diffusion in the visceral mass. For example, in *Spondilus calcifer* the mean number of spawned oocytes per female has been estimated at 48.9 million [75], whereas the number of eggs per female has been put at 4.15 million in *Ruditapes philippinarum* [41] and at 1.65 million in *Anadara antiquata* [38]. The order of magnitude of the mean number of spawned eggs per female, reported in these studies, is up to two times higher than the one we calculated. However, egg number strongly depends on the species, its size range and the estimation method.

The reproductive strategy of *C. gallina* results in high fecundity. As near-sessile organisms, their lifecycle is strongly affected by environmental factors [36]. To ensure reproductive success, large amounts of gametes are released in the water column and, after fertilization, develop into planktotrophic larvae [15, 76]. However, as demonstrated by Beninger et al. [72] in *Cerastoderma edule* using Neutral Red vital staining, not all the oocytes released during a spawning event are viable, as dead/non-viable oocytes accounted for 34-85%. Moreover, oocytes age after spawning and 4-8 h after their release they can no longer be fertilized [77]: this involves that synchronization of gamete release in the environment is crucial for the reproductive success of the species [7]. Egg number is further reduced by predation by filter-feeding organisms in the water column. In addition, early offspring mortality is also substantial, due to oceanographic and ecological factors (e.g. food availability, current transport to unsuitable habitats, predation; [78]) as well as to biological (e.g. reproductive strategy of the species, larval duration and larval behaviour; [79]) and genetic factors [43].

Management implications

Italian clam fishery is the sole fishery where the number of vessels and operators has not declined in the past four decades [6]. Despite the reduction in landings also due to the adoption of more restrictive management measures over time, the biological and management factors that allowed the clam fishing stocks to withstand the high fishing effort include:

i) the high reproductive potential (clams of 22 mm TL produce 1.4×10^5 oocytes/female, a fairly high fecundity whose order of magnitude is shared with 25 mm clams) and the multiple spawning events occurring within the same reproductive season;

ii) the early maturation, since all clams > 15 mm TL are sexually mature within the first year of life;

iii) the closure of the area within 0.3 NM of the coast (Regulation (EC) 1967/2006 [10]) to dredging activity; this measure has halved the area previously suitable for clam harvesting and provides a large area (581.7 km^2) where a huge amount of breeders contribute to the reproductive output of the population;

iv) the daily quota (reduced to 400 kg/vessel from the previous 600 kg/vessel; Delegated Regulation (EC) 2016/2376 [12]) has strongly reduced the fishing effort, because the boats take less time to achieve the predetermined quota;

v) the two-months fishing closure adopted in summer during the peak of reproduction;

vi) the technical measures set for the fishing gear (for both the dredge and the sieve on board) reduce the catch of juveniles and the fraction below 22 mm TL almost to zero [80];

vii) the setting of restocking areas, entered into force in 2017, where fishing is banned and where fishermen are required to discard undersized specimens harvested elsewhere (Delegated Regulation (EC) 2016/2376 [12]);

viii) the high survival rate of *C. gallina* (higher than 95% [81]); the specimens returned to the sea can grow and contribute to the spawning fraction of the population;

ix) the seeding and fishing area rotation applied by Management Consortia, the bodies responsible for fishery management, make the exploitation more sustainable and responsive.

The Scientific, Technical and Economic Committee for Fisheries (STECF), in the Joint Recommendation 20-01, reported that since the first implementation of the new MCRS in 2017 (Commission Delegated Regulation (EC) 2016/2376 [12]) an increase of abundance of > 22 mm individuals has been observed in the stock in certain areas of the Adriatic Sea. STECF also noted that the status of the stocks seems to have been stable or improving depending on the areas. Furthermore, it concluded that, since the reduced MCRS for Venus shells is still larger than the size at maturity (previously reported between 15-17 mm), it will probably not be detrimental to the reproductive capacity of the stock and is likely to have little effect on the exploitation rate on juveniles [82]. Therefore, our estimated TL_{50} at an even smaller size supports what stated by STECF.

Moreover, the EU Commission has considered that, based on information available in the Joint Recommendations and STECF assessments [82, 83], the derogation to the MCRS is in line with the objectives of the sustainable exploitation of the Venus shells stock in the Italian territorial waters. The lower MCRS also contributed to reduce the impact of the fishing activity on the marine ecosystem by allowing a significant decrease in fishing time and in the area being dredged as the quota is reached faster. On this basis, it appears that the proposed reduced MCRS would comply with the requirements established for technical measures in Article 15 and Article 18 of Regulation (EC), 2019/1241 [84].

However, there is necessity to:

(i) collect accurate fishery data on fishing effort through the implementation of automatic monitoring system (GPS device) onboard each vessel; the boats' movement control by the bodies in charge for the inspections (Coast Guard) would allow the coastal area within 0.3 NM of the coast to be preserved in an inexpensive way from illegal fishing activities, and therefore to safeguard a large fraction of the reproductive stocks;

(ii) conduct at least two annual samplings. This would allow to constantly monitor the resource and to relate the biomass landed with those present at sea (exploitation rate). This index, calculated for each Consortium, would be essential to ensure rational and sustainable exploitation. When a threshold value is exceeded, effort management measures and targeted closures should be put in place [6]. Such close monitoring would make it possible to immediately verify any situations of overexploitation.

Populations of marine bivalves are subject to large interannual fluctuations as a result of their sensitivity to unfavourable environmental conditions [85]. Along the Italian Adriatic coasts, extensive dying-off phenomena for *C. gallina* took place several times in the last thirty years [6]. Although it is not always easy to identify the causes of these mass mortality events, they are generally caused by sudden changes in the coastal environment (e.g. hypo-anoxia, fresh water inputs, sea storms, pollution, sudden temperature and seabed grain size variations) and presence of pathogen agents [6]. Considering that the physical and chemical parameters of the seas are changing due to water acidification, global warming, sea level rise and decreased nutrient availability [86–88], the environmental perturbations are likely to frequently raise increasing the pressure on the species. For example, Huntley & Scarponi [89] found an association between sea level rise and increasing prevalence of digenean trematodes in *C. gallina* fossil records from a Holocene shallow marine succession in the Po coastal plain. Moreover, Delgado & Silva [90] noted that, where levels of prevalence of diagenetic-trematode-like parasite were higher they induced castration in the wedge clam (*Donax trunculus*) specimens. However, at present, the possible effects induced by climate change on the life-history traits of *C. gallina* are mainly unknown. For this reason, its main biological traits (e.g. growth, size at sexual maturity, reproductive potential) should be constantly monitored in relation to a changing environment, to guarantee the adoption of suitable management actions for a responsive fishery. Therefore, a careful periodic review of the adopted technical measures based on the biology of the species should be warrant for its protection over time.

Nevertheless, genetic studies [91] confirmed that, despite the fluctuations exhibited by the species in the last four decades, its high level of genetic diversity has not been negatively affected, conferring to this species a good adaptive potential to face the environmental perturbations.

Conclusions

In conclusion, this study provides some crucial biological information that can help adjust fishery management measures to clam biology. It also confirms that in the Adriatic Sea *C. gallina* reproduces in spring-summer, thus supporting the adoption of fishing closures in this period: closures ensure that the larger individuals contribute to reproduction and that the offspring attach to the substrate. *Chamelea gallina* reaches sexual maturity in the first year of life and partial fecundity is size-related. Even though the MCRS reduction to 22 mm TL affects partial fecundity (specimens measuring 25 mm TL produce 40% more oocytes per female), we suggest that the ability of Adriatic clam stocks to withstand the strong fishing pressure of the past 40 years and the present one is due to their high reproductive potential, multiple spawning events and high genetic variability combined with the effect of management measures (closed areas/ seasons, quota, MCRS) and technical constraints on the gear and the sieve on board.

Abbreviations

MCRS : Minimum Conservation Reference Size; NM: Nautical miles; TL: Total length; TL_{50}: Size at sexual maturity; BST: Bottom seawater temperature; Chl-a: Chlorophyll-a; PF: Partial fecundity; SD: Standard deviation; d_{max}: Maximum diameter; G_v: Gonad volume; G_a: Gonad area; O_v: Oocyte volume; EC: European Commission; EU: European Union; HSD: Honestly significant difference; CI: Confidence interval; STECF: Scientific, Technical and Economic Committee for Fisheries; Repr. season: Reproductive season; Repr. traits: Reproductive traits; CW: Central-western; NE: North-eastern; N: Northern; C: Central; CS: Centralsouthern; S: Southern; SW: South-western; NW: North-western; MPS: Multiple partial spawner; Inter-IA: Interindividual asynchrony; Intra-IA: Intraindividual asynchrony; histo: Histological analysis; micro: Microscopic analysis.

Supplementary Information

The online version contains supplementary material available at https:// doi. org/ 10. 1186/ s40850- 021- 00096-4.

Additional file 1: Suppl. Mat 1. Summarizes the reproductive traits of *Chamelea gallina* described in the present study and by other authors in different geographical areas.

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Authors’ contributions

G.B.1 wrote the manuscript with contributions (in order of importance) from G.B.2, M.V. and A.L.. G.B.1 and F.D. conducted the laboratory activities. G.B.2 and P.P. performed the statistical analysis. A.L. was the Scientific Responsible of the study. The author(s) read and approved the final manuscript.

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

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Publisher’s Note

Supplementary Material 1. The table summarizes the reproductive traits of Chamelea gallina found in the present study and by other authors in different geographic areas. (Rep. Seas. = reproductive Season, Rep. Traits = Reproductive Traits, CW = central-western, NE = north-eastern, N = northern; C = central, CS = central-southern, S = southern, SW = south-western, NW = north-western, MPS = multiple partial spawner, Inter-IA = inter-individual asynchrony, Intra-IA = intra-individual asynchrony, histo = histological analyses, micro = microscopic analyses, blank spaces = info not available)

Reference	**Studied Area**	**Period of investigation**	**Onset gametogenesis**	**Spawning period**	**Rep. Seas.**	**TL50**	**Rep. Traits**	**Methods**
Present study	CW Adriatic (Ancona)	Nov 2018-Oct2019	Nov	May-Sept	prolonged	11.2 (F), 11.8 (M)	MPS, Inter-IA, Intra-IA	**histo + micro**
Bratoš Cetiniæ et al. 2007	NE Adriatic (Croatia, river Neretva)	Jan 2003 - Jan 2004	Oct-Nov	Jan-Aug	prolonged	12		histo
Salvatorelli 1967	N Adriatic (Chioggia Lagoon)	1967	Oct (F), Dec (M)	Jul-Aug	limited		Inter-IA	histo
Corni et al. 1985a	N Adriatic (Cesenatico)	May-Jun 1980		Jun	prolonged	10 (F), 11 (M)	MPS, Inter-IA	histo + micro
Corni et al. 1985b	N Adriatic (Cesenatico)	Sept-Dec 1982	Oct (F), Oct-Dec (M)	Sept-Oct			Inter-IA, Intra-IA	histo
Poggiani et al. 1973	N Adriatic	Jul 1968 - Mar 1970	Nov	Jun-Jul	limited	16-18	Inter-IA	histo
Valli & Zecchini-Pinesich 1981	N Adriatic (Trieste)	Apr 1975-Apr 1976	Oct	Apr-Sept	prolonged		MPS, Inter-IA	histo
Valli et al. 1985	N Adriatic (Grado)	1981-1982	Sept	Apr-Jul	limited			histo
Ambrogi et al. 1997	N Adriatic (Po' delta)	1997		May-Nov	prolonged			
Rizzo et al 2011	N Adriatic (Chioggia Lagoon)	Jun 2009 - May 2010	Sept-Oct	Jun-Aug	limited			micro
Moschino & Marin 2006	N Adriatic (Chioggia and Venezia)	2000-2001		spring - summer				CI
Franceschini e Bernarello 2013	N Adriatic (Porto Viro)	2013	Apr-Oct	Apr-Oct	prolonged			
Froglia 1975	C Adriatic			May-Jul, Sept-Oct	limited			micro
Giansante et al. 2006	C Adriatic (Pescara)	2005-2006		summer	prolonged	12		
Cordisco et al. 2003	CS Adriatic	2000-2002		late spring - summer			Inter-IA	
Cordisco et al. 2005	CS Adriatic (Molise)	2000-2001	late-winter/early spring	late spring +autumn	prolonged	13	MPS	micro
Romanelli 2009	Adriatic Sea			Apr-Oct	prolonged	13-15		
Scopa et. al 2014	CS Adriatic (Abruzzo, Molise)			spring-summer		13-15		
Marano et al. 1982	S Adriatic (Gulf of Manfredonia)	1978-1979	Aug-Sept	Jun-Aug	prolonged		MPS, Intra-IA	histo + micro
Cordisco et al. 2005	C Tyrrhenian Sea (Lazio)	2003	-	Aprl-Nov	prolonged		MPS, Inter-IA	micro
Gaspar & Monteiro 1998	S Portugal	Jun 1992 - May 1993	Oct - Nov	Apr-Aug			Inter-IA	histo
Joaquim et al. 2014	S Portugal (Algarve coast)	2009	Dec	May-Sept	prolonged		Inter-IA	histo
Vizuete et al. 1993	S Spain (Mazarrón Bay)	Sept 1987 - Nov 1988	Dec	May-Sept	prolonged		Inter-IA	micro
Delgado et al. 2013	SW Spain (Gulf of Cádiz)	May 2010 - Apr 2011	Nov-Feb	May-Aug	prolonged	10.29 (F); 8.41 (M); 9.34 (TOT)	MPS, Inter-IA, Intra-IA	histo
Silva & Juàrez 2009	SW Spain (Gulf of Cádiz)					9		
Rodriguez de la Rùa et al. 2003	S Spain (Atlantic)	Jun 1999 - May 2000	Nov	Jan-Sept	prolonged		Inter-IA	histo
Rodriguez de la Rùa et al. 2003	S Spain (Mediterranean)	Jun 1999 - May 2000		almost all year	prolonged		Inter-IA	
Ramon Herrero 1990	NW Mediterranean (Valencia, Spain)	May 1988 - May 1990	Dec	Jun-Aug	limeted		Inter-IA	micro
Dalgic et al. 2009	Black Sea	Dec 2002 - Nov 2003	Mar-May	Jun-Aug	limited		Inter-IA	histo
Oray et al. 1991	Marmara Sea			Jun-Jul				
Erkan 2002	Marmara Sea					18	MPS	

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Chapter 3

Reburial potential and survivability of the striped venus clam (*Chamelea gallina*) in hydraulic dredge fisheries

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Reburial potential and survivability of the striped venus clam (*Chamelea gallina*) in hydraulic dredge fisheries

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Abstract

The striped venus clam (*Chamelea gallina*) is the main edible bivalve living in Italian waters. According to Regulation (EU) 2020/2237, undersized specimens (total length of the shell, <22 mm) must be returned to the sea. *C. gallina* specimens of different size classes that had undergone hydraulic dredging and mechanized sorting were analysed for reburial ability in a laboratory tank and for survivability in the laboratory (135 clams, 21 days) and at sea (320 clams, 15 days). In the tank experiments, the reburial times (T_{50} and T_{90}) and the upper (+) and lower (-) confidence intervals (CIs) of the whole sample were about 4 h (CI+ 4.4, CI- 3.6) and 8 h (CI+ 8.2, CI- 7.7), respectively, and were significantly shorter for the medium-sized clams (22-24.9 mm) than for the smallest (<21.9 mm) and the largest (>25 mm) specimens. For the field survivability experiments, clams under and above the minimum conservation reference size (MCRS) were placed in separate metal cages. Survival rates were 94.8% and 96.2% respectively in the laboratory and at sea, without significant differences between the two experiments or among size classes. These findings conclusively demonstrate that *C. gallina* specimens returned to the sea have a very high survival probability and that they can contribute to mitigate the overexploitation of natural populations.

Keywords: *striped venus clam, discarding practices, reburying capacity, survivability, laboratory tanks, cages at sea*

Introduction

The striped venus clam *Chamelea gallina* (Linnaeus, 1758), an edible and commercially valuable bivalve, is an infaunal filter-feeding clam of the family Veneridae. It is widespread in the Mediterranean and the Black Sea and along the eastern Atlantic coasts, where it inhabits the fine well-sorted sand biocoenosis described by Pérès and Picard[1]. It thrives in sandy and muddy–sandy sediments and tolerates narrow salinity and temperature variations[2]. In particular, a study of its presence as a function of sediment type, conducted in the north-western Adriatic Sea near Venice[3], has reported that *C. gallina* does not inhabit substrata with a redox potential lower than + 50 mV (i.e. it does not tolerate reduced conditions) or with a sand fraction lower than 90% (as it does not tolerate anoxic conditions). Accordingly, the species is concentrated in limited areas 0–12 m in depth and up to 1–2 nautical miles (NM) from the coastline[4]. Its high abundance in the central and northern Adriatic Sea is determined by the large amount of nutrients, particles and organic matter which are supplied by the massive outflow of the river Po and by the coastal flows, which are carried along the Italian Adriatic coast by the Western Adriatic Current[5,6].

In Italy, this bivalve fishery involves 626 hydraulic dredgers that provide about 1500 jobs; its annual landings in 2018 and 2019 of ≈ 19,000 metric tonnes are worth about €50 million[7]. Since the 1970s, technological innovations such as hydraulic dredges and mechanized sorting equipment have considerably increased the fishing effort and exploitation level of *C. gallina* beds in the northern Adriatic Sea, resulting in overexploitation in some areas[8]. Another consequence has been the loss of the largest specimens (> 25 mm in shell total length; TL), owing to the efficiency and size selectivity of the gear, which has been estimated to catch nearly 100% of commercial-sized clams[4], as well as to inadequate stock management and protection measures[9]. On the other hand, monitoring surveys performed in the past 20 years have detected a massive amount of juveniles, with catches of undersized specimens exceeding 90% in 2016[10]. Because the species reaches the size at first maturity at 15–17 mm TL in the first year of life (Atlantic Ocean[11], Marmara Sea[12], Adriatic Sea[13]), in Italian territorial waters the Minimum Conservation Reference Size (MCRS) of 25 mm TL (Regulation (EC) 1967/2006[14]) has been reduced to 22 mm TL (Delegated Regulation (EU) 2016/2376[15], Regulation (EU) 2020/3[16], and Regulation (EU) 2020/2237[17]). Notably, according to Regulation (EU) 1380/2013[18], the obligation of landing all specimens under the MCRS does not apply to "species for which scientific evidence demonstrates high survival rates, taking into account the characteristics of the gear, of the fishing practices and of the ecosystem"; in such cases, fishers are required to return undersized specimens to the sea immediately after sorting.

Whereas gear efficiency has been studied extensively (e.g. Refs.[19,20]), data on the effects of fishing on population sustainability are more limited[8,9,21,22]. Clams harvested with hydraulic dredges are hauled up from the seabed, dumped into a collecting box on board and conveyed to a mechanized sieve for sorting. Since the smaller specimens that pass through the sieve are returned to the sea through a waste exhaust pipe, discarded clams undergo considerable physical stress[23]. Even though discards are believed to mitigate the overexploitation of natural populations, the mechanical stress to which they are subject has the potential to reduce their survivability[22,24].

The survivability of the striped venus clam (e.g. Refs.[2,22,25]) and other bivalve species (e.g. Ref.[26]) has largely been studied in terms of the natural ability of bivalves to survive periods of aerial exposure[26]. The present study is the first attempt to assess the survivability of *C. gallina*, (a) by reproducing as closely as possible the sea habitat conditions in the laboratory and (b) through field tests in the natural environment. The possible differences in reburying and survivability capacity across sizes were examined by studying undersized individuals (discards) as well as commercial-sized specimens.

Materials and methods

Gear characteristics and sample collection.

Clams were harvested by a commercial hydraulic dredger (LOA, 15.8 m; tonnage, 9.7 GT; engine power, 110 kW) using standard gear and sorting methods in two fishing trips carried out in the Ancona Maritime District (central Adriatic Sea, Figure 1). Dredging was conducted at ≈ 3 m depth 0.3 NM off Ancona on a fishing ground characterized by fine, well-sorted sandy bottoms. The tank experiments were performed in June 2019 and the experiments in the sea in October 2019.

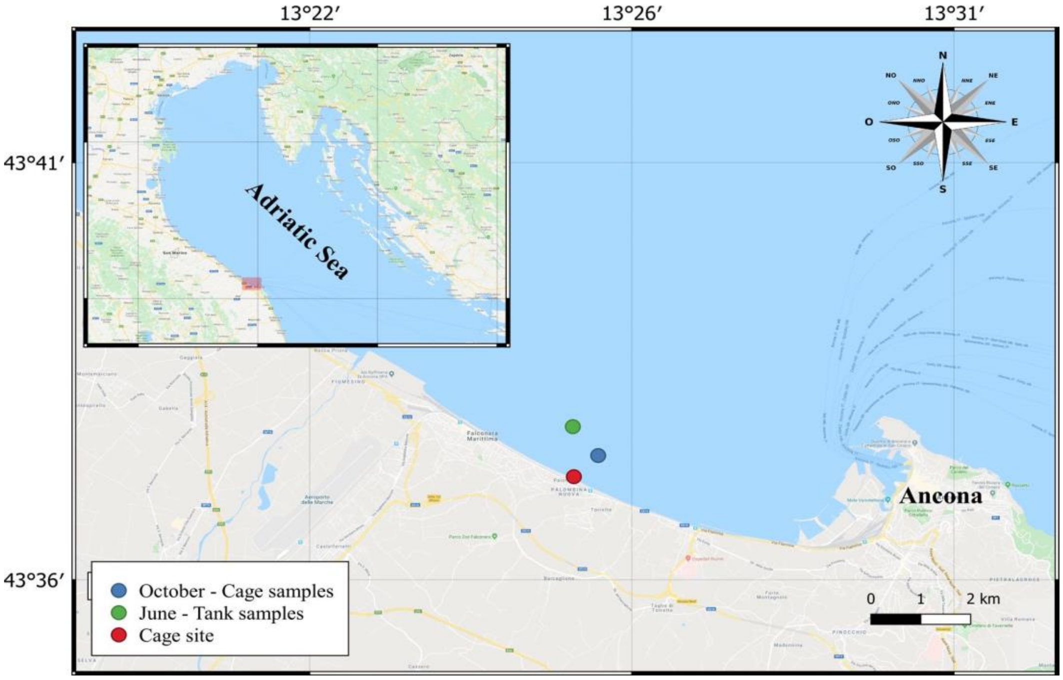

Figure 1. Map of the sampling and field experiment areas generated through the QGIS software version 3.10.10 (www. qgis. org).

The hydraulic dredge used for this study consists of a metal cage 2.8 m wide whose bottom is made of metal rods placed at 12 mm intervals to retain the clams. A blade is fitted at the dredge mouth to cut the soft bottom, whereas a hose connected to a centrifugal water pump ejects pressurized seawater from nozzles to fluidize the sediments. The cage is mounted on two sledge runners to prevent it from digging into the sediment. When the cage is hauled up, the catch is dumped onto a collecting box and conveyed to a mechanized vibrating sieve for sorting. The sieve consists of 4 stacked sorting grids with hole diameters decreasing from 32.5 to 20.3 mm (see Sala et al.[20] for details). The specimens used in the present experiments were collected directly from the vibrating sieve (≥ MCRS) or from the waste exhaust pipe (< MCRS). Clams of all sizes were used for the experiments, provided that they were intact (i.e. without shell damage, scratches, chipped edges or crushed umbos). To mimic their being returned to the sea after sorting while minimizing the stress induced by aerial exposure, all the clams used for the survival experiments were immediately placed into a plastic tank containing seawater collected during the fishing hauls. To avoid further stress, the plastic tank was handled gently until the clams were placed in the laboratory tanks/sea cages.

To reproduce the clams' environmental conditions in the laboratory, seawater temperature and salinity were recorded during fishing operations using a CTD probe (model YSI 30).

Laboratory experiments

Clam reburying capacity and survivability under controlled conditions were assessed following the guidelines established in the Workshop on Methods for Estimating Discard Survival (WKMEDS—ICES, 2015).

The captivity study used a glass tank divided into 9 communicating sub-compartments, each measuring 30 × 30 × 35 cm, connected to a sump (180 l, 90 × 60 × 35 cm), forming a closed system (Figure 2a). The tank was filled with osmotic water—obtained by a purification process using a partially permeable membrane system to remove ions, larger particles and unwanted molecules—added with salt to achieve a salinity of 35 ppm; water temperature was set at 20.0 ± 1.0°C and maintained by a cooler connected to the sump through a pump with a flow rate of 1500 l/h. Constant temperature, salinity and dissolved oxygen were ensured throughout the experiment. Aeration was provided by 3 aerators (8 W, 550 l/h) through silicon tubes ending with an air stone (Figure 2b). Water quality, i.e. ammonia, nitrate, nitrite, and phosphate concentrations and pH, was measured with reagent tests (SERA or Jbl) at weekly intervals, to exclude stress due to non-optimal or sub-toxic conditions. About 7 cm of sand collected from the harvesting area (43.6198 N; 13.4252 E) was placed on the bottom of each sub-compartment, after sieving to remove shell fragments and benthic macrofauna (e.g. bivalves, gastropods, crustaceans, echinoderms), thus avoiding clam overestimation and potential predation. Water recirculation was ensured by 3 pumps (flow rate, 950 l/h) installed in the sump, with the water flowing from the tank into the sump by gravity, falling on sponges that served as mechanical and biological filters. The filter was previously matured by adding 10 vials (each 1 ml) of nitrogen cycle bacteria one week before beginning the experiment. A skimmer system (flow rate, 520 l/h) was installed in the sump to remove organic particulate matter. Clams were fed daily ad libitum with marine gel phytoplankton (Easy booster 25) consisting of 31% *Nannochloropsis*, 33% *Isochrysis*, 18% *Tetraselmis* and 18% *Phaeodactylum*.

For the tank experiments, clams were divided into 3 size classes: 1, 19.0–21.9 mm TL; 2, 22.0–24.9 mm TL; and 3, 25.0–27.9 mm TL. A total of 15 specimens with 3 replicates per size class were placed in each sub-compartment.

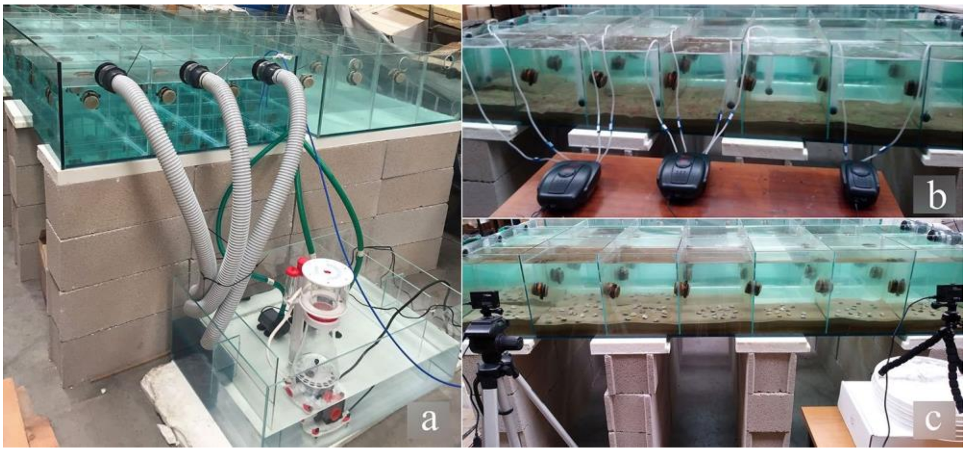

Figure 2. Experimental glass tank for clam reburial and survival experiments. (**a**) The sump (centre) was connected to the tank through 3 pumps, forming a closed circuit. The sump was equipped with sponges serving as mechanical and biological filters (left) and with a skimmer system to remove the organic particulate matter (middle). (**b**) Aerators placed outside the tank (picture taken on day 15). No clams are visible on the sediment surface, since by 21 h all had reburied. (**c**) GoPro cameras placed outside the tank and programmed to shoot at 15-s intervals. At the beginning of the reburial experiment, all clams are still visible on the sediment surface.

Reburying capacity

Reburial ability was assessed by time-lapse monitoring (shots taken at 15-s intervals) using two GoPro 5 Black cameras positioned outside the tank (Figure 2c). When clams were no longer visible on the sediment surface the cameras were switched off. The time required for clams to become invisible was estimated using shots taken at 30-min intervals, the number of clams still visible in each shot being counted and recorded. Data were processed separately for each size class.

Survivability in captivity

The laboratory survivability experiment lasted 21 days. In the morning and late afternoon, the tank was examined for dead specimens (clams with open valves), which were removed and measured. At the end of the experiment, the surviving clams were extracted from the sand, counted and measured. The percentage of clams under and above the MCRS was calculated and compared.

Survivability at sea

The survivability experiment at sea lasted 15 days and was performed off Palombina beach, near the clam beds (Figure 1). Two metal cages (100 × 120 × 40 cm) marked on the surface by floats were anchored to the bottom at ≈ 1 m depth. The cage surfaces were covered with netting: the bottom panel had a nominal mesh size of 5 mm, whereas the wider mesh size of the other panels (20 mm) ensured water circulation and prevented predation. The clams, 160 specimens < MCRS and 160 ≥ MCRS (Supplementary Video 1), were placed in the respective cages through an opening on the top and left undisturbed for 15 days to avoid further stress. Dead specimens were counted at the end of the experiment.

Data treatment and statistical analysis

A generalized linear model (GLM) with a binomial distribution was applied to analyse clam reburial time and survivability. The factors for the former analysis comprised time (continuous variable) and size class (3 levels); their interaction, indicating a significant difference among factor levels, was also investigated[27]. Model selection was based on Akaike's Information Criterion (AIC). The log-likelihood ratio test (based on χ^2 distribution) was used to assess factor significance in the model. Whenever a factor was significant, a Wald z-test based on χ^2 distribution was applied to determine the significance of pairwise estimates[28]. After model selection, over-dispersion and residuals were analysed to further validate the selected model.

For the reburial study, the time when none of the clams were still visible on the sediment surface was recorded; the times at which 50% (T_{50}) and 90% (T_{90}) of the specimens were likely reburied and their upper (+) and lower (−) 95% Confidence Intervals (CIs) were computed both for the whole sample and for the 3 size classes.

For the survivability study, the proportions of survivors under and above the MCRS at the end of the trials were calculated for the laboratory and the field experiments. Moreover, to compare survivability as a function of the MCRS (< 22 and ≥ 22 mm TL), the GLM considered the "Experiments" (Sea and Laboratory tests) and the "Size Classes" (under and above MCRS) as two-level factors. Condition (2 levels: number of live and dead individuals) was used as a response variable and the whole dataset was treated as a contingency table[27]. All analyses were performed using the *stats* package of the freely available software R (version 3.6)[29].

Results

Reburying capacity in the tank

By 21 h, all specimens had reburied regardless of their size (Supplementary Video 2). However, the χ^2 test highlighted a significantly different ($p < 0.01$) reburial ability depending on size class (Table 1). The Wald z-test detected a significant difference between size classes 1 and 2 ($p < 0.01$) and 2 and 3 ($p < 0.01$), but not between classes 1 and 3 ($p = 0.32$) (Table 2). Medium-sized clams were the fastest to rebury (Figure 3); their T_{50} was 3.0 h (CI+ 3.4, CI− 2.7) and their T_{90} was 6.0 h (CI+ 6.3, CI− 5.8). The T_{50} of the smallest and the largest clams was 4.8 h (CI+ 5.3, CI− 4.5) and 4.1 h (CI+ 4.5, CI− 3.8), respectively, whereas their T_{90} was 9.4 h (CI+ 9.7, CI− 9.2) and 8.4 h (CI+ 8.7, CI− 8.2), respectively. The T_{50} and T_{90} for the whole sample (135 clams) were ≈ 4 h (CI+ 4.4, CI− 3.6) and 8 h (CI+ 8.2, CI− 7.7), respectively.

Table 1. Log-likelihood ratio test showing significant differences in reburial ability for each size class.

Effects	**df**	**Deviance**	**AIC**	**LRT**	***p* (> χ^2)**
Log-likelihood ratio test					
Time	1	1945.7	2170.3	1774.1	< 0.0001
Size class	2	244.7	467.2	73.1	< 0.0001
Time × size class	2	171.6	398.2	18.3	0.0001
Full model		153.3	383.2		

Table 2. Wald z-test showing significant differences in reburial time in size class 1 versus 2 and size class 2 versus 3.

Pairwise interactions	**df**	**χ2**	***p***
Wald z-test			
1 versus 2	1	15.3	< 0.0001
1 versus 3	1	0.98	0.32
2 versus 3	1	10.4	0.0012

Survivability experiments in the tank and at sea.

By the 21st day in the laboratory tank, 7 of the 135 specimens (2 < MCRS and 5 ≥ MCRS) had resurfaced and died. Deaths were recorded from day 4 to day 10 and showed no size dependence (Figure 4). The survival rates of commercial-sized and undersized specimens were respectively 94.4% and 95.5% (mean, 94.8%).

By the end of the 15th day in the sea cages, 12 of the 320 specimens (4 < MCRS and 8 ≥ MCRS) had died. The survival rate of the commercial-sized and the undersized specimens was respectively 95.0% and 97.1% (mean, 96.2%), again without any size dependence.

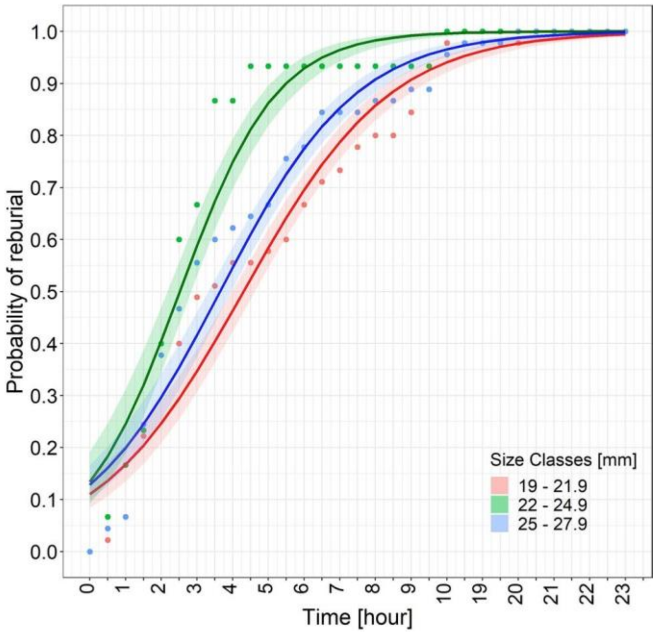

Figure 3. Logistic curves of the reburial probability calculated for the three size classes. Coloured areas around the lines: confidence intervals of the theoretical model; points: experimental observations.

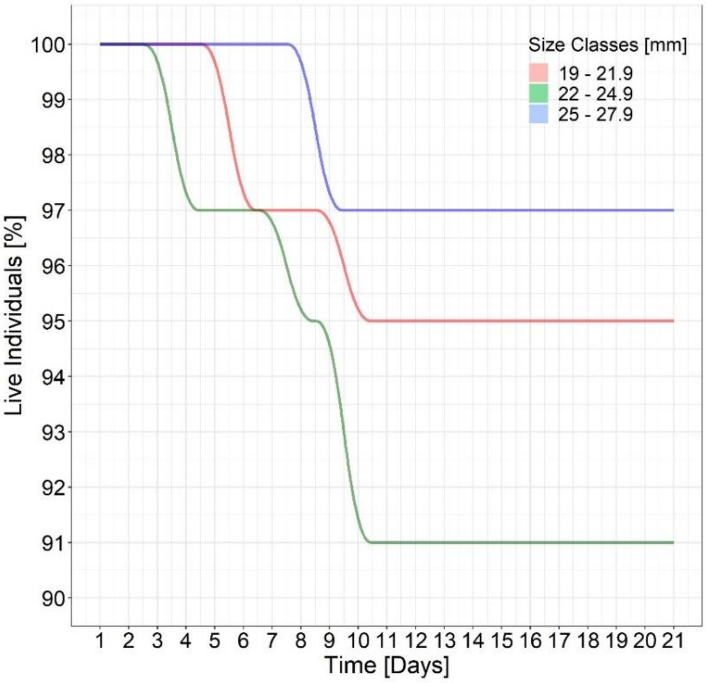

Figure 4. Percentage of clams of each size class surviving during the tank experiments.

According to the χ^2 test, mortality in the tank and field experiments and among size classes was not significantly different (p = 0.90) (Table 3).

Table3. Log-likelihood ratio test showing the absence of significant differences between survival in the laboratory and at sea and between size classes.

Effects	**df**	**Deviance**	**AIC**	**LRT**	***p*** **(> χ2)**
Log-likelihood ratio test					
Experiments	1	0.3	17.5	0.3	0.583
Size class	1	0.4	17.6	0.4	0.526
Experiments × size class	1	0.1	19.2	0.1	0.904
Full model		0.1	21.1		

Discussion

Despite the economic importance of *C. gallina*, data on the reburial ability and survival of discarded clams returned to the sea are scarce. A tank study of reburial ability by Morello et al.[30] reported an overall T_{50} of about 3 h, which is very similar to the one (T_{50} < 4 h) estimated in the present study for the whole sample (135 clams), despite in the previuos study clams were kept at both 12°C and 20°C. Even when reburial time was calculated separately for the three size classes, in our study the T_{50} ranged from 3 to 4.8 h. Morello et al.[30] also found that less than 35% of clams were still visible after 4 h, whereas by 8 h, 90% of our sample had reburied and by 21 h no clams were visible any longer. Differences in reburial time may be due to the different energy stores of specimens of different size classes. Indeed, Moschino and Marin[2] have reported that larger clams store more energy whereas smaller clams consume more energy per unit of volume. Accordingly, even though larger clams should rebury faster because of their larger energy stores, they also have a larger surface area to be reburied, whereas smaller specimens have a smaller surface area to rebury, but less stored energy per unit of volume. These considerations may explain the absence of significant differences in reburial time between the larger (size class 3) and the smaller clams (size class 1) of our sample. These data suggest that medium-sized clams (size class 2) may have a more favorable balance between surface area and stored energy, since they reburied significantly faster than the other size classes. Henderson and Richardson[31] have sought potential relationships between shell size and the time required to rebury in other bivalve species (*Ensis siliqua* and *Ensis ensis*), using time-lapse video to analyse burying behavior in different fine and coarse sediment types. They found a relationship only for *E. siliqua* in fine sediment (smaller individuals reburied comparatively faster). This suggests that shell shape may play an important role in burying time in relation to specimen size; notably, in some cases the elongated shell of the smaller razor clams may provide an advantage on the more globous shell of the smaller striped venus clams. Bivalve reburial ability has also been studied in situ following dredging. For example, Chícharo et al.[32] tested the reburial time on the seabed of *Spisula solida* specimens dislodged by the dredge or hand-collected by divers. By 12 min, all the hand-collected specimens had reburied, whereas those that had been dislodged by the dredge took more than 30 min to rebury completely. Leitão et al.[33] tested the burying response of discarded undersized cockles (*Cerastoderma edule*) that had been hand-dredged or harvested with a knife; they found that 83% of specimens had reburied within 15 min irrespective of the collection method, whereas only 10% were still visible on the sediment surface 1 h after being discarded. In an underwater study comparing a traditional dredge to an innovative dredge for *Callista chione*[34], the macrobenthic species that had escaped through the metal rods of the new dredge, which included bivalves with and without commercial value (*C. chione, Pharus legumen, E. ensis, Solen marginatus, E. siliqua, Mactra glauca, Lutraria anguistor, Laevicardium crassum, S. solida, Venus striatula, Dosinia exoleta*), reburied soon after they escaped. These studies describe a relatively faster reburial ability of bivalves tested directly at sea or replaced on the bottom soon after dredging compared to those transferred into containment facilities (present study and Morello et al.[30]). This observation may lead to even more reassuring considerations on the reburial ability of undersized *C. gallina* specimens discarded directly at sea during commercial fishing operations. However, aerial exposure exceeding 1 h has been reported to involve a significant reduction of reburial ability and of the physiological response to dredging-induced stress in *S. solida*[35].

Clam mortality in our laboratory experiments was low (≈ 5%) and did not correlate with shell size, whereas other studies have found that the smallest clams are more likely to die[36,37]. Moreover, at variance with the finding that clams may be more likely to die immediately after being placed in the tanks or around the end of experiments due to containment[38], the mortality of our captive *C. gallina* specimens was not related to a particular time. In our study, neither the harvesting and sieving process nor captivity in the tank induced significant mortality, suggesting that other factors (e.g. disease, parasites) may have caused the death of weaker or less healthy specimens.

Although Breen et al.[39] recommend monitoring the key environmental parameters (e.g. depth, temperature, salinity) during captivity, the high survival rate of our specimens suggests that the slight depth difference (1-1.5 m) between the fishing ground and the cage site did not affect survivability. Similarly, specimen size did not affect survivability, since only 7 individuals died in captivity (2 <MCRS and 5 ≥MCRS) and 12 individuals died in the sea trials (4 <MCRS and 8 ≥MCRS).

This was the first study investigating the survival of discarded striped venus clams in environmental conditions mimicking the natural habitat. The similar mortality recorded in the laboratory and the field experiments demonstrates the ability of our conditions in captivity to closely mimic those at sea. Studies of clam survival in relation to aerial exposure have found L_{50} values of 4 days[25], 5–6 days[2] and 6.2 days[34]. The season, together with other biotic (e.g. gonadal development and energy storage) and abiotic factors (e.g. seawater temperature and salinity), influences clam conditions[22,40] hence survivability in air. A study of survival in air of *Mytilus edulis* from the Dutch coast[26] has found that pollutants accumulated in clam tissue reduce survival time in air. Exposure to different pollutant concentrations for different times inhibited bivalve reburial ability, leading to death (e.g. Refs.[41,42]).

Another stress factor that influences the survival potential and condition of captured clams is the dredging fishing effort[21,43]. Clam beds are subject to extremely high fishing pressure, as demonstrated by the Side Scan Sonar surveys in the Adriatic Sea[44], and to high discard rates[19,23]. Notably, Petetta et al.[19] have estimated that the first size selection performed by the dredge on the seabed does not spare undersized individuals, since more than 58% of the clams caught are under the former MCRS of 25 mm TL and undergo sieving, which retain less than 5% of undersized individuals[20]. Mechanical sorting and discarding into the sea may cause a physiological stress and physical damage to small clams, which may be harvested as many as 20 times a year[8,21].

Moschino et al.[22] have examined the effect of hydraulic dredging on the physiological response of *C. gallina* from the north-western Adriatic Sea, both in the laboratory and at sea. In laboratory experiments, mechanical stress was simulated by vortexing the clams in a mixer, whereas field experiments included four levels of stress, the lowest involving manual sampling by scuba divers and the highest involving exposure to high water pressure and mechanized sorting, mimicking collection by commercial gears. The laboratory specimens showed a lower physiological response than controls and a shorter survival in air (L_{50}, 6 days vs. 10 days), whereas those undergoing the sea trials exhibited a declining physiological response and survival in air (L_{50}, ≈ 5 days) as the stress level increased. At variance with these findings, our clam sample exposed to high water pressure and mechanized sorting showed very high survival rates, also considering the additional stress due to handling and transport to the tank or the sea cages. A study of mortality related to hydraulic dredging[24] has reported a rate of 2 to 20% (mean, ≈ 10%) corresponding to a survival rate of at least 80%. Considering that the water pressure used in the study was higher than the regulation 1.8 bar (DM 22/12/2000[45]), the mortality rate using the legal water pressure should be lower. A 7-day captivity study has assessed the survivability of three undersized commercial bivalve species (*Donax trunculus*, *S. solida* and *C.*

gallina) harvested with hydraulic dredgers without recreating the natural sea bottom habitat. Undersized and commercial-size individuals of the three species were divided into those with intact shells and shells with the edge chipped. At the end of the experiments, the survival rate of the intact specimens ranged from 86 to 100% irrespective of species and size, in line with the survival rate of the undamaged clams analysed in our study. The survival rate of the chipped specimens ranged from 24.2 to 60%[46], suggesting the need for additional work on the survivability of damaged individuals.

Altogether, previous findings and the present data - documenting that a very large proportion of clams survive harvesting and sorting and that they show a high reburial ability and survival rate after reburying - demonstrate the high survival potential of *C. gallina* and support the claim that undersized specimens of this bivalve can be returned to the sea *as per* Regulation (EU) 2020/2237[17]. The present data suggests that a very high proportion of discarded *C. gallina* survive and grow to the commercial size (MCRS), which is reached on around 2 years of age[47]. The common observation of clams with repaired shells further testifies to their survival ability. Longerterm studies are clearly needed to understand the extent of the ecological disruption induced by dredge-fishing and discarding on the feeding, growth and reproduction of discarded specimens. Further work is also required to improve our understanding of the impact of fishing gears on damaged clams if a more rational management of this important resource is to be achieved.

Data availability

The datasets generated and/or analysed during the study are available from the corresponding author upon reasonable request.

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Author contributions

G.B. wrote the manuscript with contributions (in order of importance) from M.V. and A.L. G.B., A.P. and M.V. conducted the laboratory and sea activities under the coordination of G.B. C.V. performed the statistical analysis and A.P. generated the map using the software QGIS. A.L. was the Scientific Responsible of the study.

Competing interests

The authors declare no competing interests.

Additional information

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Chapter 4

Evaluation and quantification of shell damage and related survivability in the striped venus clam (*Chamelea gallina*) hydraulic dredging fisheries

Submitted paper

Evaluation and quantification of shell damage and related survivability in the striped venus clam (*Chamelea gallina*) hydraulic dredging fisheries

Abstract

1. The impact of hydraulic dredging was assessed on *Chamelea gallina* populations in the mid-western Adriatic Sea by evaluating and quantifying the damage exerted on the harvested (non-sieved) and sorted (sieved: commercial or discarded) individuals and by estimating the survival probability of discarded clams.
2. The results showed that the large majority of individuals remained intact after both dredging (86.3%) and sorting (79.4%) processes with clams with one or two chipped valves as the most represented damage classes. This highlights that the effect of the dredge was higher than the mechanical vibrating sieve in determining shell damage.
3. The analyses also revealed that the damage probability was significantly associated with shell length in both non-sieved and sieved samples, but the effect of shell length was significantly greater in discard samples as the longer the individuals remained in the sieve the higher was the probability of being damaged.
4. Survival tests carried out at sea on discarded damaged individuals allowed to estimate an overall survival probability of the whole discarded fraction (damaged + undamaged) of 91.7%.
5. The introduction of modified gears, as modified dredges and coated grids and walls of the vibrating sieve, are discussed as a potential mitigation measures to further reduce the damage caused on bivalve shells during the fishing process and to better manage this valuable economic resource.

Keywords: *discards, EU regulations, hydraulic dredging, shell damage, striped venus clam, survivability*

Introduction

The striped venus clam, *Chamelea gallina* (Linnaeus, 1758), is one of the most economically and ecologically important species in the Adriatic Sea. *Chamelea gallina* dominates coastal well-sorted fine sands (Pérès and Picard (1964) between 2 and 12 m (Morello et al., 2006; Lucchetti and Sala, 2012). In 2018 and 2019, the overall Italian production was around 19,000 tons per year, representing 11% in weight and 6% in revenue (around € 51.4 million) of all fisheries production in Italy (DGPEMAC, 2019). Clams harvesting has a long history in Italy, and fishing techniques evolved hand in hand with improvements in technological innovations. Since the 1970s, the exploitation of *C. gallina* beds increased due to the transition from hand-operated fishing gears (hand dredge) to hydraulic dredges and mechanical vibrating sieves on-board. This system allows to achieve a high selectivity and to return at sea undersized individuals (Sala et al., 2017). At present the Minimum Conservation Reference Size – MCRS – is set at 22 mm total length (TL) by Delegated Regulation (EU) 2016/2376, Regulation (EU) 2020/3 and Regulation (EU) 2020/2237, which is reached on around two years of age (Bargione et al., 2020). On the other hand, hydraulic dredges raised concern over the years as this fishing technique can leads to the death of target and non-target species, alter habitats and disturb seabed sediments (Hall-Spencer and Moore, 2000; Tuck et al., 2000; Gaspar et al., 2003b; Constantino et al., 2009; Lucchetti and Sala, 2012). Operating on the seabed inevitably alter the physical and biological settings of the bottom often resulting in a decrease in habitat complexity and negatively influencing on benthic communities (Kaiser et al., 2000; Carbines et al., 2004; Gilkinson et al., 2005; Vergnon and Blanchard, 2006; McLaverty et al., 2020). Recent efforts to understand the impact of dredges have focused on deep water fisheries and less is known about the impact of dredges in the costal environment (Soon and Ransangan, 2019). In particular, in costal environments studies have been mainly performed to assess the impact of the dredge on by-catch and macro-benthic communities (Gaspar et al., 2002, 2003a, 2003b; Hauton et al., 2003; Urra et al., 2017; Anjos et al., 2018; Baeta et al., 2021a, 2021b) and little is known of the impact exerted on the target species. Different authors found that, despite the great reduction in abundance caused by dredging, biological communities present in the fishing areas are the typical ones that live in low-depth and high-energy environments (Tuck et al., 2000; Gaspar et al., 2001; Constantino et al., 2009; Vasapollo et al., 2020) and recolonization by the majority of the species usually occurs from 2 to 6 months (Pranovi and Giovanardi, 1994; Pranovi et al., 1998; Vasapollo et al., 2020).

Shell damage caused by fishing is widely recognized, and represents a threat on discarded individuals as well as on dislodged ones left on the dredge passage (e.g. Robinson and Richardson, 1998; Gaspar et al., 2003a, 2003b; Hauton et al., 2003; Moschino et al., 2003; Baeta et al., 2021a; Urra et al., 2021). Evaluation of scarring on shells surface has also been adopted as a method to evaluate fishing intensity in dredged areas compared to pristine ones, especially where fishing effort data were lacking (Witbaard and Klein, 1994; Klein et al., 1995; Ramsay et al., 2000; Schejter and Bremec, 2007). The shell damage suffered by the target and non-target species can take place *i)* in the sediment due to compaction of the sand, *ii)* inside the dredge due to the collisions between bivalves and other organisms, debris, and the metallic grid, *iii)* when tipped from the dredge to the collecting box, or *iv)* due to the mechanized vibrating sieve during the sorting process (Gaspar et al., 2001). Nevertheless, not all the damaged individuals die as repaired shells and abnormal calcifications are commonly observed in bivalve mollusks (Lomovasky et al., 2005; Schejter and Bremec, 2007), indicating that organisms can repair the damaged shells (Day et al., 2000; Alexander and Dietl, 2001; Harper et al., 2009).

Multiple factors may influence the proportion of damaged clams, such as shell thickness, burrowing depth, shell TL, tow speed and duration, and gear characteristics (e.g. tooth length and spacing, inclination of the blade) (Gaspar et al., 1994; Gaspar and Monteiro, 1998; Ramsay et al., 2000; Vasconcelos et al., 2011). Nevertheless, the damage induced during fishing operation may cause, in addition to direct fishing mortality, also indirect fishing mortality on discarded and dislodged clams. This threat has been ignored for a long time, however for managing purposes and to support Regulation (CE) 1380/2013 which establishes that where applicable discards must be returned to the sea, it is of pivotal importance to understand the effects of dredging and sorting operations on the discarded fraction and its survival rates. Following the Commission Delegated Regulation (EU) 2020/2237 in the Venus clam fishery discards can be released to the sea until December 31th 2022 unless scientific evidence clearly prove once and for all their survival.

For these purposes, focusing on the target species, were assessed: (*i*) the effect of the dredge on the non-sieved fraction of clams, (*ii*) the effect of the sieve on sorted clams (*i.e.* commercial and discarded), and (*iii*) the survival rate of discarded clams in relation to their damage level.

Methods

Sampling design

To evaluate the damage inflicted by hydraulic dredging on *C. gallina* specimens harvested during commercial fishing operations, a total of 8 trials were carried out in the Ancona Maritime District (Figure 1) on fine, well-sorted sandy bottoms at a depth ranging between 3 and 4 m. The activity at sea was conducted in October 2020 on board a commercial vessel (LOA, 15.8 m; tonnage, 9.7 GT; engine power, 110 kW). Each haul lasted 3 minutes, from the moment when the lateral breeches entered into tension until the tension of the same ceased. At the end of the haul the dredge was hoisted and its content dropped into the collecting box. The total weight of the catch (non-sieved; NSV) was recorded and subsequently sorted in commercial (COM, ≥22 mm TL) and discard (DIS, mainly < 22 mm TL) samples through the on-board mechanical vibrating sieve (for details see Sala et al., 2017). The total weight of each group (NSV, COM, DIS) was taken through a marine-type compensation scale (Mod. *Marelec W50/50-D4 marine scale*) with accuracy of 50 g.

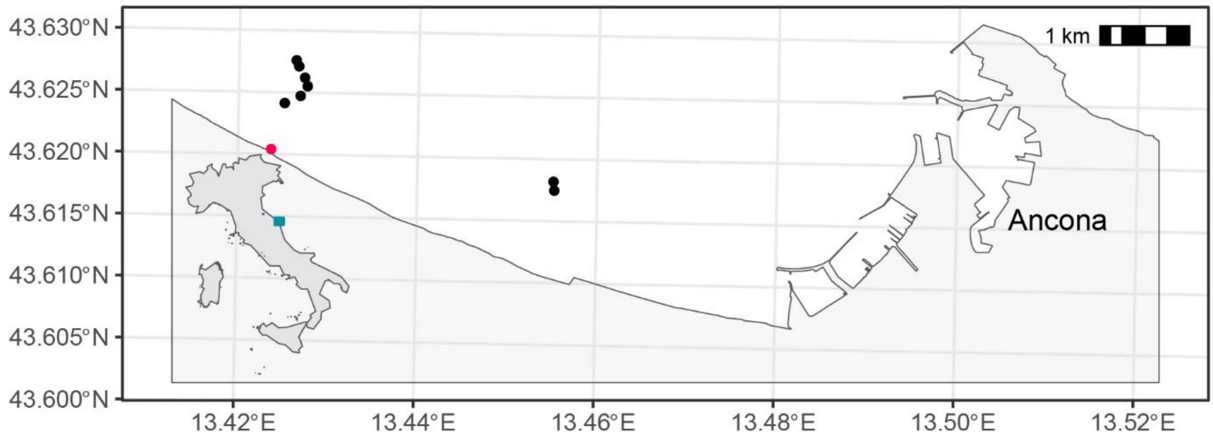

Figure 1. Geographic representation of the study area. Black dots denote the sampling site, the red dot indicates the site in which the survival experiment was carried out.

Examination of shell damage

To estimate the effects of the dredge and the mechanical vibrating sieve on *C. gallina* specimens integrity, a sub-sample of about 2 kg was collected from each group: NSV taken from the collecting box, where individuals were only subjected to high water pressure (1.8 bar *as per* Ministerial Decree 22/12/2000); while COM (individuals exited the vibrating sieve at the end of the first and second selection grids) and DIS (individuals collected by blocking the waste exhaust pipe) samples had undergone also to mechanized sorting.

All samples were carefully handled to avoid additional damage before and after being frozen at -22°C for further investigation in the laboratory. All clams present in each group were visually inspected to identify potential damage. Damage was defined as any area of the shell in which some parts of the valve/s were missing or broken or there were missing layers of the shell surface. Seven category damage classes was adopted: *a*) intact, *b*) repaired, *c*) one valve chipped, *d*) two valves chipped, *e*) broken umbo, *f*) crushed shell, and *g*) peeled (Figure 2). Each damage class implied an increasing damage level and soft tissue exposure, except for the last one (*g*).

For each sample, all the individuals belonging to each damage category were measured by mean of a video analysis system, according to Stagioni (2010) protocol. Clams were placed on a backlit table to be photographed by a digital camera mounted at a fixed distance above the table. Photographs were then processed with ImageJ software (Rasband, 2018), allowing for the estimation of the TL for each clam. For each picture, the central clam was manually measured with the caliper, to calibrate the analysis and minimize any error due to any lens movement or distortion among different images. Measures were performed with an accuracy of 0.2 ± 0.1 mm.

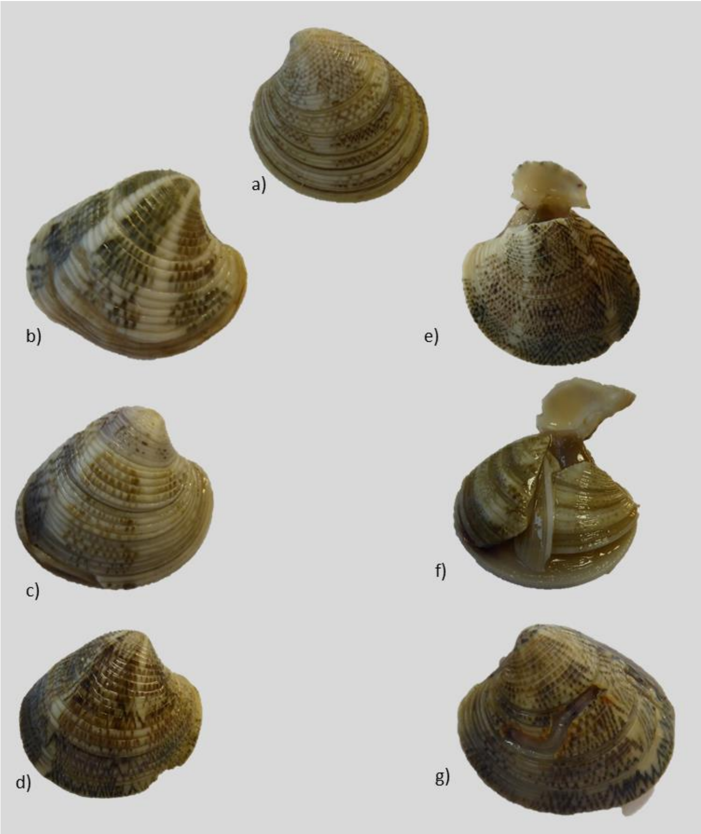

Figure 2. Damage levels detected on *C. gallina* specimens: *a*) intact, *b*) repaired, *c*) one valve chipped, *d*) two valves chipped, *e*) broken umbo, *f*) crushed shell, and *g*) peeled.

Survival experiment

To estimate the survival probability of all discarded clams, an additional 3-minutes haul was carried out in the same area the day after to collect the damaged individuals (Figure 1). These specimens had undergone both high water pressure as well as mechanized sorting and were directly collected from the waste exhaust pipe. After sorting on-board about 50 kg of clams a total of 262 damaged individuals were found (of which 25, 61, 117 and 59 in damage classes *b*, *c*, *d* and *g*, respectively). No individuals belonging to class *e* and *f* were considered for the experiment as they were poorly represented. Clams were then immediately placed into a plastic tank containing seawater collected during the fishing haul. To avoid further stress, the plastic tank was handled gently until the clams were placed in sea cages. Subsequently, clams were randomly placed into two metal cages (100 x 120 x 40 cm) anchored to the bottom at ≈ 1 m depth off Palombina beach, near the collection site (Figure 1), as described in Bargione et al. (2021). Cages were left for 21 days, after which dead and alive clams in each damage classes were sorted and counted.

The mean survival rate of discarded clams was estimated by applying the survival rates obtained from the experiment to the DIS samples. The average population structure of DIS samples was obtained by averaging clam abundance in each length class over the number of samples at time zero (T_0), viz when immediately harvested. Likewise, the survived DIS average population structure was obtained by averaging the net clam abundance at the end of the survival experiment (T_1), derived by multiplying the initial clam abundance at T_0 for the respective survival rates in relation to the damage classes, over the eight samples. Survivability of intact individuals (damage class a) was considered to be 96.2% as reported in the study conducted by Bargione et al. (2021), while no probability to survive was assumed for individuals belonging to class *e* and *f*. Hence, the mean survival rate was derived by taking the difference between the mean total individuals discarded at T_0 and the mean total individuals survived at T_1.

Data analyses

Summary statistics were calculated on the eight hauls to provide mean and standard deviation (SD) about the proportion of individuals in NSV and SVD (i.e. sieved = COM + DIS) groups and each damage class. Damage class *b* was considered not to be caused by the dredging nor the sieving process and was therefore pooled together to damage class a, as repaired individuals were considered intact as well.

To explore the effect of TL on the damage probability in the three groups we fitted a multilevel generalised linear model as follow:

$$DMG_{ijk} | \beta_{0j}, \beta_{1k} \sim Bern(\pi_{ijk})$$

$$logit(\pi_{ijk}) \sim \beta_{0j} + \beta_{1k} \times TL_{ij}$$

$$\beta_{0j} | \beta_0, \sigma \sim N(\beta_0, \sigma^2)$$

$$\beta_0 \sim N(0, 2.5^2)$$

$$\beta_{1k} \sim N(0, 2.5^2)$$

$$\sigma \sim Exp(1)$$

Where the damage probability DMG follows a binomial distribution (1 if damaged and 0 if intact) with parameter π_{ijk}. Letting π_{ijk} be the damage probability of the individual i for the treatment k (NSV, COM, DIS), at site j (S1 to S8). The model was fitted with the stan_glmer function in rstanarm, in a way similar to the glmer function in R's lme4 package (S1). However, rstanarm uses Stan's MCMC (Markov Chian Monte Carlo) algorithm to draw from the posterior distribution of the model parameters rather than estimating them via the frequentist approach taken by glmer. Prior specification, were left as default as the weakly informative priors automatically assigned by stan_glmer were sufficient (S1).

Before assessing model results, sampling quality was assessed. $\hat{R}$ values were very close to 1, effective sample sizes were large, and Monte Carlo standard errors were smaller than the posterior standard deviations, indicating that the chains were stable and explored the whole distribution (S1). Trace plots of the MCMC chains exhibited good mixing and show no signs of convergence problems (S1).

All data analyses and visualizations were carried out in R (v: 4.1.2; R Core Team, 2021) availing of "tidyverse" and "gridExtra" for data management and basic data visualization, "rstanarm" and "bayesplot" for modelling and simulations, and "sf" for mapping (Baptiste, 2017; Pebesma, 2018; Goodrich and Gabry, 2020; R Core Team, 2021; Gabry and Mahr, 2022).

Results

Overall catches

On average the total catch of a single 3 min haul was 114.7 ± 5.2 kg (mean ± SD), where 92.0 ± 41.5 kg (80.3 ± 2.9%) were made of clams, while 22.7 ± 10.8 kg (19.7 ± 2.9%) were made of by-catch species. The mean commercial fraction weighted 44.7 ± 20.6 kg (39.0 ±10.4% of the total catch), while discarded clams weighted 50.4 ± 27.7 kg (41.3 ±8.5% of the total catch) (Figure 3). The relative mean number of individuals was $25.8 \times 10^3 \pm 12.1 \times 10^3$ in the NSV treatment, $9.0 \times 10^3 \pm 4.2 \times 10^3$ (34.6 ± 10.9%) in the COM treatment - representing the fraction removed for commercial purposes - and $17.1 \times 10^3 \pm 8.7 \times 10^3$ (65.4 ± 11.0%) in the DIS treatment. This information, shows that almost 2 times more individuals of clams are returned at sea.

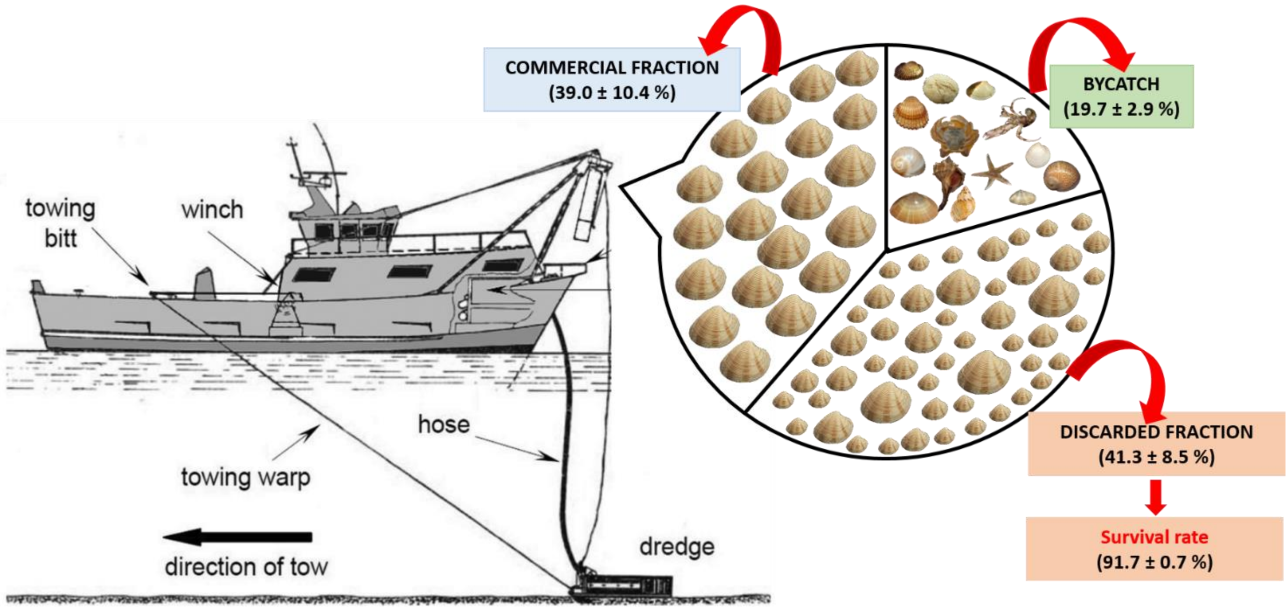

Figure 3. Summary of the mean fraction (± SD) of the parts of the catch (non-sieved, bycatch, commercial and discarded fraction) harvested during common fishing operations. All the percentages refer to the total catch, except for the one estimating the survival rate which is relative to the total discarded fraction of clams (figure adapted from Lucchetti and Sala, 2012).

Damage classes

Intact individuals (damage classes *a*+*b*) accounted for the largest proportion of the samples independently from the group: NSV = 0.863 and SVD = 0.794. Of these estimates, repaired individuals ranged on average between 0.150 and 0.200 in both NSV and SVD groups. Aside from intact individuals, the proportion left was mainly represented by chipped clams, damage classes *c* and *d*, which accounted for about 0.058 and 0.059 in NSV samples and 0.114 and 0.064 in SVD samples (Figure 4 and Table 1). The majority of individuals in these classes presented a very small damage, which in most of the case did not subsisted in more than 1 to 2 mm missing chip, and no tissues exposed, hence it was rare to observe chipped calms with serious damage and exposed tissues. The remaining damage classes cumulatively failed to account for more than 0.008 and 0.009 in NSV and SVD samples, respectively. However, the damage class *g* (peeled shell) was identified in all the treatments as ascribable to the endolithic activity by Polychaeta of the genus *Polydora*, which dig tunnels on the shell’s surfaces and was not caused by dredging.

Assuming that all individuals were intact before collection, NSV samples displayed a largest reduction of the proportion of intact individuals (0.137) compared to SVD samples (+0.068). This result highlighted that the dredge had a greater causal effect on shell damage than the sieve.

Table 1. Mean fraction (± standard deviation, SD) of clams in each damage class (*a*-*g*) in relation to the kind of group (non-sieved, and sieved). Here damage class *b* is pooled together to damage class *a*.

Group	Damage class	AM	SD	Q0.1	Q0.5	Q0.9
NSV	**a**	0.863	0.034	0.812	0.879	0.896
NSV	**c**	0.058	0.017	0.044	0.051	0.079
NSV	**d**	0.059	0.022	0.036	0.059	0.083
NSV	**e**	0.006	0.003	0.003	0.005	0.009
NSV	**f**	0.014	0.005	0.008	0.013	0.019
NSV	**g**	0.004	0.002	0.002	0.005	0.006
SVD	**a**	0.794	0.035	0.751	0.794	0.835
SVD	**c**	0.114	0.018	0.097	0.113	0.132
SVD	**d**	0.064	0.021	0.041	0.063	0.085
SVD	**e**	0.006	0.003	0.003	0.006	0.009
SVD	**f**	0.016	0.007	0.008	0.017	0.024
SVD	**g**	0.006	0.006	0.001	0.004	0.011

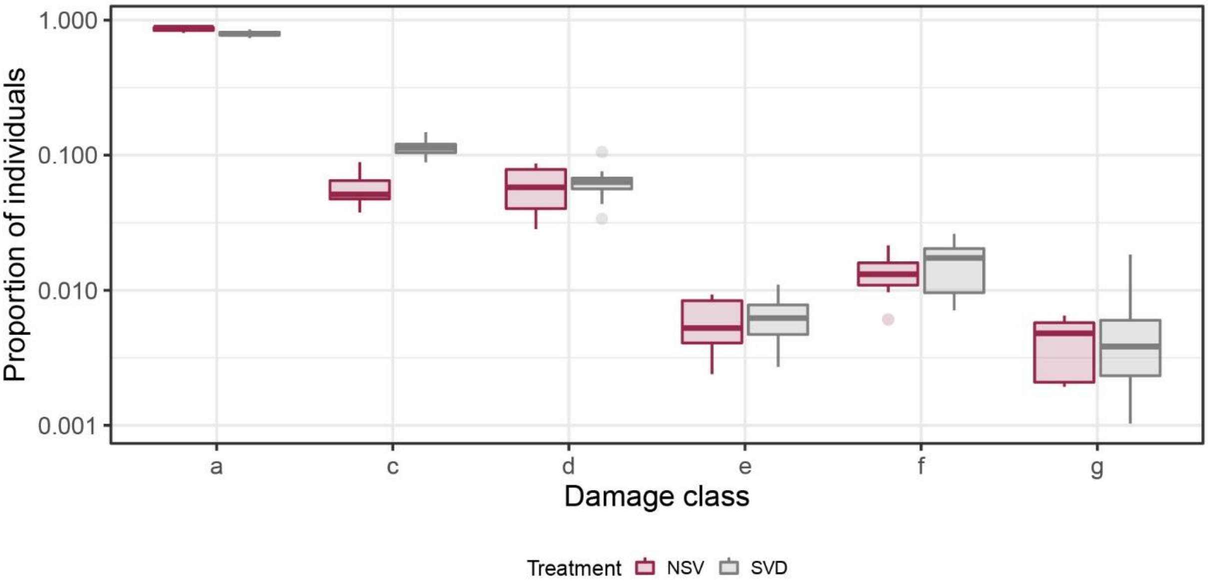

Figure 4. Boxplots showing the proportion of clams in each damage class (*a*-*g*) in relation to the sample treatment (non-sieved, commercial and discard). Here damage class *b* is pooled together to damage class *a*. The thick line inside the box denotes the median. The height of the box marks the interquartile range. The whiskers show the 95% interval and the dots the outliers.

Damage probability

Testing for the association between damage probability and TL revealed a significant positive relationship between the two parameters for all treatments (Figure 5a,b): β_{NSV}~N(0.163, 0.011) β_{COM}~N(0.176, 0.009) and β_{DIS}~N(0.188, 0.011). These results indicate that the dredge was the most important factor determining the damage probability by length class as the difference between the β_{NSV} and β_{COM}, or β_{DIS}, was always negative and smaller than the β_{NSV}. That is, SVD samples always showed the greatest damage probability by length class likely resulting from the additional mechanical vibrating process, but overall the sieving process added only a slight damage probability. Between the two sieved groups, DIS samples showed the greatest damage probability by length class suggesting that this probability increased with the time and distance spent inside the vibrating sieve. Therefore, the longer individuals were retained inside the vibrating sieve the higher was the probability of larger individuals of being damaged.

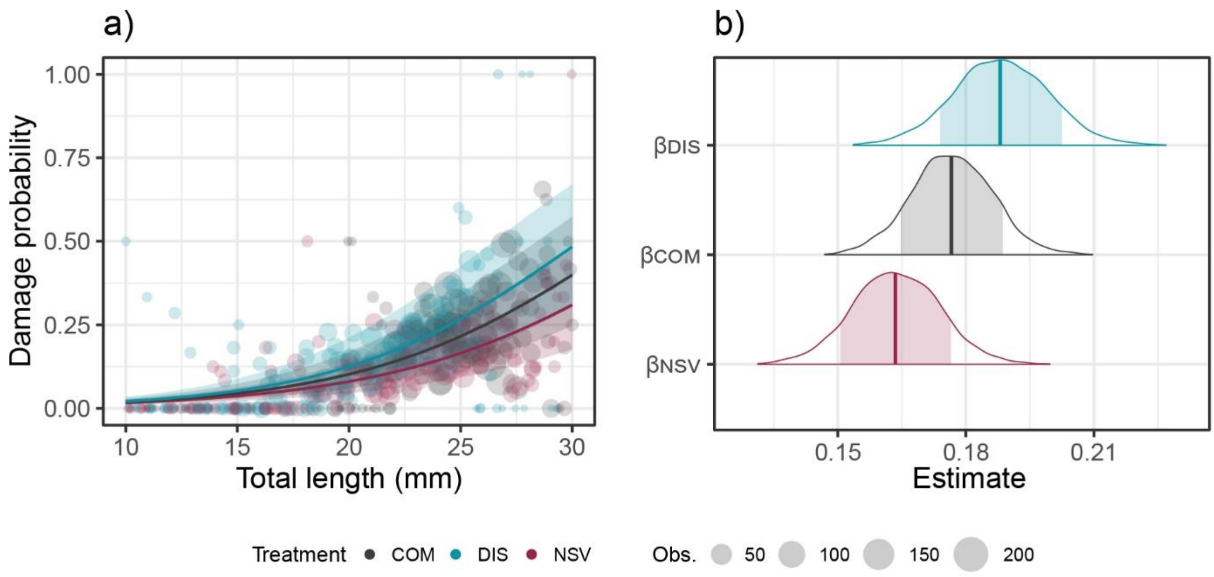

Figure 5. Visual model output. a) logistic regression, b) estimates of the slope for each treatment. Estimates are represented showing the whole range, while the colored portion inside the shape denotes the 95% interval.

Survival experiments

At the end of the 21 days experiment a total of 203 out of 262 damaged clams were still alive. In details, clams in damage class *b* showed the highest survival probability (0.96) supporting previous results about the survival probability of intact clams, followed by clams in damage classes *c*, *d* and *g* (0.84, 0.74 and 0.71, respectively) (Table 2).

Table 2. Number of individuals placed in the sea cages at the beginning of the survival experiment (T_0) and the number of the ones survived (T_1) at the end of the experiment, together with the relative survival probability in relation to the tested damage classes.

Damage class	**T_0**	**T_1**	**Survival probability**
b	25	24	0.96
c	61	51	0.84
d	117	86	0.74
g	59	42	0.71

The survival rate of the average discarded fraction was estimated to be 91.7 ± 0.7%. In discard samples the majority of the individuals was less than 23 mm TL, since most of the large individuals have been mainly removed through the sieving process. The average size distribution had a skewed distribution centered around 23 mm TL for both discarded and survived groups at time T_0 and T_1, respectively (Figure 6). Comparing the two curves, the largest mortality was observed to occur between 21-24 mm TL resulting from the great number of damaged individuals - positively related to shell size - reducing the survivability in these classes.

Overall, the estimated mortality rate represented 8.3 ± 0.7% of the entire discarded fraction. However, considering that discarded clams represented 65.4 ± 11.0% in number of all harvested clams, the mortality rate related to the total catch translates into 5.6 ± 0.5% plus 34.6 ± 10.9% representing the fraction removed for commercial purposes (total: 40.2 ± 10.2 %).

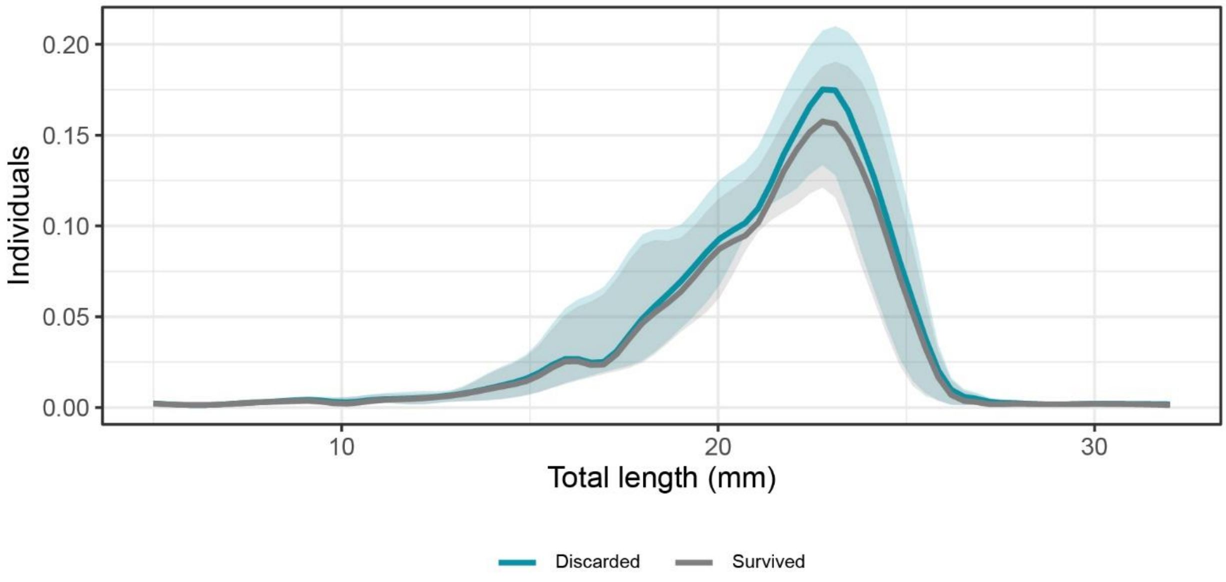

Figure 5. Mean size distribution of discarded and survived individuals with 80% Confidence Intervals (CIs).

Discussion

For managing purposes, it is of primary importance to have a sound knowledge of the biology of the species, the selectivity of the gears and the impact exerted by dredging on the whole environment, so that it is possible to evaluate the effectiveness of the management measures undertaken aimed at guaranteeing the conservation of the species and the persistence of the fishing activity over time.

This study aimed at estimating the damage caused by the dredge and the mechanical vibrating sieve currently used by the Italian fishing vessels targeting *C. gallina*. First, it was observed that the majority of the catch was made up by the target species (both commercial and undersized fractions), which in other bivalve fisheries targeting *Donax trunculus*, *Spisula solida* and *C. gallina* was also found to be high (> 70%) (Urra et al., 2017, 2021; Anjos et al., 2018; Baeta et al., 2021a); whereas in the fishing activity targeting the razor clam, *Ensis spp.*, it only represented the 23.0% of the total catch (Hauton et al., 2003). Nevertheless, the discarded clam fraction reported by Urra et al. (2017, 2021) and Anjos et al. (2018) was lower than the one here reported and it only ranged between 13.2 – 22.1% of the total catch. In this study, discarded clams contributing to promote the maintenance of the natural populations were on average almost 2 times higher in number than the commercial fraction despite the similar size in weight. Overall, on average the striped venus clam accounted for 80.3% in weight of the total catch indicating that the area was principally dominated by the target species forming a "*facies* à *C. gallina*" (Pérès and Picard, 1964). Indeed, clam dredging generally occurs in areas where the target species is the dominant one in macro-benthic communities, and thus often the proportion of discarded non-target species is relatively low (Butcher et al., 1981).

Hydraulic dredgers are towed scraping the seabed collecting anything larger than the distance between the metal rods of the dredge so that during towing the material inside the dredge gradually get tight clogging the dredge (Petetta et al., 2021), reducing shaking and consequently shell damage. In the 3 min hauls, dredging and mechanized sorting were both responsible for the damages inflicted on clam shells and the probability of shell damage was positively related to shell size. About 14% of clams tipped into the collecting box were already damaged and it was mainly assumed that those damages were ascribable to the dredging activity. However, other factors rather than fishing may cause shell damage such as predatory attacks, burrowing processes, parasitism and physical events as storms or moving rocks (Day et al., 2000; Ramsay et al., 2000; Alexander and Dietl, 2001, 2005; Lomovasky et al., 2005; Silina, 2006; Mondal et al., 2014). Failed predation attempt by decapods (e.g. crabs), representing one of the main predators for clams are highlighted by chips, scrapes and gouges on the outer shell surface (Hillard and Walters, 2009). These studies assessing different sources of damage, especially those trying to quantify the damage inflicted by each factor, are still few (Ramsay et al., 2000; Urra et al., 2021). Although is not always possible to ascertain the cause of the different types of damage, in this study it was only possible to ascertain that to a very low extent some shells were peeled, indicating a damage inflicted by a ectoparasite and not by the dredge (*Froglia C. personal communication 2021*). Notwithstanding, dredging has always been recognized as the major cause of shell damage in commercially fished areas by several authors (e.g. Gaspar et al., 1994; Witbaard and Klein, 1994; Moschino et al., 2003; Ragnarsson et al., 2015).

Dredging activity targeting a variety of bivalve species worldwide differently affects the integrity of shells. The severity of injuries inflicted by dredging on different bivalve species is related to their species-specific characteristics (e.g. morphology, body size, structure, fragility) (Bergmann et al., 2001; Leitão et al., 2009; Urra et al., 2019), behavior (e.g. burrowing depth), fishing gear and tow characteristics, as well as by seasonality (Gaspar and Chícharo, 2007; Dalgıç and Ceylan, 2012; Urra et al., 2013, 2021). A study carried out on the smooth clam (*Callista chione*) revealed that during dredging activity most of the shell damage was attributable to compaction (i.e. an evenly distributed force/pressure on the shell within the sediment) rather than to the direct impact of the dredge teeth on the shell (Vasconcelos et al., 2011). Moreover, testing a new dredge for *C. chione* where the net bag of the traditional one was replaced by a semicircular metallic grid showed that the impact exerted on the target species, as well as on the macro-benthic communities, was reduced of about 50% due to its greater efficiency capture (Gaspar et al., 2001). These results highlighted the importance of testing different gears to mitigate damage. Dredge damage is also documented for the razor clams (*Ensis spp.*) where shell margin breaks, consisting of deep clefts in the outer shell layer in which sand grains are embedded, were detected using the shell sectioning technique of the acetate peel replicas (Gaspar et al., 1994; Robinson and Richardson, 1998). For this species, the increase in tow duration and decrease in the dredge tooth length were reported to increase the proportion of damaged razor clams (Gaspar and Monteiro, 1998). Moreover, damaged individuals, both caught and dislodged, were attributed to the impact with the blade of hydraulic dredges (Hauton et al., 2003). Instead, Urra et al. (2017) found that the discarded specimens of the wedge clam *Donax trunculus* harvested with mechanized dredgers were, for less than 10 %, severely damaged, otherwise intact, whereas Urra et al. (2021) found that only 2.4% of all commercial sized wedge clams suffered any type of damage and the proportion of damaged individuals was positively correlated to bottom features and towing speed.

Crushed and broken shells are the result of compaction forces inside the sediment and of dumping the catch of the haul into the collecting box, while chipping and disjoint umbo commonly derive from collisions with hard items inside the dredge, and the former even during the sorting process inside the vibrating sieve (Gaspar et al., 2001). Consistently to these observations, in this study crushed shells did not vary between SVD and NSV samples, while the proportion of chipped clams was greater in SVD samples. Such outcome can be attributed to the large number of collisions between clams and the vibrating sieve. Notwithstanding, most of the damage was attributable to dredge itself, which alone was responsible for about 14% of the damaged individuals, while the sieving process caused the damage of an additional 6.8%. These results are consistent with the hypothesis that increasing levels of stress (i.e. high water pressure and mechanized sorting) are directly related with the proportion of damaged shells in both captured and discarded samples (Marin et al., 2003; Moschino et al., 2003).

Given that the size of a particle is related to the magnitude of the collisions we hypothesized that clam total length could be a driver of the damage probability. In this study, damage probability was positively related to size, supporting previous observations (Ramsay et al., 2000; Schejter and Bremec, 2007; Vasconcelos et al., 2011; Soon and Ransangan, 2019). The difference in the damage probability across size is likely due to changes in the physical characteristics of the shell and on the ratio between the weight and the volume of clams during growth. Shell resistance to breakage depends on shell length, width, height, volume, thickness, shape microstructure and sculptural features (Zuschin et al., 2003), all of which change along the life-cycle. Mancuso et al. (2019) found that smaller individuals have a more porous shell than bigger ones, and micro-density (i.e. mass per unit volume of the shell material, excluding the volume of pores) as well as bulk density (i.e. shell mass/volume ratio, including the volume of pores) are positively related to shell size. However, the impact of large and heavy individuals is more violent resulting in a higher damage risk compared to the collisions to which small individuals are exposed despite the more fragile nature. Additionally, larger individuals have a higher probability to hit the walls of the grids holes rather than passing through without any collision. In fact, the evaluation of the magnitude at which TL influence the damage probability allowed for estimating a greater effect in the DIS samples as the retention within the vibrating sieve was longer (for details see Sala et al., 2017), and thus likely increase the number of hits resulting in a higher damage probability. The only study experimentally testing the relationship between damage, and mortality, on discarded clams and sorting equipment found a positive relationship between the free falling distance of the cockle *Cerastoderma edule* in the rotary sieve and the number of damaged clams (Coffen-Smout, 1998). On the other hand, most common are the studies testing the effect of the dredge only on shell damage (e.g. Gaspar et al., 2001, 2002, 2003a; Leitão et al., 2009).

In the present study it was found that damaged individuals accounted, on average, for about 21% of the SVD fraction, whereas in the northern Adriatic Sea Moschino et al. (2003) found that up to 27% of the discarded clams only were damaged, indicating a higher damage estimate than ours. These differences are likely linked to different sediment features (i.e. rocks and pebbles) and abundance per length classes considering the diverse areas where samplings carried out. However, not all damaged clams die as indicated by the high proportion of repaired shells found in the samples. Their sensitivity to survivorship is related to shell size, entity and type of damage, as well as to seasonal variation in both environmental and endogenous conditions (Moschino et al., 2003; Jenkins et al., 2004). Indeed, high water temperature and reproduction are reported to lower survivorship in bivalves (Eertman et al., 1993). The loss of shell integrity could lead to an increased probability of post-fishing mortality from either predators or physiological stress (Robinson and Richardson, 1998; Chícharo et al., 2002; Maguire et al., 2002; Gaspar et al., 2003a; Broadhurst et al., 2006; Gaspar and Chícharo, 2007), while sub-lethal effects likely affect growth and reproduction on repaired damaged shells (Gaspar et al., 1994; Hillard and Walters, 2009; Watson et al., 2009). Notwithstanding, available evidence failed to detect differences in growth rates of damaged and undamaged individuals of the mollusk *Laternula elliptica* (Harper et al., 2012), but the possible consequences of the induced damage on biological traits remain largely understudied.

Overall, intact individuals, independently of shell size, were reported to be capable for reburying with a survival probability at sea of about (96.2%) (Bargione et al., 2021b). In this study, repaired clams showed almost an identical survival probably to what reported by Bargione et al. (2021b), while chipped and peeled clams showed survival rates between 71 and 83 %. Contrarily, a study carried out to assess the survival rate on both undamaged and chipped individuals of three commercial bivalve species (*D. trunculus, S. solida* and *C. gallina*) found lower survival rates for damaged individuals (24.2 - 60.0%) but not for undamaged individuals (86.0 – 100.0%), irrespective of species and size (Anjos et al., 2018). However, additional works should be carried out for investigating and deepening survival rates of discarded species which are still scant.

Based on the present and previous studies, the striped venus clam demonstrated to be a species with a high survival rate supporting the claim that discarded specimens of this bivalve can be returned to the sea *as per* Regulation (EU) 2020/2237. The present data suggests that only a small number of discarded individuals is damaged and of this the majority will likely survive reaching the MCRS set at 22 mm TL by Delegated Regulation (EU) 2016/2376, Regulation (EU) 2020/3 and Regulation (EU) 2020/2237. Overall, mortality of the discarded clams, predation excluded, was 8.3%, and to further reduce the impact exerted by the fishing activity on the species research and development should target mitigation measures to prevent the damage associated with the dredge, while the development and implementation of coated grids and walls of the vibrating sieve with soft materials, similar to those used for fishing bivalve mollusks in brackish and mixed waters, should be tested to reduce the damage associated with the mechanical vibrating sieve.

Longer-term studies are clearly needed to fully understand the extent of the ecological disruption induced by dredge-fishing and discarding on the feeding, growth and reproduction of discarded and even dislodged damaged specimens for a better management of the resource. Further work is also required to improve the understanding of the impact of fishing gears on the macro-benthic communities if an ecosystem approach to fisheries must be achieved.

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Chapter 5

Damage assessment on the discarded macro-benthic fauna in the Italian striped venus clam (*Chamelea gallina*) fisheries

The study was carried out with the collaboration of the Instituto Português do Mar e da Amosfera (IPMA), Division of Fisheries Modeling and Management of Olhão (Faro, Portugal) with the support of Dr. Paula Moura, during my overseas period conducted telematically because of the pandemic.

Damage assessment on the discarded macro-benthic fauna in the Italian striped venus clam (*Chamelea gallina*) fisheries

Abstract

The striped venus clam fishery in Italy is carried out by means of hydraulic dredges and it is one of the most important socio-economic activities in the Italian fisheries sector. Dredging has traditionally been considered among those fishing activities with a greater impact at the ecosystem level and we investigated the similarity of the benthic communities and the disturbance level exerted by dredging on the studied area, and the impact and the mortality exerted by dredging, using a four-level damage scale, on the discarded macro-benthic fauna living associated with *Chamelea gallina* during the summer season. The most represented faunal groups in both abundance and number were crabs, bivalves, and sea urchins. The analysis of the benthic communities' structure between hauls revealed that in all the hauls (except one) the species composition was very similar to each other (ca. 70%) and ABC plots together with the Warwick Statistics (WS) revealed a moderately disturbed macro-benthic community. Overall, 61.0% of the individuals were undamaged, whereas 16.1%, 3.7%, and 19.2% displayed slight, intermediate, and severe damage, respectively. We found that soft-shelled or soft-bodied species were the most affected by the harvesting process, whereas thick-shelled or thick-bodied species suffered minor damage. In particular, the species suffering major damage were the sea urchin *Echinocardium cordatum* (>69%), the bivalves *Mactra stultorum* and *Polititapes aureus* (>35% and >13%, respectively), the crab *Liocarcinus vernali*s (>7%), and the sea star *A. irregularis* (>2%). The mortality rate was 22.9% of all the discarded individuals with *E. cordatum* showing the highest mortality rate of 95.4%. These findings highlight the importance of guaranteeing the integrity of the entire ecosystem, therefore new or modified gears should be adopted to reduce the impact on unwanted species.

Keywords: *hydraulic dredging, Chamelea gallina, striped venus clam, associated macrofauna assemblages, damage level, mortality rate, discard*

Introduction

Discard comprises the accidental capture of non-target organisms that is returned to sea after a fishing operation (Hall, 1999) because they are unmarketable species, highly damaged species, surplus to quota or individuals below the Minimum Conservation Reference Size (MCRS) (Kelleher, 2005; Tsagarakis et al., 2014). Discard amount is highly variable depending on the métier and sometimes can represent a large fraction of the total catch (Veale et al., 2001; Kelleher, 2005). Concern has raised over years on the impact exerted by the different fishing activities which generate discards (Pranovi et al., 2001; Urra et al., 2017). So far, most of the researches has mainly focused on the impact caused by deep-sea trawling fisheries on bycatch species (Bergmann and Moore, 2001a; Bergmann et al., 2001; Thrush and Dayton, 2002) as well as on commercial ones (Bergmann and Moore, 2001b). On the contrary, albeit attention is now increasing in several parts of the world, a minor number of studies assessed the impact caused by bivalve dredging on shallow costal fishing grounds on the target species (Moschino et al., 2003; Vasconcelos et al., 2011; Soon and Ransangan, 2019) and on the macro-benthic communities (Gaspar et al., 2002, 2003a, 2003b; Morello et al., 2005a; Urra et al., 2017; Anjos et al., 2018; Vasapollo et al., 2020; Baeta et al., 2021a, 2021b). Dredging has traditionally been considered among those fishing activities with a greater impact on costal benthic ecosystems (Collie et al., 2000). However, impact also depends on many other factors such as the time scale (i.e. short and long term) (Piersma et al., 2001), the technical features of dredges (e.g. mesh size, tooth length, water jects, etc.), the fishing effort, the local conditions (e.g. depth, type of sediment, benthic community composition, other stress factors) (Collie et al., 2000) and seasonality (Urra et al., 2017; Baeta et al., 2021a). On the long term, removing species and individuals from their habitat through the generation of discards during common fishing practices – viz changing the species relative abundance and size, together with the population structure of prey and/or predators – can lead to structural and functional disturbances in the ecosystem (Pauly et al., 2002; Thrush and Dayton, 2002). For example, the accidental catch of non-target species can modify the diversity, biomass and productivity of the associated biota (Jennings and Kaiser, 1998), disrupt trophic interactions (e.g. removal of prey and/or predators; Pauly et al., 1998) with subsequent modifications to food webs (Gaspar et al., 2001), change the structure of the benthic communities in the short and long term (Hall-Spencer and Moore, 2000; Jenkins et al., 2001; Morello et al., 2005b; Ragnarsson et al., 2015), alter species foraging behavior (FAO, 2003), reduce the ratio of large-to-small bodied species (Bianchi and Morri, 2000) and affect fishing yields in other fisheries (Clark and Hare, 1998). For this reason, it is of key importance to have a sound knowledge on the effects derived from fishing at ecosystem level, in order to adopt suitable management plans and actions with the aim to achieve a responsible and sustainable fishing activity.

In this context, the striped venus clam *Chamelea gallina* (Linnaeus, 1758) fishery in Italy is carried out by mean of a hydraulic dredge, a fixed-mouth metal cage, equipped with a scraper blade on the lower part, towed over the seabed ejecting pressurized water from nozzles placed at the dredge mouth and inside the cage to dislodge the marine organisms living in sediment and facilitate their catch (Lucchetti and Sala, 2012), inevitably catching and damaging also non-target organisms that occur in the same fishing grounds of the target species. The fleet targeting *C. gallina* consists of 626 active hydraulic dredges mainly concentrated along the Adriatic coasts where the species thrives at depths of 2 – 12 m (Morello et al., 2006; Lucchetti and Sala, 2012). *Chamelea gallina* is an important faunal component of the shallow soft bottoms in exposed sandy beaches, and, in the Adriatic Sea, the sub-littoral biocenosis of well-sorted fine sands is characterized by a *C. gallina* facies (Vatova, 1949; Froglia, 2000). This is an area subjected to intense hydrodynamism and environmental fluctuations, and the benthic community associated with the species have an inherent resilience to natural physical disturbance (Macdonald et al., 1996; Kaiser, 1998).

In recent years (2018-2018), the Italian annual production of the striped venus clam fishing sector has been around 19,000 tons (~ €50 million), accounting for 11% of fishery production in weight and for 6% in revenues (DGPEMAC, 2019). Taking into consideration the valuable socio-economic importance of *C. gallina* for the Italian fishing sector a variety of studies investigating a very broad spectrum of aspects have been carried out. Until now, new and updated studies have been recently carried out in the Adriatic Sea on *C. gallina* population deepening some biological aspects such as the age and growth (Mancuso et al., 2019; Bargione et al., 2020), fecundity and reproductive cycle (Bargione et al., 2021a), survivability of undamaged discarded clams (Bargione et al., 2021b), as well as the dredge (Petetta et al., 2021) and sieve selectivity (Sala et al., 2017) and the impact exerted by the dredge on the sediment (Lucchetti and Sala, 2012). On the contrary, in the Adriatic Sea, studies related to the impact of hydraulic dredging on macro-benthic communities (Morello et al., 2005b), the damages inflicted on them (Morello et al., 2005a) and on the target species (Marin et al., 2003; Moschino et al., 2003) are older and less updated.

The striped venus clam fishery is currently regulated by detailed European and Italian management plans. The Regulation (CE) 1380/2013 establishes that in the Mediterranean Sea, where applicable, all catches of species which are subject to catch limits or to a MCRS must be retained on board fishing vessels, recorded, landed and counted against the quotas. These rules are to be followed unless scientific evidence demonstrates high survival rates of discards, “taking into account the characteristics of the gear, of the fishing practices and of the ecosystem”, that apparently is the case of bivalve molluscs.

The discard generated by bivalve dredging would not constitute a major problem if the discarded individuals survive after returning to the sea (Gaspar and Chícharo, 2007). However, it is known that dredging affect the benthic communities either directly or indirectly (Pranovi et al., 2001; Gaspar et al., 2002). Indeed, discarded or dislodged organisms left on the dredge path may be killed outright, suffer different damage levels which make them susceptible to predation or the ones with minor damage should be able to recover and survive (Mensink et al., 2000; Gaspar et al., 2003b). The injuries suffered by benthic organisms, which may result into death, can occur during the towing of the dredge on the seabed as specimens hit against the bars of the gear or because of abrasion inside the dredge, or during the sieving and discarding processes (Veale et al., 2001). Therefore, it is crucial to analyse the composition of discard in order to propose new strategies to minimize their impact (Urra et al., 2017). The present work, whose original sampling plan has changed significantly due to Covid-related restrictions, should be considered as a preliminary study which aimed at *i*) qualitatively and quantitatively describe the macro-benthic fauna living associated with *C. gallina* in one of the most important Italian fishing ground for clams harvesting during the summer season, *ii*) investigate the similarity of the benthic communities and the disturbance level exerted by dredging on the studied area, and *iii*) estimate damage and mortality on discarded non-target species by mean an *ad hoc* damage scale.

Materials & Methods

Study area and biological sampling

Field work was carried out in June 2021 off the mid-western Adriatic Sea in Porto San Giorgio, one of the most important *C. gallina* fishing grounds along the Italian coasts (Figure 1). Only one-day fishing trip was carried out on a potential commercial fishing ground with a total of 9 hauls conducted onboard a commercial hydraulic dredge at 0.3 NM off the coast at a depth ranging between 4-5 m. However, the control area was not tested because of Covid-related reasons, which did not allow to conduct the second day fishing-trip. Every tow lasted 3 minutes and was conducted at 1.9 knots, the speed currently used by the commercial Italian dredge fleet. At the end of the haul the dredge was hoisted and its content tipped into the collecting box. The total weight of the catch (Not sieved) was recorded and subsequently sieved through the on-board mechanical vibrating sieve which allowed to sort for i) discard, the fraction retained by the top filter containing the bulkier material (hereafter named in this way excluding the undersized target species returned to the sea), ii) commercial sized clams (≥22 mm TL), the fraction caught by grid 1 and 2, and iii) non-commercial sized clams (≤22 mm TL), the fraction caught by grid 3, the bottom filter and potentially released to the sea through the waste exhaust pipe which, on this occasion, was blocked (for details on the vibrating sieve see Sala et al. (2017)). Potential non-target small individuals that passed through the grid bars were manually removed and added to the discard fraction. At the end of the sieving process also the total weights of these three compartments were recorded. All the weights were taken through a marine-type compensation scale (Mod. *Marelec W50/50-D4 marine scale*) with accuracy of 50 g.

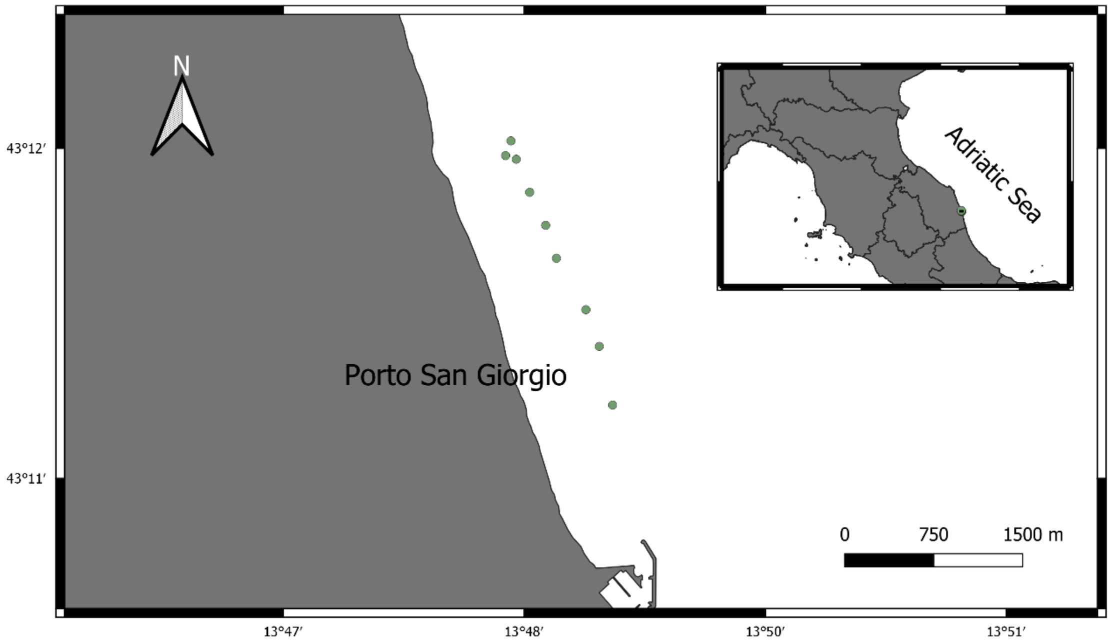

Figure 1. Map of the sampling area.

In order to estimate the catch composition in abundance (number of individuals/haul) and biomass (g/haul) a sub-sample of about 2 kg was collected from the commercial and non-commercial fraction of clams, whereas the fraction of debris was entirely collected. Before any kind of laboratory analyses the samples were stored at -22°C and handeled gently all through the process in order to avoid additional damage. The two kinds of subsamples of clams taken (commercial and undersized) were processed to count for the total number and fraction of clams present by mean of a video analysis system (Image J) according to Stagioni (2010) protocol. In the laboratory, to assess the discard composition and the damage inflicted by dredging on non-target species, for each sample of discard, every specimen was identified to the lowest taxonomic level (whenever possible) and quantified (abundance and biomass [±0.1g wet weight]). To assess the damage rate, the damage caused to all caught individuals of non-commercial species was assessed using a four-level damage scale: D_0 – intact, D_1 – slightly damaged, D_2 – moderately damaged, D_3 – severely damaged (Table 1). Mortality rate was calculated assuming damage classes D_2 and D_3 expecting to die. Indeed, according to the methodology proposed by (Gaspar et al., 2001) the damage rate corresponds to the proportion of damaged individuals (i.e. assigned with damage scores D_1 to D_3), whereas the mortality rate corresponds to the proportion of individuals with high likelihood of death (damage score D_2) and dead specimens (damage score D_3).

Table 1. Damage scale and criteria adopted for scoring different taxa caught as bycatch species during the dredging activity (adapted from Gaspar et al. 2001).

	Class damage			
Taxa	D_0	D_1	D_2	D_3
Bivalves	In good condition, intact/repaired	Edge of shell chipped	Hinge broken	Crushed / dead
Gastropods	In good condition, intact/repaired	Edge of shell chipped	Shell cracked/punctured	Crushed / dead
Crabs	In good condition, intact	1-3 legs missing / minor carapace cracks	>3 legs missing / major carapace cracks	Crushed / dead
Heremit crabs	In good condition, intact	Out of shell and intact	Out of shell and damaged	Crushed / dead
Sea urchins	In good condition, intact	-	Minor cracks	Crushed / dead
Sea stars	In good condition, intact	Arms missing	Worn and arm missing /minor disc damage	Major disc damage/dead
Ribbon worms	In good condition, intact	-	-	Sectioned
Elasmobranchs	In good condition, intact	-	-	Dead

Data analysis

Every single haul was treated as a single replicate within the same area and depth, therefore no differing conditions sussisted between hauls. As already mentioned due to Covid-related reasons it was not possible to carry out the second day fishing-trip in the control area, therefore the analysis of similarities (ANOSIM) could not be applied. However, the species composition of discards (excluding *C. gallina*) was preliminary qualitatively analyzed for differences in the number of individuals between hauls using non-metric multidimensional scaling (MDS) and average group linkage cluster analysis both based on Bray-Curtis similarity matrices of fourth-root transformed data. The SIMPER routine (Similarly Percentages; Clark and Warwick, 1994) was used to investigate the contribution of each species among hauls dissimilarities, with a similarity cut-off of 90%.

Also, preliminary investigations to determine levels of disturbance on the benthic communities' structure were carried out by means of the abundance-biomass comparison (ABC) plots (Clarke et al., 2014) and the Warwick Statistics (W-S; Clarke et al., 2014). Algebraically, WS takes values in the range -1 (severely disturbed) to +1 (undisturbed) (Clarke and Warwick, 1994).

Results

Catch description

During the 3-min haul the weight of the total catch was highly variable and ranged between 33,000 to 266,000 g/haul. The mean total catch weighted 127,000 ± 23,900 g/haul (mean ± Standard Error, SE), the mean clam commercial fraction 28,055 ± 2,299 g/haul, the mean clam non-commercial fraction 92,866 ± 22,859 g/haul, and the mean discarded non-commercial species 6,077 ± 868 g/haul (Table 2).

Each haul was mainly represented by the target species (commercial + non-commercial fraction) both in weight and number, whereas the discarded non-commercial fraction only represented 3.2% in weight and 1.3% in number (Table 3). However, the discard ratio, calculated as undersized clams + discarded non-commercial species / total catch by weight, showed high values ranging between 0.48 and 0.88, being on average 0.72 and highlighting that the great majority of the catch was always returned to the sea (Table 2).

Table 2. General data of capture of the single hauls and their mean values (± SE). Data refer to the weights (g) of each compartment (total catch, commercial clams, undersized clams and discard), their relative fraction and the discard ratio (DR).

Haul	Total catch	Commercil clams	Undersized clams	Discard	Commercil clams	Undersized clams	Discard	DR
1	100,000	21,000	68,800	10,200	21.00	68.80	10.20	0.79
2	186,000	36,000	144,500	5,500	19.35	77.69	2.96	0.81
3	266,000	31,500	231,100	3,400	11.84	86.88	1.28	0.88
4	175,000	30,000	135,000	10,000	17.14	77.14	5.71	0.83
5	135,000	27,500	102,500	5,000	20.37	75.93	3.70	0.80
6	65,000	34,000	23,500	7,500	52.31	36.15	11.54	0.48
7	33,000	13,500	14,600	4,900	40.91	44.24	14.85	0.59
8	88,000	30,000	54,900	3,100	34.09	62.39	3.52	0.66
9	95,000	29,000	60,900	5,100	30.53	64.11	5.37	0.69
Mean	127,000 (±23,899)	28,055 (±2,299)	92,866 (±22,858)	6,077 (±868)	27.50	65.92	6.57	0.72

Table 3. Mean abundance and biomass (± SE) of the commercial and undersized fraction of clams and discard harvested with hydraulic dredges. The biomass (%B) and abundance (%N) fraction for each compartment are also reported.

Fraction	**Abundance (N/haul)**	**Biomass (g/haul)**	**%N**	**%B**
C. gallina (commercial)	4258.6 (±401.5)	28055.5 (±2299.5)	11.9	22.5
C. gallina (undersized)	31208 (±8788.3)	92866.6 (±22859.0)	86.9	74.3
Discard (non-target species)	451.2 (±82.9)	4000.1 (±465.0)	1.3	3.2

Discard

Within the discarded non-commercial species, a total of 8 faunal groups and 21 species have been identified: bivalves (9 species), gastropods (5 species), crabs (2 species), hermit crabs, sea urchins, sea stars, ribbon worms and fish all with 1 species identified per group. Discard was dominated by crabs (45.7% and 54.8% of total abundance and biomass, respectively), bivalves (30.6% and 24.1%) and sea urchins (16.0% and 17.2%) (Table 4, Figure 2). The grey swimming crab (*Liocarcinus vernalis*) and the common heart urchin (*Echinocardium cordatum*) were the only species mainly contributing to the respective fraction of abundance and biomass. In bivalves *Anadara demirii*, *Acathocardia tuberculata*, *Mitylus galloprovincialis* and *Mactra stultorum* represented the majority of the biomass fraction, whereas for the abundance fraction also *Donax semistriatus* respresented an important species which contributed in determining it. Other taxa were less representative and displayed a lower dominance such as sea stars (2.1% in weight and 5.2% in number), gastropods (1.3% in weight and 1.5% in number), hermit crabs, ribbon worms and fish (both ≤1.0% in weight as well as in number) (Table 5, Figure 3). The frequency of occurrence was calculated for each discarded species and there were only 4 ubiquitous species (i.e. *A. tuberculata*, *M. stultorum*, *L. vernalis* and *Astropecten irregularis*) and others mainly common (i.e. *D. semistriatus*, *A. demirii*, *Diogenes pugilator*, and *E. cordatum*); despite each of them had a different weight in determining the mean abundance and biomass fraction (Table 5).

Table 4. Mean abundance and biomass (±SE) of the faunal groups collected during dredging as non-target discarded species. The biomass (%B) and abundance (%N) fraction for each faunal group are also reported.

Taxon	**Abundance (N/haul)**	**Biomass (g/haul)**	**%N**	**%B**
Bivalves	138.3 (±48.6)	967.1 (±181.6)	31	24
Gastropods	6.8 (±2.3)	51.7 (±15.0)	1.5	1.3
Crabs	206.8 (±16.0)	2202.9 (±132.1)	46	55
Hermit crabs	4.7 (±2.3)	16.5 (±8.6)	1	0.4
Sea urchins	72.3 (±29.6)	692.9 (±299.4)	16	17
Sea stars	23.4 (±5.7)	86.2 (±19.6)	5.2	2.1
Ribbon worms	0.1 (±0.1)	1.1 (±1.1)	<0	<0
Elasmobranchs	0.1 (±0.1)	0.8 (±0.8)	<0	<0

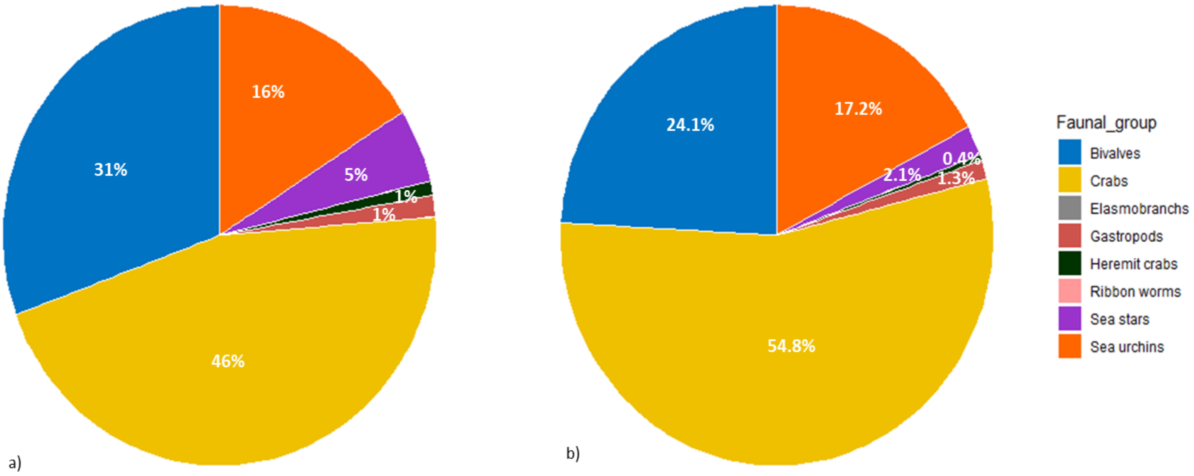

Figure 2. Percentages in a) number and b) weight of the faunal groups collected as non-target and discarded in the striped venus clam fishery.

Table 5. Mean abundance and biomass (±SE) of all the non-target discarded species collected during dredging. The biomass (%B) and abundance (%N) fraction and the frequency of occurrence (FO) for each species are also reported.

	Non-target species	**Abundance (N/haul)**	**Biomass (g/haul)**	**%N**	**%B**	**FO**
Bivalves	*T. planata*	0.1 (±0.1)	2.6 (±2.6)	<0.1	<0.1	11
	P. aurea	1.4 (±0.6)	2.6 (±1.3)	0.3	<0.1	67
	D. lupinus	3.7 (±2.3)	0.7 (±0.4)	0.8	<0.1	33
	D. semistriatus	12 (±5.3)	2.5 (±1.0)	2.7	<0.1	89
	A. demiri	19.4 (±11.4)	164.6 (±52.0)	4.3	4.1	89
	A. tuberculata	16.9 (±5.5)	334.3 (±130.7)	3.7	8.4	100
	M. galloprovincialis	35.7 (±33.8)	85.6 (±72.6)	7.9	2.1	56
	M. stultorum	47.3 (±6.4)	352.2 (±44.1)	10.5	8.8	100
	O. edulis	0.4 (±0.2)	3 (±2.0)	<0.1	<0.1	22
Gastropods	*N. mutabilis*	0.6 (±0.5)	1.8 (±1.4)	0.1	<0.1	22
	N. josephinia	3.2 (±1.3)	25.1 (±10)	0.7	0.6	67
	N. stercusmuscarum	1.8 (±1)	14.5 (±7.4)	0.4	0.4	44
	B. brandaris	0.6 (±0.2)	8.2 (±3.5)	0.1	0.2	44
	F. aperta	0.7 (±0.7)	2.2 (±2.2)	0.2	<0.1	11
Crabs	*I. nucleus*	0.5 (±0.5)	1.6 (±1.6)	0.1	<0.1	11
	L. vernalis	206.4 (±16.1)	2201.4 (±132.3)	45.7	55.0	100
Hermit crab	*D. pugilator*	4.6 (±2.3)	16.2 (±8.7)	1	0.4	78
Sea urchin	*E. cordatum*	72.3 (±29.6)	692.9 (±299.4)	16	17.3	89
Sea star	*A. irregularis*	23.4 (±5.7)	86.2 (±19.6)	5.2	2.2	100
Ribbon worms	*Nemertea spp.*	0.1 (±0.1)	1.1 (±1.1)	<0.1	<0.1	11
Elasmobranchs	*Raja spp.*	0.1 (±0.1)	0.8 (±0.8)	<0.1	<0.1	11

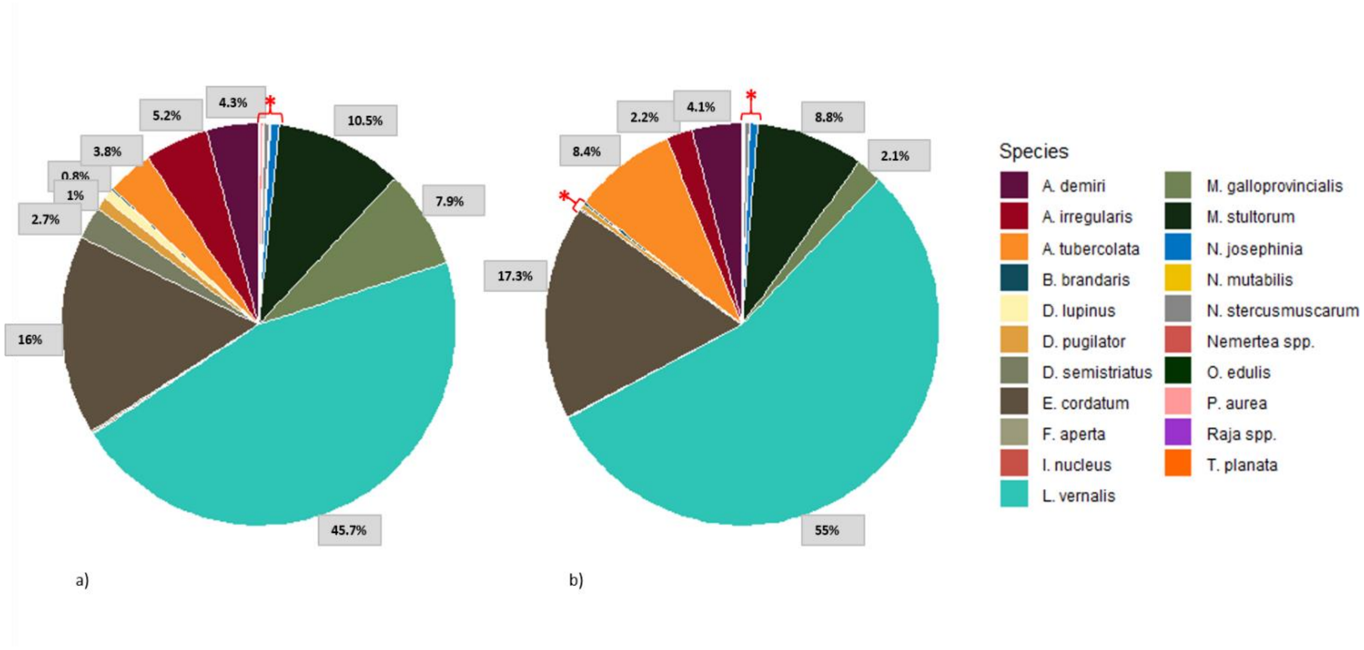

Figure 3. Percentages in a) number and b) weight of each non-target discarded species collected in the striped venus clam fishery. Species indicated with star (*) accounted for less than 0.5% and the exact value is not shown.

The analysis of the benthic communities' structure between hauls revealed that in all the hauls (except haul 8) the species composition was very similar to each other, being this similarity of about 70% (Figure 4).

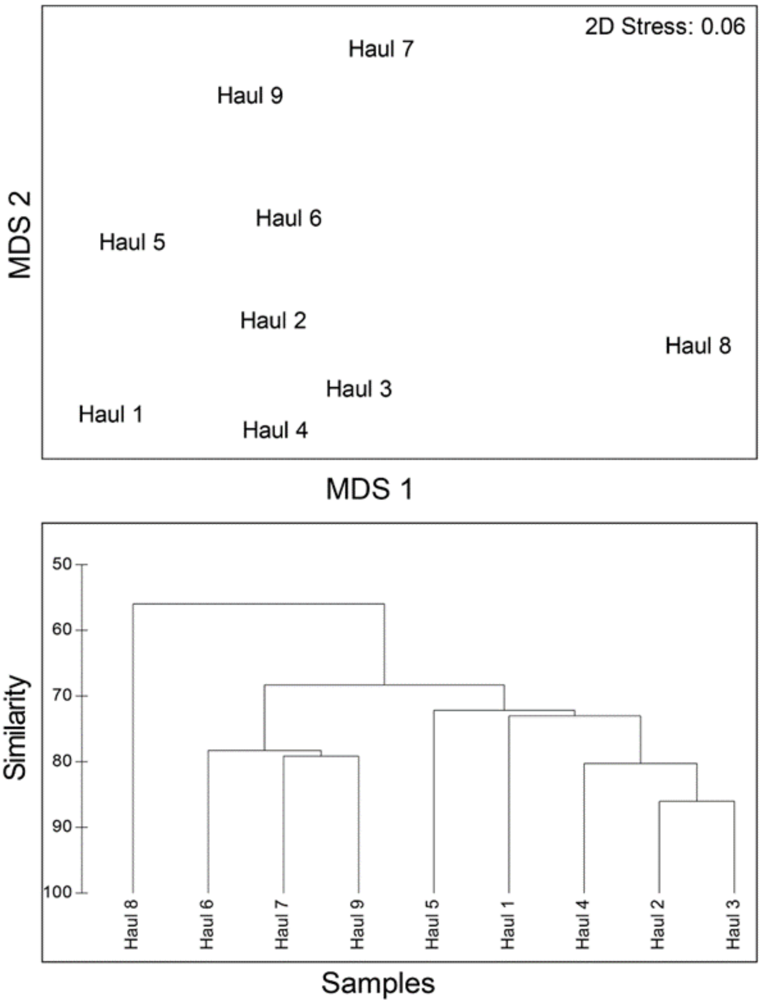

Figure 4. MDS plot representing the benthic community structure in each haul of the striped venus clam fishery in San Benedetto Maritime Districts (on the top). Average group linkage clustering of the benthic community structure (on the bottom).

SIMPER analysis carried out on abundance data showed an average similarity between hauls of 68.4% and indicated that the species that mainly contributed to similarity were the crab *L. vernalis*, the bivalves *M. stultorum*, *A. tuberculata*, *A. demirii*, *D. semistriatus*, the sea star *A. irregularis*, the sea urchin *E. cordatum*, the hermit crab *D. pugilator* and the gastropod *N. josephinia* (Table 6).

Table 6. Similarity percentages analysis (SIMPER) of discard species abundance in San Benedetto Maritime District. Average similarity between hauls = 68.4%. Av.: average, Abund.: abundance, Sim.: similarity, Contrib %: percentage contribution, Cum %: cumulative percentage.

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum%
L. vernalis	3.77	16.51	7.97	24.15	24.15
M. stultorum	2.58	10.98	6.79	16.06	40.21
A. irregularis	2.12	8.76	6.08	12.8	53.01
E. cordatum	2.29	6.52	1.51	9.53	62.54
A. tuberculata	1.76	5.92	1.77	8.66	71.2
A. demiri	1.62	4.87	1.72	7.12	78.32
D. semistriatus	1.52	4.7	1.75	6.88	85.2
D. pugilator	0.97	2.25	0.8	3.3	88.49
N. josephinia	0.94	2.12	0.82	3.11	91.6
					Average similarity: 68.38

Of the 9 hauls carried out all showed signs of moderately disturbed macro-benthic communities as shown from ABC plots in which the biomass is constantly slightly above the abundance curve, intersecting only at higher species ranks (Figure 5 and Figure 6). The moderate disturb is also confirmed by the Warwick Statistics (WS) (Figure 7).

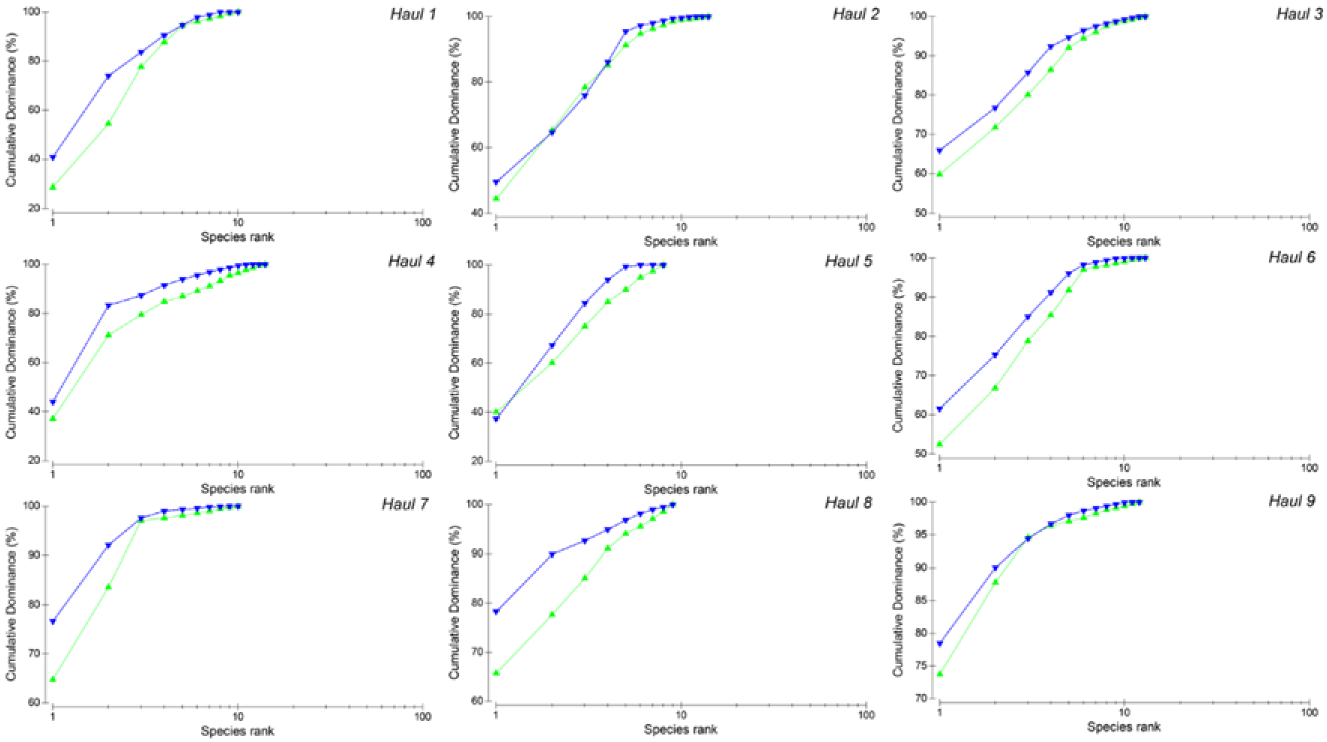

Figure 5. ABC plots generated from the combination of abundance (green lines) and biomass (blue lines) data for each haul.

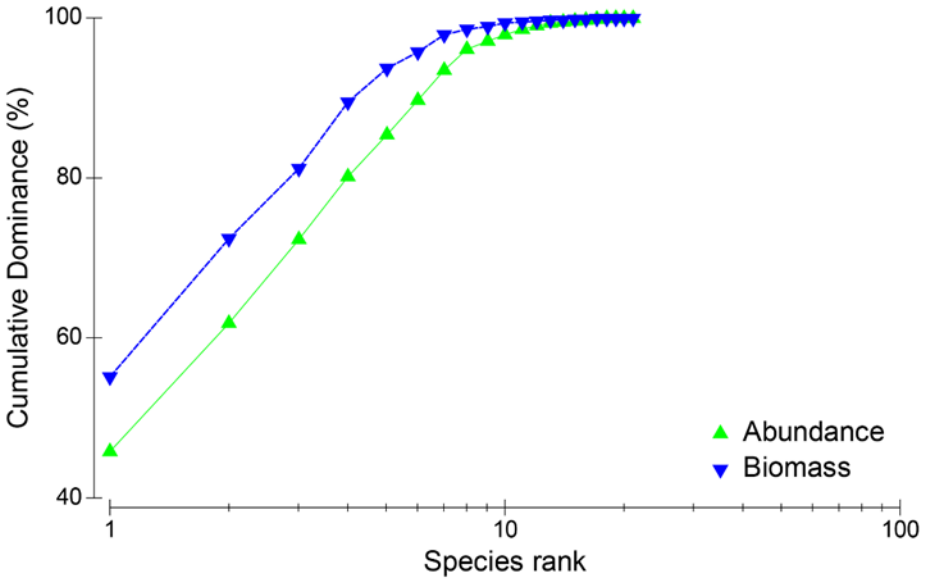

Figure 6. Average ABC plots generated from the combination of mean abundance and biomass data relatively to the 9 hauls.

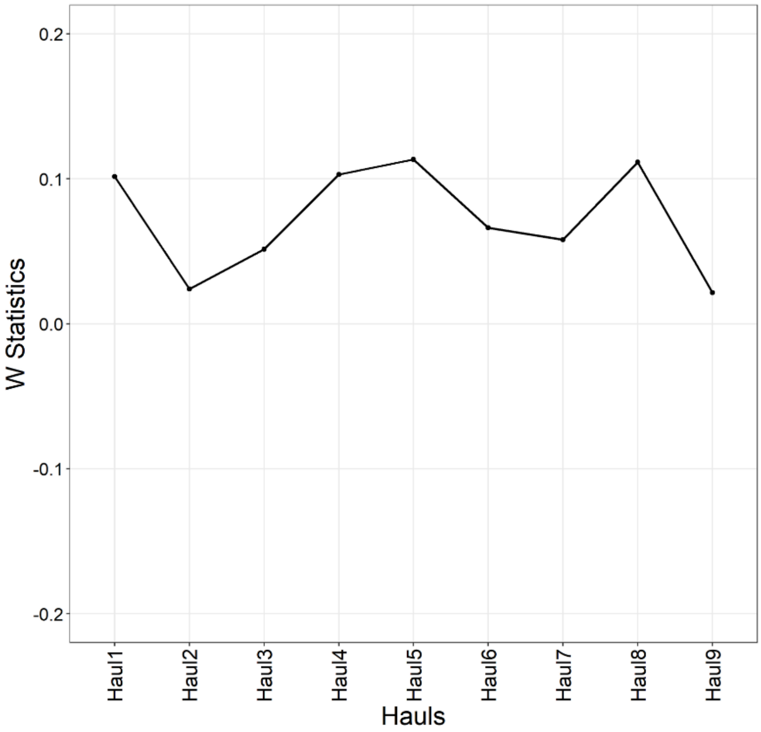

Figure 7. WS plot of the hauls. The index never goes in the negative field but does not differ much from zero, confirming the trend of the ABC plots of a moderate disturbance.

Discard damage and mortality rate

Overall, 61.0% of the individuals were undamaged, whereas 16.1%, 3.7% and 19.2% displayed slight, intermediate and severe damage, respectively. Intact individuals in damage class D_0 were dominated by the grey swimming crab *L. vernali*s (47.2% and 59.2% of total abundance and biomass, respectively) and bivalves (42.1% and 33.5%), followed by less dominant species of different taxa as the sea star *Astropecten irregularis* (5.7% and 2.2%), gastropods (2.1% and 1.9%), the hermit crab *D. pugilator* (1.6% and 0.7%) the sea urchin *E. cordatum* (1.2% and 2.5%) and ribbon worms and fish (≤0.1% and ≤1.1%). Among bivalves it is noteworthy *A. demiri* where 100% of the collected individuals were classified as intact although frequent signs of previous damage, subsequently repaired, were detectable on the shells. Slightly damaged individuals in class D_1 were dominated exclusively by the grey swimming crab *L. vernali*s (87.6% and 93.0%) despite a minor dominance by the sea star *A. irregularis* (7.3% and 2.6%), bivalves (3.7% and 3.5%) and gastropods (1.2% and 0.9%) was also present. Within this damage class, for bivalves *Ostrea edulis* was the species mainly damaged (50% of the total collected individuals) showing chipped edges of the shell, whereas for gastropods *Bolinus brandaris* (40%) and *Neverita josephinia* (20.7%) were the ones showing major damage with chipped edges of the shell. Intermediate damaged individuals in class D_2 were dominated by the sea urchin *E. cordatum* (52.1% and 59.0% of total abundance and biomass, respectively), the grey swimming crab *L. vernali*s and pebble crab *Ilia nucleus* (35.6% and 31.2%), followed by the bivalves *M. stultorum* and *A. tuberculata* (10.6% and 8.8%) and the sea star *A. irregularis* (1.8% and 0.9%). For *I. nucleus* only an individual was recorded on the whole sampling showing major carapace cracks and all legs missing. Severely damaged individuals in class D_3 were dominated by the sea urchin *E. cordatum* (69.3% and 73.5% of total abundance and biomass, respectively), bivalves (20.5% and 16.3%) and the grey swimming crab *L. vernali*s (7.8% and 8.1%), whereas the sea star *A. irregularis* contributed marginally (2.3% and 2.0%) (see Figure 8 and Figure 9). Among bivalves *M. stultorum* and *Polititapes aureus* (13.3%) were the ones mainly damaged within this class. These results show that soft-shelled or soft-body species (i.e. *E. cordatum*, *I. nucleus*, *M. stultorum* and *P. aureus*) were the most affected by the harvesting process, whereas thick-shelled or thick-body species suffered minor damage. The greatest sensitivity to the catch is shown by the sea urchin *E. cordatum* where 83.3% of the total individual caught were completely smashed, while 12.1% suffered minor but unrepairable cracks. The second most damaged species was the soft-shelled *M. stultorum* followed by the soft-shelled *P. aureus* with 33.5% and 13.3% of the total individuals caught completely crushed, respectively (Figure 9).

Overall, mortality rate was 22.9% of all the discarded individuals. The sea urchin *E. cordatum* was extremely sensitive to dredging with a mortality rate of 95.4%, whereas bivalves and *L. vernalis* showed a mortality rate of 14.2% and 6.2%, respectively. Among the lower abundant discarded species, the sea star *A. irregularis* had a mortality rate of 9.9%.

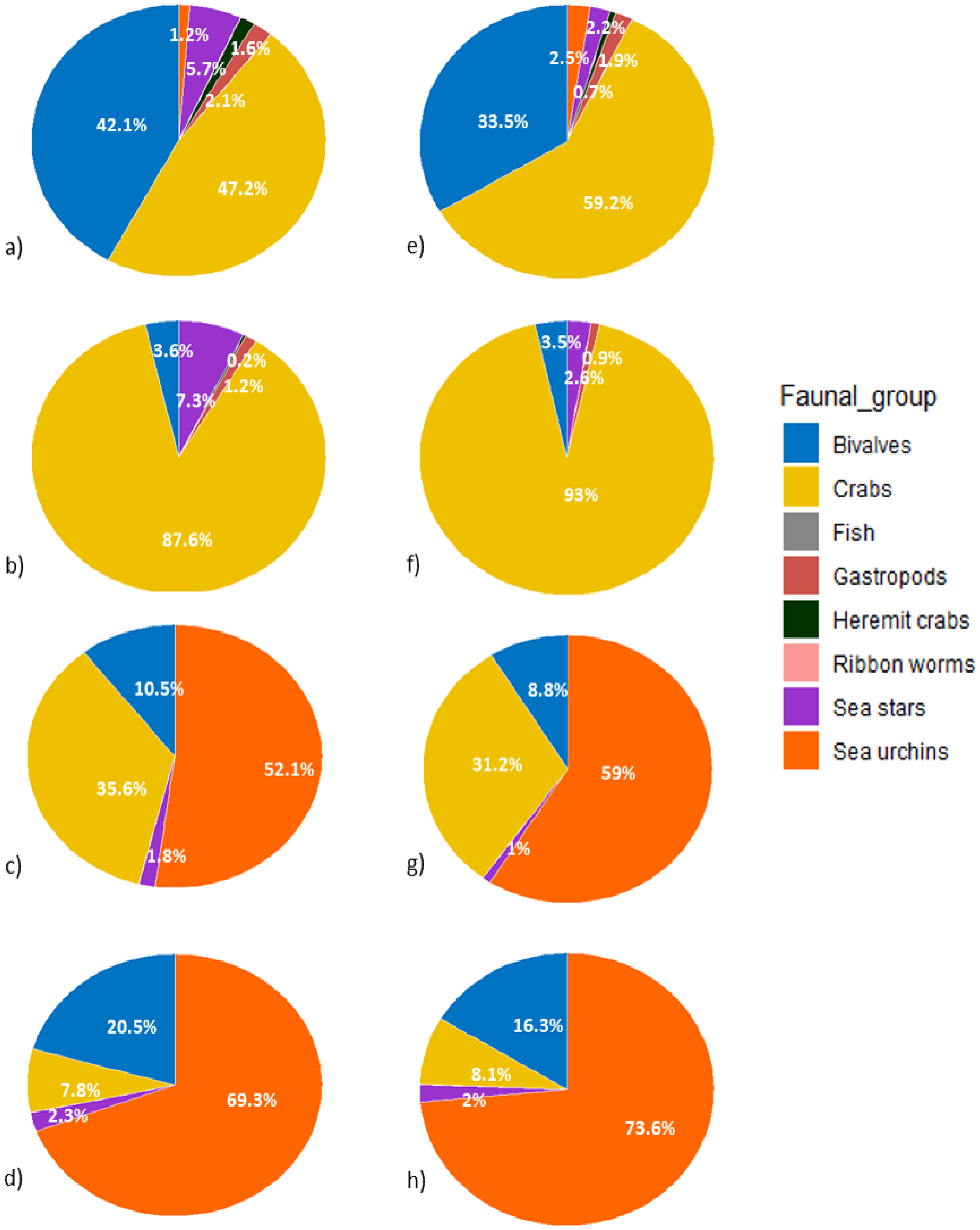

Figure 8. Overall percentage of higher taxa individuals displaying different damage level (from D_0 to D_3) for all the non-target species bycaught as discard in the striped venus clam fishery in terms of abundance (a-d) and biomass (e-h).

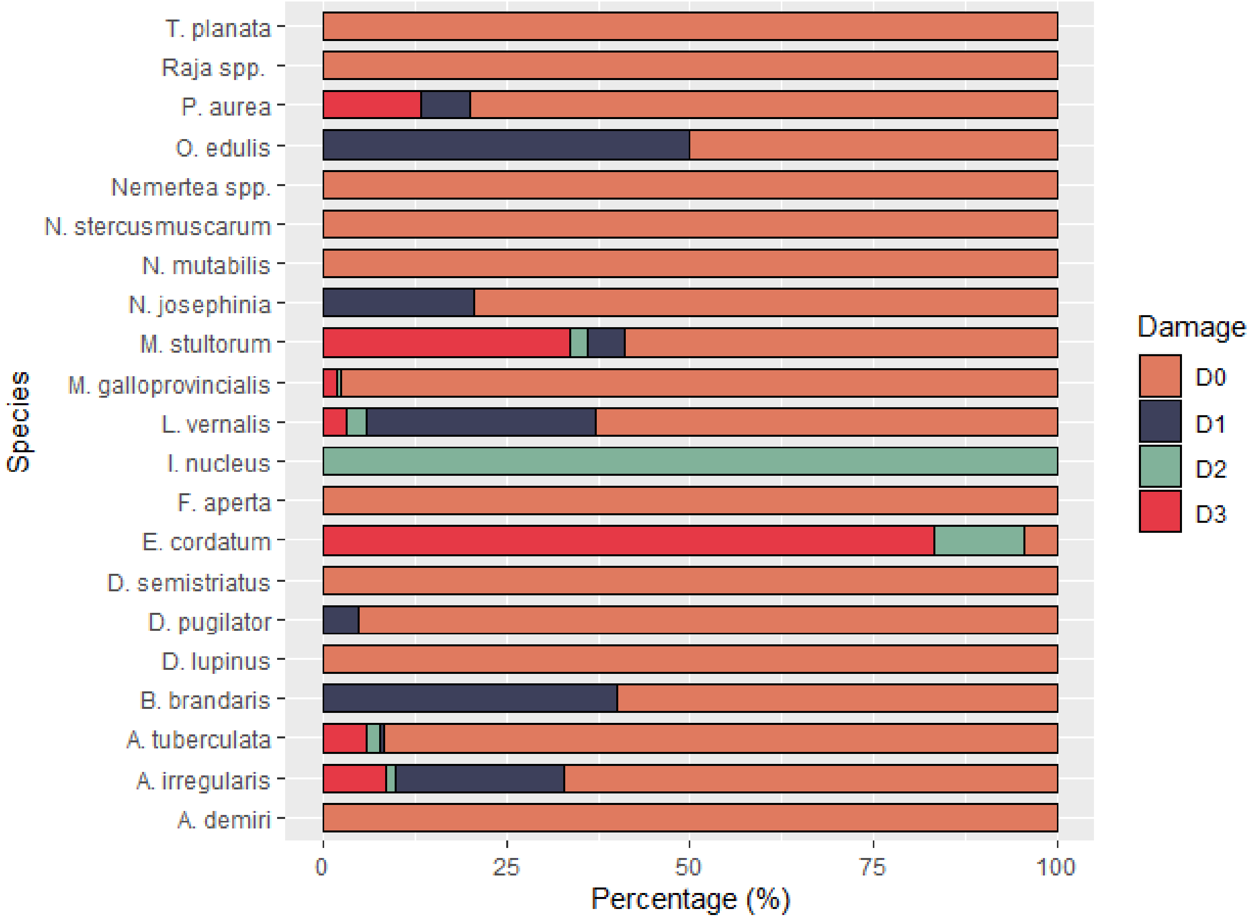

Figure 9. Overall percentages in number of each non-target discarded species collected in the striped venus clam fishery in relation to damage level (D_0-D_3).

Discussion

Discard analysis has raised much attention in the recent years as the issue has been pointed as an important aspect for fisheries management, especially after the establishment of the ecosystem approach to fisheries (FAO, 2003; Garcia et al., 2003; Pikitch et al., 2004) and the implementation of diverse European directives and regulation (e.g. Commission Regulation 1581/2004, Regulation (EC) 1380/2013). However, the assessment of bycatch/discard is a critical issue for assessing the sustainability of any fishery. Studies aimed at assessing the impact of dredging (hydraulic or mechanized) on macro-benthic communities structure are still few (Morello et al., 2005b; Constantino et al., 2009; Vasapollo et al., 2020; Baeta et al., 2021a) because data before the impact are often lacking as well as the time series on the composition of discards to detect patterns of change of faunal communities. Nevertheless, some studies have evaluated the discard catch composition (Dalgıç and Ceylan, 2012; Başçinar et al., 2020; Urra et al., 2021b) and, especially along the Spanish and Portuguese coasts, also started to focus on the damage exerted by dredging on non-target species and the consequent mortality derived (Gaspar et al., 2002, 2003b, 2003a; Urra et al., 2017, 2021a; Anjos et al., 2018; Baeta et al., 2021a).

In this study the fraction of non-target species rejected to the sea (discard) was extremely low in both abundance (1.3%) and biomass (3.2%) indicating that the area was principally dominated by the target species forming a "*facies* à *C. gallina*" (Pérès and Picard, 1964). Indeed, clam dredging generally occurs in areas where the target species is the dominant one in macro-benthic communities, and thus often the proportion of discard is relatively low (Butcher et al., 1981). However, if considering within discards even the target species, an extremely large fraction of the striped venus clam was rejected at sea as "undersized" individuals (86.9% and 74.3% in abundance and biomass, respectively) showing the presence of abundant resource promoting the maintenance of clam population on this fishing ground. Such high values can be explained by the fact that sampling occurred during the middle of the reproductive season (Bargione et al., 2021a) and as observed in the samples they were full of newborn accounting for the high number of rejected clams. On the other hand, the high abundance of newborn cannot also explain the high biomass value returned to the sea, which is instead justified by the fact that on Porto San Giorgio fishing grounds (which belong to the Consortium of San Benedetto del Tronto) commercial clams at the time of sampling had a very large size (CAMEL Project, 2021), therefore fishermen sell only the ones sorted by the largest grid of the sieve (grid 1, for details on the sieve see Sala et al., 2017), leaving the ones potentially marketable sorted by grid 2 and 3 to return to the sea, and this harvesting method was followed in the present study as well. In this way the fraction of rejected clams is highly composed even by the ones above the MCRS having a higher weight in biomass rather than the newborn.

Different authors reported variable percentages of discards depending on the gear and on the fishing grounds ranging from 6.3% to 82% (Morello et al., 2005b; Anjos et al., 2018; Baeta et al., 2021a). Also, the undersized commercial species frequently accounted for more than half of the discarded fraction. For example, in the striped venus clam fishery of the Black Sea, Dalgıç and Ceylan (2012) found that 36% of the catch was unwanted (19% undersized clams and 17% non-target species), whereas in the wedge clam fisheries of the Alboran Sea (Urra et al., 2017, 2021a) found that 42% (25% undersized clams and 17% non-target species) and 22.7% (13.2% undersized clams and 9.5% non-target species) of the catch was unwanted in years 2013-2014 and 2018, respectively. On the other hand, other studies recorded considerable amount of discards in the bivalve dredge fisheries along the Algarve coast, even exceeding the catch of the target species in some time of the year (Gaspar and Chícharo, 2007).

Many factors can influence the amount of discard. For instance, by comparing two different types of dredges the “DDredge” targeting *Donax trunculus* and the “SDredge” targeting *Spisula solida* and *C. gallina,* along the Algarve coast, it was found that the former collected a minor fraction of discard in weight (6.3%) compared to the latter (32.9%) (Anjos et al., 2018). Seasonal trends have also been observed in the discards composition, abundance and biomass related to abiotic and biotic factors (Gaspar and Chícharo, 2007; Dalgıç and Ceylan, 2012; Urra et al., 2013, 2021b). In our study carried out in summer the discarded fraction of non-target species was very low, whereas Başçinar et al. (2020) found higher abundances in summer followed by the spring season, Urra et al. (2021b) in winter and Dalgıç and Ceylan (2012) in autumn. These peculiar patterns can be explained by different environmental conditions over seasons and to populations dynamics of the dominant species related to their biology and ecology (i.e. reproductive or feeding strategies). Moreover, where fishing intensity is higher discards could be lower as a direct result of large-body and slow growing species removal (Urra et al., 2021b), even if the non-homogenous distribution of a species within a fishing ground could also explain the variable amount of discards (Pranovi et al., 2001), which in our study was low.

The MDS revealed a similar benthic community structure between hauls, with except for haul 8 which differed from the others. This might be due to the highest fraction of muddy sediment observed and recorded on this haul, and we can speculate that the presence and/or transitional formation of a siltier sands belt could explain the difference in the benthic community structure found. On the other hand, in all the other sampling points a similar benthic community structure was found possibly because of the same bathymetry at which hauls were conducted. Indeed, great variability in the catch composition of the striped venus clam fishery was found at different bathymetry in the western Adriatic Sea, near the area of our sampling, revealing a clear segregation of the benthic community according to depth (Morello et al., 2005a).

However, the faunal composition documented here is quite similar to those observed in discards in closed by or other Mediterranean areas (Morello et al., 2005b, 2005a; Anjos et al., 2018; Urra et al., 2021a), although the total number of species detected was much lower indicating an important difference in species richness potentially related to the above mentioned factors. It is not uncommon that discards are represented by relatively few families, like a consequence of dominance by only a few species (Malaquias et al., 2006). Crabs were the most represented faunal group in terms of abundance and biomass, while mollusks were the most diversified one in agreement with what observed by the previous authors. Including also the target species within discard, it’s composition was dominated by the presence of undersized target individuals and mainly by benthic species with large dimensions and morphological features that prevented their passage through the rods of the gear, such as larger bivalves, crabs and heart urchins, although representatives of a number of small-bodied species were also retained since, as reported by Petetta et al. (2021), in a short time the dredge fills up preventing the passage of specimens independently from their size. As stated by Kaiser et al. (2000) the heavily fished areas are dominated by higher abundances of smaller bodied organisms, whereas the less intensely fished area are dominated by fewer, larger-bodied biota. The ABC plots together with the WS revealed moderately disturbed macro-benthic communities within the investigated area, as also indirectly confirmed by the high abundances of large and/or soft-bodied individuals composing discard, in particular of *L. vernalis, E. cordatum* and *M. stultorum* which accounted for much of the total number and weight of discard. Indeed, samples were collected in proximity to the 0.3 NM, the limit within which clam fishing is banned, therefore even if this area is interdicted to fishing GPS data confirm that the area despite its proximity to the harbor is sometimes dredged explaining the level of disturbance here investigated (*unpublished data*). In the smooth clam fishery Baeta et al. (2021b) observed that the abundance of *E. cordatum* was higher in unexploited bottoms after 5 years of fishing closure and species with fragile shells as *M. stultorum* also appeared on those grounds in the absence of fishing activity. By comparing the macro-benthic species composition between control and dredged plots before and after a fishing disturbance in a fishing area pretty close to our (Maritime District of Ancona, Mid-western Adriatic Sea) Morello et al. (2005b) found that the swimming crab *L. vernalis* was associated with control sites. Moreover, the scavenging gastropod *Nassarius mutabilis* had a strong difference in its biomass, although not significant, between dredged and control plots, with the higher in dredged ones, indicating that the ground we explored was not previously intensively fished. Therefore, our findings compared to those of other authors seem to support our inference on assessing the moderate fishing intensity previously experienced by the grounds where we conducted the sampling activity. Based on the kind of species (morphology, size and fragility) detected within the area, it is also derived that the low amount of discard can be mainly due to the patchy distribution of the macro-benthic assemblages rather than to species removal due to high fishing intensity (*see above*).

In the Mediterranean Sea clam dredging fisheries frequently occurs on shallow costal area, which are high-energy habitats, and benthic communities seem to be well-adapted to short and medium-term perturbations showing a high level of resilience (Tuck et al., 2000; Constantino et al., 2009; Ragnarsson et al., 2015; Vasapollo et al., 2020). However, is also expected that fragile, near surface dwelling and larger species are more impacted by fishing activity. We found that soft-bodied or soft-shelled species (i.e. *E. cordatum, I. nucleus, M. stultorum, P. aureus*) were the most sensitive to clams dredging as widely reported by other authors (e.g. Hall-Spencer and Moore, 2000; Pranovi et al., 2001; Urra et al., 2017, 2021b). *Echinocardium cordatum*, whose exoskeleton is formed by very thin plates fused together, was the most affected species experiencing the highest percentages in number of severe damage >83.0% and mortality rate > 95%, in agreement with other authors which reported it to be the most vulnerable species to different types of clam dredging (Tuck et al., 2000; Urra et al., 2017; Baeta et al., 2021b). However, also lower damage and mortality rates (<70-30%) were reported for the species (Tuck et al., 2000; Hauton et al., 2003; Anjos et al., 2018). The only individual of *I. nucleus* found suffered intermediate damage and was intended as dead, similarly Baeta et al. (2021b) found that it suffered 50% severe damage indicating its high probability of being cracked due to its thin carapace. The thin shell *M. stultorum* was the second most species suffering severe damaged > 33% and a mortality rate > 36%, even if higher percentages of damage and mortality have been detected for the genus *Mactra* (>75% and 60%, respectively) (Anjos et al., 2018). The bivalve *P. aureus* was the third most impacted species albeit showed moderate severe damage and mortality (>13%); it's vulnerability was attributable to the fragile thin shell (Morello et al., 2005b). On the other hand, we detected that thicked-shells or individuals with strong protections were less sensitive to dredging. Among the most representative thicked-bivalves *A. demiri* suffered no damage, whereas *A. tuberculata* suffered minor severe damaged and mortality rates (7.8% for both), thanks to their shell thickness (Urra et al., 2017). Also gastropods and the hermit crab *Diogenes pugilatror* showed only slight damage with no mortality rate thanks to their robust, thick shells unlikely to break as similarly previously reported by different authors (Bergmann et al., 2001; Pranovi et al., 2001; Gaspar et al., 2002; Anjos et al., 2018). The swimming crab *L. vernalis* occurred in high abundances and mainly suffered slight damage (31%) and a mortality rate of 6% in line with the other study where damage ranged between 25-39% whereas the mortality rate between 0.0-0.14%. The mobile species *A. irregularis* mainly suffered minor damage (ca. 23%) and a low mortality rate (ca. 10%), however it has been reported as a species that can suffer high severe damage as are generally widely distributed and not restricted to a certain sediment type being caught by different trawling activity increasing its damage probability (Bergmann et al., 2001; Pranovi et al., 2001). Our results are in line with the slight damage detected by Pranovi et al. (2001) on *A. irregularis* and *L. vernalis* in the flatfish fishery, whereas they suffered major severe injuries in the scallop fishery probably because of the large amount of hard shells harvested which macerate the catch during towing and hauling.

Overall, a large fraction of the total non-target species discarded was damaged (40%) of which more than half suffered the higher damage levels (3.7% intermediate and 19. 2% severe damage, respectively). Similar estimates of the damaged discarded total fraction (about 40 %) where found for the smooth clam fishery by (Baeta et al., 2021b) even if with different proportions between the intermediately and severely damaged fraction (ca. 14% and 26%, respectively). Lower estimates were instead found in other studies accounting for other different clam fishery activities. For instance, in the fisheries targeting *C. gallina* 4.5% and 11% of discarded individuals exhibited intermediate and severe damage, respectively, whereas in the one targeting *D. trunculus* 15% and 12% exhibited intermediate and severe damage (Urra et al., 2017, 2019). These notable differences in the proportion of discards suffering damage may be the result of different factors (e.g. clam dredge technical design, the fishing operation, intensity and frequency of fishing activity, catch efficency, local environmental conditions, grain size, depth, quantity of the catch, species behavior) (Gaspar and Chícharo, 2007).

The estimated direct mortality of the total discarded fraction was moderately high (ca. 23%), regardless of the fact that it might have been underestimated as high levels of mortality can occur independently from the level of damage (Bergmann and Moore, 2001a, 2001b). Unobserved post-fishing mortality can occur in both damaged and undamaged individuals depending on dredge-induced stress, air exposure, the time needed to reach the sea bottom and rebury (for infauna) or resume normal activity (epifauna) which can affect predation (Chícharo et al., 2002; Maguire et al., 2002; Gaspar et al., 2003a; Broadhurst et al., 2006; Gaspar and Chícharo, 2007). In the venus clam fishery the catch is rapidly sorted on the deck and non-target and undersized target species are returned to the sea near the natural beds in a short time, probably not affecting mortality too much. Predators and scavengers have been observed to aggregate very quickly along the dredge tracks, preying not only on damaged organisms but also on undamaged ones before they had the opportunity to rebury (Hall-Spencer and Moore, 2000). This aggregation can last from few minutes (Gaspar et al., 2003b) to a few days (Jenkins et al., 2004). All these factors accounting for indirect-post fishing mortality should be taken into consideration for the calculation of more real mortality estimates, therefore survival experiments should be carried out especially directly at sea. At present only few studies carried out survival experiments on discarded macro-benthic species returned to the sea after fishing operations. For instance, Anjos et al. (2018) conducted survival experiments into containment facilities finding diverse vulnerability of taxa to survivorship, however confirming the influence of damage score on mortality rate, whereas Gaspar et al. (2003b) assessed survivorship directly at sea on dislodged individuals. Increase the number of studies directed at obtaining the damage and mortality rate induced also on uncaught dislodged individuals left on the dredge path is a future challenge to have a full view of the effects caused by dredging activity. Since the present results should be considered as preliminary, an ad hoc study aimed at investigating the effects of dredging on benthic communities both on a spatial and temporal scale is needed for the future.

Moreover, on the eye of an Ecosystem Approach to Fisheries is therefore essential to safeguard the macro-benthic communities living associated with *C. gallina* by reducing the impact exerted by dredging on the entire ecosystem. Good attempts to marginally solve the problem have been made in the western Mediterranean Sea by Gaspar et al. (2003a) which tested different types of clam dredges observing different percentages of partially or severely damaged individuals; whereas Gaspar et al. (2002) did not find any differences in these percentages by making small changes in the design of clam dredges (i.e. tooth spacing and mesh size). Also in the Adriatic Sea Italian Mollusks Consortia in these years have been involved in developing more selective gears thanks to the grant of the European Maritime Affairs and Fisheries Fund (FEAMP 2014/2020, Regulation (EU) 508/2014 Action 1.39). However, for a more responsive fishery a lot of work still needs to be done to develop new or modified more efficient and selective dredges. Such alternative gears should reduce both direct and indirect mortality allowing the immediate escape of bycatch individuals from the gear during towing. In this way animals will be subject to less stress, recover they activity faster and be less exposed to predation.

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Discussion

In Italy, although the hydraulic dredger sector accounts for less than 6% of the Italian fleet, the quantities yearly landed (around 20 thousand tons) reach 15% of all fisheries production, and the Venus clam is the second most important species, second only to the anchovy. Therefore, considering its high socio-economic value for a correct management of the resource it is crucial to have a sound knowledge of the biological-traits of the species. Indeed, dated or lacking primary information might compromise the current management and conservation of the stock. This three years PhD thesis work deepened and investigated some old and new biological aspects of *C. gallina*, as well as the effects of the interaction between the gear and the target and non-target specimens which return to the sea after the onboard sieving process.

To date, the knowledge of some biological aspects of venus clam was dated, while some others needed to be investigated more in depth. Detailed studies of the population age structure and growth of *C. gallina* are essential, since uncertainties in age estimation undermine the effectiveness of management actions. Two of the three techniques I adopted to estimate the age of the species resulted equally reliable (i.e. thin sections and acetate peel replicas), whereas the surface growth rings method underestimated age and was error-prone, due to inherent difficulties related to the absence/misinterpretation of rings on the shell surface. This technique has often yielded contrasting results in various bivalve species, either underestimating (Gaspar et al., 2004; Hernández-Otero et al., 2014) or overestimating age (Gaspar et al., 1995; Peharda et al., 2002), despite the occasional success (Mancuso et al., 2019). On the other hand, the other two methods where described by the same von Bertalanffy (VBF) growth curve parameters (L_∞ = 43.9, k = 0.26, t_0 = -0.84) and age estimates provided range between 0.5 to 6.5 years, although the thin sections technique revealed to be less time-consuming and therefore is suggested to be adopted to assess age. Moreover, the analyses demonstrated that shell growth is slower in the cold season and in older specimens. The annual periodicity of the growth bands on *C. gallina* thin sections has been validated in the western Adriatic Sea by Arneri et al. (1995), who determined that the translucent (dark zones) bands are laid down once a year, approximately between October and February, whereas the opaque bands (light zones) are deposited from March to September.

The two shell sectioning techniques demonstrated that the growth rate decreases as specimens become older, highlighting a very fast growth in the first year of life (of 14-15 mm TL). In the second year, the growth rate had more than halved already. Growth is the result of linear extension along the umbonal-ventral axis per unit of time, and slows down with increasing age or size (Lorrain et al., 2004), as also confirmed by isotope analysis (Keller et al., 2002; Mancuso et al., 2019). However, by comparing the resulting length at age data with previous ones in the Adriatic, the estimated length at age 1 was very similar in all the studies, independently from the technique applied, whereas differences emerged from the second year. In the past, 2-year-old clams have been reported to have a mean length of about 23-25 mm TL (Poggiani et al., 1973; Froglia, 1975b; Marano et al., 1982; Polenta, 1993a), whereas in this study it was just under 22 mm (the current MCRS), reflecting a slight reduction in shell growth rate over time that has already been reported by Biondi & Del Piero (2012) in the Gulf of Trieste. It can be assume that the fishing pressure may have induced a reduction in shell growth in the Adriatic, as in the Black Sea the growth rate has declined in areas subject to high fishing pressure compared to non-dredged areas, where clams grew faster (Dalgiç et al. 2010). However, several factors, including spawning, food availability, type of substratum, depth, light, temperature, salinity and population density may affect shell growth rate (Gaspar et al., 2004; Dalgiç et al., 2010). Consequently, the slight shell growth decline detected may be the result of the synergistic action of multiple factors. However, to quantify how much fishing affects growth studies in non-dredged areas should be carried out.

The reproductive cycle of *C. gallina* (i.e. gametogenic cycle, size at sexual maturity and partial fecundity) was investigated through microscopic, histological and video analysis techniques. *C. gallina* exhibited a cyclical annual pattern of gonads development driven by environmental parameters (BST and Chl-a). At the beginning of the sampling year in November gametogenesis had already restarted matched with high values of BST and Chl-a, even if it stopped in winter during the coldest months when BST was below 10°C. Different studies report that thermal anomalies during the autumn and winter seasons (i.e. T above 14-18 ° C) could justify the presence of clams in advanced stages of maturity and reproduction (Cordisco et al., 2003b, 2005; Rizzo et al., 2011). Notably, in temperate climates, the most common pattern in bivalves is the starting of gametogenesis when seawater temperature begins to increase reaching a threshold level (Dang et al., 2010). Congruently, when BST is lower than 10°C gonad development of clams as well as clam growth is reduced (Salvatorelli, 1967; Froglia, 1975b). When BST and Chl-a slowly restarted to increase in March, gametogenesis progressed and specimens with ripe gonad were first detected. Spawning events, highlighted by signs of partial emission and recovery of the gonad, occurred during the late spring and summer months, followed by a regression and inactive stage in late-summer and early-autumn, respectively. Several studies have demonstrated how water temperature and food availability significantly influence the reproductive cycle of *C. gallina* (e.g. Dalgiç et al., 2009; Delgado et al., 2013; Joaquim et al., 2014) as well in other bivalves (Darriba et al., 2004; Dridi et al., 2007; Enríquez-

Díaz et al., 2009). In particular, it was found that the Venus clam showed an opportunistic reproductive strategy that is gonads development and sexual maturation are closely associated with the accumulation of nutrients and therefore with the food availability in the environment (Llodra, 2002; Da Costa et al., 2013). Moreover, in temperate regions it is reported that egg laying takes place in favorable conditions for the development of *C. gallina* planktotrophic larvae, i.e. when phytoplankton and Chl-a concentration is abundant, and when the water temperature is between 18-27 ° C, to guarantee successful recruitment (Cordisco et al., 2003b).

The reproductive cycle of *C. gallina* was previously investigated by other authors inside the Adriatic which reported a similar long lasting spawning season (e.g.Valli and Zecchini-Pinesich, 1981; Cordisco et al., 2003; Scopa et al., 2014) with some exceptions of reproductive events in early autumn (Cordisco et al., 2005; Rizzo et al., 2011). The identification of signs of partial emission during the reproductive season allowed to confirm that the striped venus clam is a multiple partial spawner (Marano et al., 1982; Corni et al., 1985; Erkan, 2009; Delgado et al., 2013). It was observed a synchronous gonads development in females and males, as widely detected in different works (Salvatorelli, 1967; Bratoš Cetinić et al., 2007; Joaquim et al., 2014) as a strategy adopted to maximize their reproductive success.

For both sexes the length at sexual maturity was reached at a size of about 11.2 mm TL, within the first year of life, and all of the clams were mature above 15 mm TL. Controversial size at sexual maturity ranging from 9 to 18 mm TL is commonly reported inside and outside the Adriatic Sea (e.g. Erkan and Sousa, 2002; Bratoš Cetinić et al., 2007; Delgado et al., 2013; Scopa et al., 2014). The different or similar TL_{50} reported for this species from the same or different areas could be attributed to intrinsic reproductive variability of clams, driven by different local environmental conditions such as seawater temperature and food availability, as well as to anthropogenic, genetic and physiological factors (Da Costa et al., 2013). However, its thorough knowledge is crucial to evaluate the spawning fraction and fecundity of the papulation not harvested by hydraulic dredges, contributing to the overall reproductive output of the species (Delgado et al., 2013).

The estimation of potential annual fecundity is a key element, yet seldom studied due to the widespread gonad tissue inside the visceral mass, for understanding bivalve production and population dynamics (Beninger et al., 2021). Despite the estimations of PF reported are related to a single event of eggs emission, in multiple-partial-spawner bivalves the number of spawning events occurring within the same reproductive season is unknown, as it is for the intensity of each reproductive peak (Park and Choi, 2004), which also changes over years (Morvan and Ansell, 1988).

Beyond the results presented in my thesis work, only another study assessed the fecundity of *C. gallina* in the Gulf of Cadiz, south-western Spain (Delgado et al., 2013), finding a higher PF estimate than what I found, even if the order of magnitude of the oocytes number related to TL is the same. I also found that the fecundity of clams is positively related to size, as observed by other authors (e.g. Mzighani, 2005; Soria et al., 2010; Johnson and Smee, 2012). The allocation of energy from growth to reproduction characterizes the older bivalves, while in the younger the growth is rapid during the first periods of life and the investment in reproduction is lower (Honkoop et al., 1998; Johnson and Smee, 2012). Nevertheless, of all the emitted eggs not all are fertilized as a portion is non-viable (Beninger et al., 2021), another undergo ageing, therefore fertilization cannot take place any longer (André and Lindegarth, 1995), and a portion also faces predation as they are filtered by filter-feeding organisms present in the water-column. It follows that the number of released oocytes is therefore not equal to the number of offspring, which in turn suffer substantial mortality early in the life cycle due to oceanographic and ecological factors (e.g. food availability, current transport to unsuitable habitat, predation; Cushing, 1990), as well as to biological (e.g. reproductive strategy of the species, larval duration and larval behaviour; Cowen et al., 2006) and genetic ones (Plough et al., 2016). All these factors complicate the fisheries management as it cannot be known in advance the strength of recruitment supporting the population structure at sea basing the estimates on the reproductive output of the species (Plough et al., 2016), especially if the species is a multiple partial spawner as *C. gallina*.

Data on the effects of fishing on population sustainability is still limited (Froglia, 2000; Marin et al., 2003; Morello et al., 2005b; Moschino et al., 2008), whereas gear efficiency has been studied extensively (*e.g.*Sala et al., 2017; Petetta et al., 2021). Since the smaller specimens that pass through the sieve are returned to the sea through a waste exhaust pipe, discarded clams undergo considerable physical stress (Morello et al., 2005a). Even though discards are believed to mitigate the overexploitation of natural populations, the mechanical stress to which they are subject has the potential to reduce their survivability (Moschino et al., 2003, 2008). However, data on the reburial ability and survival of discarded clams returned to the sea are scarce, despite their economic importance. By comparing my results of undamaged clams reburial ability with the one of Morello et al. (2006) they reported a T_{50} of about 3 h, which is very similar to the one I estimated ($T_{50} < 4$ h) for the whole sample (135 clams); and even when reburial time was calculated separately for the three size classes, the T_{50} ranged from 3 to 4.8 h. Morello et al. (2006) also found that less than 35% of clams were still visible after 4 h, whereas by 8 h, 90% of my sample had reburied and by 21 h no clams were visible any longer. Bivalve reburial ability has also been studied *in situ* following dredging (Gaspar et al., 2001; Chıcharo et al., 2002), or after discarding (Leitão and Gaspar, 2011), and all of them describe a relatively faster reburial ability of bivalves (12-60 min) compared to those transferred into containment facilities (my study and Morello et al., 2006). This observation may lead to even more reassuring considerations on the reburial ability of undersized *C. gallina* specimens discarded directly at sea during commercial fishing operations. However, aerial exposure exceeding 1 hour has been reported to involve a significant reduction of reburial ability and of the physiological response to dredging-induced stress in *S. solida* (Chícharo et al., 2003).

Clam mortality in the laboratory experiments was low (≈ 5%) and did not correlate with shell size, whereas other studies have found that the smallest specimens are more likely to die (Broadhurst et al., 2006; Uhlmann and Broadhurst, 2015). Moreover, at variance with the finding that clams may be more likely to die immediately after being placed in the tanks or around the end of experiments due to containment (ICES, 2015), the mortality of the captive *C. gallina* specimens was not related to a particular time. In the study I carried out, neither the harvesting and sieving process nor captivity in the tank induced significant mortality, suggesting that other factors (*e.g.* disease, parasites) may have caused the death of weaker or less healthy specimens.

Clam mortality in the experiments at sea was low as well (≈ 5%), and did not correlate with shell size. Although Breen et al. (2007) recommend monitoring the key environmental parameters (*e.g.* depth, temperature, salinity) during captivity, the high survival rate of the specimens suggests that the slight depth difference (1-1.5 m) between the fishing ground and the cage site did not affect survivability. Similarly, specimen size did not affect survivability, since only 7 individuals died in captivity (2 <MCRS and 5 ≥MCRS) and 12 individuals died in the sea trials (4 <MCRS and 8 ≥MCRS).

This was the first study investigating the survival of discarded striped venus clams in environmental conditions mimicking the natural habitat. The similar mortality recorded in the laboratory and the field experiments demonstrates the ability of the conditions recreated in captivity to closely mimic those at sea. Moreover, the season, biotic (*e.g.* gonadal development and energy storage) and abiotic factors (*e.g.* seawater temperature and salinity; Boscolo et al., 2003; Moschino et al., 2008) together with the dredging-fishing effort (Ballarin et al., 2003; Marin et al., 2003) influence clam conditions and hence their survivability. Mechanical sorting and discarding into the sea may cause a physiological stress and physical damage to small clams, which may be harvested as many as 20 times a year (Marin et al., 2003; Morello et al., 2005b). A study of mortality related to hydraulic dredging (Moschino et al., 2003) has reported a rate of 2 to 20% (mean, ≈ 10%) corresponding to a survival rate of at least 80%. Considering that the water pressure used in the study was higher than the regulation 1.8 bar (DM 22/12/2000), the mortality rate using the legal water pressure should be lower. Indeed, in my experiments the clams exposed to regulatory water pressure and mechanized sorting showed a higher survival rates, without even considering the potential additional stress suffered by clams due to handling and transport before their introduction in the tanks or sea cages. A 7-day captivity study has assessed the survivability of three undersized commercial bivalve species (*Donax trunculus, S. solida* and *C. gallina*) harvested with hydraulic dredgers without recreating the natural sea bottom habitat. At the end of the experiments, the survival rate of the intact specimens ranged from 86% to 100% irrespective of species and size (Anjos et al., 2018), in line with the survival rate of the undamaged clams here analysed.

Altogether, previous findings and the present data – documenting that a very large proportion of undamaged clams survive harvesting and sorting and that they show a high reburial ability and survival rate after reburying – demonstrate the high survival potential of *C. gallina* and support the claim that undersized specimens of this bivalve can be returned to the sea *as per* Regulation (EU) 2020/2237.

Dredging activity targeting a variety of bivalve species worldwide differently affects the integrity of shells. The severity of injuries inflicted by dredging on different bivalve species is related to their species-specific characteristics (e.g. morphology, body size, structure, fragility) (Bergmann et al., 2001; Leitão et al., 2009; Urra et al., 2019), behavior (e.g. burrowing depth), fishing gear and tow characteristics, as well as by seasonality (Gaspar and Chícharo, 2007; Dalgıç and Ceylan, 2012; Urra et al., 2013, 2021a). The study I carried out aiming to estimate the damage caused by the dredge and the mechanical vibrating sieve currently used by the Italian fishing vessels targeting *C. gallina* revealed that the high water pressure and mechanized sorting were both responsible for the damages inflicted on clam shells, and the probability of shell damage was positively related to shell size. About 14% of clams tipped into the collecting box were already damaged and it was mainly assumed that those damages were ascribable to the dredging activity. A study carried out on the target bivalve species *Callista chione* revealed that during dredging activity most of the shell damage was attributable to compaction (i.e. an evenly distributed force/pressure on the shell within the sediment) rather than to the direct impact of the dredge teeth on the shell (Vasconcelos et al., 2011). To reduce the damage inflicted by the dredge on this species an experimental new dredge (NDD) was tested compared to a traditional one (TD), finding that the impact exerted on the target species, as well as on the macro-benthic communities, was reduced of about 50% by the NDD due to its greater efficiency capture (Gaspar et al., 2001), highlighting the importance of testing different gears to mitigate damage.

Crushed and broken shells are the result of compaction forces inside the sediment and of dumping the catch of the haul into the collecting box, while chipping and disjoint umbo commonly occur at the dredge level because of abrasion, whereas when sorted clams are chipped (Gaspar et al., 2001). This explains why, in my work, the fraction of crushed and broken shells substantially did not vary after sorting, while it was detected an effect of the sieve which significantly reduced the fraction of intact shells while the chipped ones increased. Increasing levels of stress on clam individuals (i.e. from low water pressure to high water pressure + mechanized sorting) are known to increase the fraction of damaged shells on both captured and discarded clams (Marin et al., 2003; Moschino et al., 2003). In particular, Moschino et al. (2003), in two different sites of the north-western Adriatic Sea, found that in one of the two (Lido) damage was mostly due to the action of the mechanized sorter while in the other, (Jesolo) the effect of the high water pressure was most clearcut, in agreement with my results. These differences are likely linked to differing bottom features, which have been seen to be responsible of differing damage extent in bivalves in relation to the greater content of hard particles in the bottom sediment (Urra et al., 2021a). In my study, on average, it was estimated that the dredge alone is responsible for damaging 14% individuals, on which the sieving process add +6.8% more individuals. Giving these numbers the mechanical sieve causes a damage 0.5 times higher than the one already induced by the dredge on *C. gallina.* To reduce shell damage and mortality of undersized discarded cockles, *Cerastoderma edule*, due to the onboard sorting equipment, Coffen-Smout (1998) positively tested a reduction of the free falling distance (<0.3 m) of clams in the rotary sieve. However, studies investigating the effect of the vibrating sieve on shell damage are still few and unbalanced in relation to the ones testing the effect of the dredge (e.g. Gaspar et al., 2001, 2002, 2003a; Leitão et al., 2009).

I detected that the probability of damage was positively related to size, as already widely recognized in bivalves by different authors (Ramsay et al., 2000; Schejter and Bremec, 2007; Vasconcelos et al., 2011; Soon and Ransangan, 2019). The difference in the damage probability across size is likely due to changes in the physical characteristics of the shell and on the ratio between the weight and the volume of the clam during growth. Shell resistance to breakage depends on shell length, width, height, volume, thickness, shape microstructure and sculptural features (Zuschin et al., 2003), all of which change along the life-cycle. However, I also found that the magnitude of damage was higher in the discarded fraction, a possible explanation can be found if considering the path throughout these discarded clams undergo inside the sieve, which is longer and more tortuous rather the one experienced by the commercial clams (for details see Sala et al., 2017), and thus likely increase the number of hits resulting in a higher damage probability.

Not all damaged discarded clam die, sure enough, the high mean percentage of repaired shells I found in non-sieved and sieved samples (15.0 – 20.0%) indicate that a wide fraction of damaged discarded clam is able to recover. Their sensitivity to survivorship is related to shell size, entity/type of damage, as well as to seasonal variation in both environmental and endogenous conditions (Moschino et al., 2003; Jenkins et al., 2004). Indeed, high water temperature and reproduction are reported to lower survivorship in bivalves (Eertman et al., 1993). Overall, in my work, the discarded clams had a high survival rate (91.7%), despite the possible different levels of shell damage and the associated survival rate. On the contrary, a study carried out to assess the survival rate on both undamaged and chipped individuals of three commercial bivalve species (*D. trunculus*, *S. solida* and *C. gallina*) found, irrespective of species and size, a lower survival rate for damaged individuals (24.2 - 60.0%) compared to the one of undamaged individuals (86.0 – 100.0%) (Anjos et al., 2018).

The data presented in this PhD thesis suggests that a very high proportion of discarded damaged and undamaged *C. gallina* specimens survive and grow to the commercial size. However, to further reduce the impact exerted by the fishing activity on the target species it is suggested that testing coated grids and walls of the vibrating sieve with rubber material may reduce the damage suffered by sieved clams. This kind of sieve is already in use for fishing for bivalve molluscs in brackish waters.

Discard analysis has raised much attention in the recent years as the issue has been pointed as an important aspect for fisheries management, especially after the establishment of the ecosystem approach to fisheries (FAO, 2003; Garcia et al., 2003; Pikitch et al., 2004) and the implementation of diverse European directives and regulation (e.g. Commission Regulation 1581/2004, Regulation (EC) 1380/2013). In the preliminary study I carried out aiming at qualitatively and quantitatively describe the macro-benthic fauna living associated with *C. gallina* in one of the most important Italian fishing ground for clams harvesting during the summer season, it was found that the discarded non-target fraction was extremely low in both abundance (1.3%) and biomass (3.2%) indicating that the area was principally dominated by the target species forming a "*facies* à *C. gallina*" (Pérès and Picard, 1964). Many factors can influence the amount of discards. For instance, by comparing two different types of dredges the "DDredge" targeting *Donax trunculus* and the "SDredge" targeting *Spisula solida* and *C. gallina,* along the Algarve coast, it was found that the former collected a minor fraction of discard in weight (6.3%) compared to the latter (32.9%) (Anjos et al., 2018). Seasonal trends have also been observed in the discards composition, abundance and biomass related to abiotic and biotic factors (Gaspar and Chícharo, 2007; Dalgıç and Ceylan, 2012; Urra et al., 2013, 2021b). In my work carried out in summer, the discarded fraction of non-target species was very low, whereas Başçinar et al. (2020) found higher abundances in summer followed by the spring season, Urra et al. (2021b) in winter and Dalgıç and Ceylan (2012) in autumn. These peculiar patterns can be explained by different environmental conditions over seasons and to populations dynamics of the dominant species related to their biology and ecology (i.e. reproductive or feeding strategies). Moreover, where fishing intensity is higher discards could be lower as a direct result of large-body and slow growing species removal (Urra et al., 2021b), even if the non-homogeneous distribution of a species within a fishing ground could also explain the variable amount of discards (Pranovi et al., 2001), which in my study was low.

The non-metric multidimensional scaling (MDS) revealed a similar benthic community structure between hauls, with except for haul 8 which differed from the others. This might be due to the highest fraction of muddy sediment observed and recorded on this haul, and I can speculate that the presence and/or transitional formation of a siltier sands belt could explain the difference in the benthic community structure found. On the other hand, in all the other sampling points a similar benthic community structure was found possibly because of the same bathymetry at which hauls were conducted. Indeed, great variability in the catch composition of the striped venus clam fishery was found at different bathymetry in the western Adriatic Sea, near the area of my sampling, revealing a clear segregation of the benthic community according to depth (Morello et al., 2005a).

The faunal composition documented in my study is quite similar to those observed in discards in other closed by and Mediterranean areas (Morello et al., 2005b; Anjos et al., 2018; Urra et al., 2021a), although the total number of species detected was much lower indicating an important difference in species richness potentially related to the above mentioned factors. It is not uncommon that discards are represented by relatively few families, like a consequence of dominance by only a few species (Malaquias et al., 2006). Crabs were the most represented faunal group in terms of abundance and biomass, while mollusks were the most diversified one in agreement with what observed by the previous authors. Discard composition was dominated by the presence of benthic species with large dimensions and morphological features that prevented their passage through the rods of the gear, such as larger bivalves, crabs and heart urchins, although representatives of a number of small-bodied species were also retained since, as reported by Petetta et al. (2021), in a short time the dredge fills up preventing the passage of specimens independently from their size. As stated by Kaiser et al. (2000) the heavily fished areas are dominated by higher abundances of smaller bodied organisms, whereas the less intensely fished area are dominated by fewer, larger-bodied biota. The ABC plots together with the WS revealed moderately disturbed macro-benthic communities within the investigated area, as also indirectly confirmed by the high abundances of large and/or soft-bodied individuals composing discard, in particular of *L. vernalis, E. cordatum* and *M. stultorum* which accounted for much of the total number and weight of discard.

In the Mediterranean Sea clam dredging fisheries frequently occurs on shallow costal area, which are high-energy habitats, and benthic communities seem to be well-adapted to short and medium-term perturbations showing a high level of resilience (Tuck et al., 2000; Constantino et al., 2009; Ragnarsson et al., 2015; Vasapollo et al., 2020). However, is also expected that fragile, near surface dwelling and larger species are more impacted by fishing activity. We found that soft-bodied or soft-shelled species (i.e. *E. cordatum, I. nucleus, M. stultorum, P. aureus*) were the most sensitive to clams dredging as widely reported by other authors (eg. Hall-Spencer and Moore, 2000; Pranovi et al., 2001; Urra et al., 2017, 2021b). Overall, a large fraction of the total non-target species discarded was damaged (40%) of which more than half suffered the higher damage levels (3.7% intermediate and 19. 2% severe damage, respectively). Similar estimates of the damaged discarded total fraction (about 40 %) where found for the smooth clam fishery by Baeta et al., (2021b) on the Catalan coast even if with different proportions between the intermediately and severely damaged fraction (ca. 14% and 26%, respectively). Lower estimates were instead found in other studies accounting for different clam fishery activities. For instance, in the fisheries targeting *C. gallina,* in the northern Alboran Sea, 4.5% and 11% of discarded individuals exhibited intermediate and severe damage, respectively, whereas in the one targeting *D. trunculus* 15% and 12% exhibited intermediate and severe damage (Urra et al., 2017, 2019). These notable differences in the proportion of discards suffering damage may be the result of different factors (e.g. clam dredge technical design, the fishing operation, intensity and frequency of fishing activity, catch efficiency, local environmental conditions, grain size, depth, quantity of the catch, species behavior) (Gaspar and Chícharo, 2007).

The estimated direct mortality of the total discarded fraction was moderately high (ca. 23%), regardless of the fact that it might have been underestimated as high levels of mortality can occur independently from the level of damage (Bergmann and Moore, 2001a, 2001b). Unobserved post-fishing mortality can occur in both damaged and undamaged individuals depending on dredge-induced stress, air exposure, the time needed to reach the sea bottom and rebury (for infauna) or resume normal activity (epifauna) which can affect predation (Chícharo et al., 2002; Maguire et al., 2002; Gaspar et al., 2003a; Broadhurst et al., 2006; Gaspar and Chícharo, 2007). In the venus clam fishery, the catch is rapidly sorted on the deck and non-target and undersized target species are returned to the sea near the natural beds in a short time, probably not affecting mortality too much. Predators and scavengers have been observed to aggregate very quickly along the dredge tracks, preying not only on damaged organisms but also on undamaged ones before they had the opportunity to rebury (Hall-Spencer and Moore, 2000). This aggregation can last from a few minutes (Gaspar et al., 2003b) to a few days (Jenkins et al., 2004). All these factors accounting for indirect-post fishing mortality should be taken into consideration for the calculation of more real mortality estimates, therefore survival experiments should be carried out especially directly at sea. At present only few studies carried out survival experiments on discarded macro-benthic species returned to the sea after fishing operations. For instance, Anjos et al. (2018) conducted survival experiments into containment facilities finding diverse vulnerability of taxa to survivorship, however confirming the influence of damage score on mortality rate, whereas Gaspar et al. (2003b) assessed survivorship directly at sea on dislodged individuals. Increase the number of studies directed at obtaining the damage and mortality rate induced also on uncaught dislodged individuals left on the dredge path is a future challenge to have a full view of the effects caused by dredging activity. Moreover, alternative gears should be tested to reduce both direct and indirect mortality of the macro-benthic fauna living associated with *C. gallina* to move towards a more responsive fishery.

Conclusion

In conclusion, the researches carried out during my PhD made it possible to deepen some fundamental knowledge in order to rationally support some fisheries management measures.

Summarizing the major results, I found that *C. gallina* reaches 1 year at a size of about 15 mm TL, while the 2^{nd} year at about 22 mm TL. The results confirmed that the growth rate decreases as specimens become older, highlighting a very fast growth in the first year of life. However, many factors can affect shell growth rate such as fishing effort, population density, food availability, type of substratum, temperature and salinity (Gaspar et al., 2004; Dalgiç et al., 2010) and future in depth studies should be carried out to test any difference in clam growth over time and space.

I also confirmed that the species in the Adriatic Sea reproduces in spring-summer, and gonads development and maturation is strictly related to seawater temperature and Chl-a concentration increase. The prolonged reproductive season is characterized by different events of gametes release of variable intensity, allowing only to assess a potential fecundity in a single egg release event. I found that PF is positively related to size, and that clams of 25 mm TL produce 40% more oocytes per female than clams of 22 mm TL. The size at sexual maturity (TL_{50}) is reached at about 11.2 mm TL for both sexes, therefore very early in the life cycle and within the first year of life, however all the specimens are mature above 15 mm TL.

Reburial ability tests in laboratory conditions showed that all the undamaged clams are able to rebury in the sediment, irrespectively of their size (T_{50} < 5h and T_{90} < 10 h), and by comparing the reburial time of bivalves tested in other studies directly *in situ* they all showed a faster time (Gaspar et al., 2001; Chıcharo et al., 2002; Leitão and Gaspar, 2011). This could suggest that clams discarded at sea soon after the on-board sieving process may require shorter time to rebury in the sediment.

The survivability tests also demonstrate a high survival rate of all the undamaged specimens placed into laboratory tanks or rejected at sea (>95%), irrespective of their size, without even considering the potential stress suffered by clams during transport before being transferred into lab tanks or sea cages. This allow to claim that undamaged clams returned to the sea are able to survive and grow contributing to the reproductive output of the stock.

This work on clam survivability has been evaluated by the Scientific, Technical and Economic Committee for Fisheries (STECF) of the European Commission through the Joint Recommendation (JR), which considered the experiment robust to demonstrate the high survival potential of the species (STECF, 2022; 2020).

Evaluation and quantification tests of shell damage demonstrate that the dredge alone accounts for about 14% of shell damage and the sieving process adds an additional 6.8% damage, being 0.5 times higher than the previous one. Damage probability increases with shell size however the magnitude of increase between treatments (not-sieved, commercial and discard) is significantly higher for the discard, possibly because of the path throughout the discarded clams undergo, which is longer and more tortuous rather the one experienced by the commercial clams, and thus likely increase the number of hits resulting in a higher damage probability. Therefore, to further reduce the impact exerted by the fishing activity on the species coated grids and walls of the vibrating sieve with rubber material should be tested. Nonetheless, the survival rate of total discarded individuals (damaged + undamaged) is still high (91.7%), once again in support of the fact that the species has a high survival potential and can be rejected to the sea.

Evaluation and quantification tests of damage on the macro-benthic communities' reveal that that soft-shelled or soft-bodied species are the most affected by the harvesting process, whereas thick-shelled or thick-bodied species suffer minor damage. The two most damaged species are the sea urchin *E. cordatum* (>69%) and the bivalve *M. stultorum* (>35%). Overall, when returned to the sea at the end of the fishing process 61.0% of the individuals are undamaged, whereas 16.1%, 3.7% and 19.2% display slight, intermediate and severe damage, respectively. The mortality rate is 22.9% of all the discarded individuals with the sea urchin *E. cordatum* showing the highest mortality rate of 95.4%. These findings highlight the importance of guaranteeing the integrity of the entire ecosystem, thus new or modified gears should be tested or adopted to reduce the impact on unwanted species accidently caught.

A responsible management plan for the striped venus clam fishery should take into account the biological aspects of the species and the effects of the gear on the species populations, and provide guidelines to ensure the persistence and conservation of the species over time, so that a suitable plan is the one able to allow the exploitation of the resource not exceeding the maximum sustainable yield.

Based on the biological results gained it is reasonable to set the two mandatory months of fishing closure during the spring-summer months, when clams are in reproduction. This could guarantee both larger individuals to contribute to the reproductive event, as well as allow offspring to catch on the substrate.

It is reasonable to reject the undersized specimen at sea following the Regulation EU 1380/2006 as scientific evidence has demonstrated that the species has a high survival rate. The rejected specimens can grow and reach the size of maturity participating in the reproductive output of the species.

Moreover, is fundamental to keep the area within 0.3 NM from the coast closed to dredging harvesting as per Regulation EU 1967/2006, as it represents a wide nursery and breeding area (581.7 km^2) in which all the individuals in reproduction, in particular larger ones, can contribute to enhance the reproductive output of the population in the environment.

Technical management measures such as the establishment of restocking areas (Delegated Regulation (EU) 2016/2376), in which harvesting is not allowed but where fishermen must reject undersized specimens, contributes to widening the area in which clams are present but cannot be harvested, although participate to the reproductive event.

Technical measures are set on the fishing gear to reduce to almost zero the catch of juveniles. Petetta et al. (2021) have estimated that the first size selection performed by the dredge on the seabed does not spare undersized individuals, since more than 58% of the clams caught are under the former MCRS of 25 mm TL. Therefore the sieving process after the harvesting is necessary and it retains less than 5% of undersized individuals (Sala et al., 2017). The Regulation (MD 27/12/2016) does not have any tolerance on undersized fraction which must not be present in the commercial product intended for sale.

To move forward a more responsive fishery, management measures that take into account not only the target species but even the surrounding environment must be supported. Therefore, is crucial to limit the quota to reduce the fishing effort, as the dredged area is reduced and consequently lesser is the impact exerted on the macro-benthic communities, as well as to adopt a rotation system of the fishery grounds to allow the macro-benthonic species to recover after the dredge disturbance. Sidescan sonar records (Lucchetti and Sala, 2012) showed evidence of considerable physical disturbance in the surveyed area, with numerous tracks crisscrossing the area, which remained traceable for 2-3 months. Furrows had an average height of 10 cm and a width of 3 m, equivalent to the dredge width. The time required to macro-benthonic species to recover ranges between 3-6-month as indicated by Pranovi and Giovanardi (1994) and Vasapollo et al., (2020), or over about 2 months for areas with predominantly sandy characteristics used for commercial fishing (Pranovi et al., 1998). The ecological effects and the recovery of the benthic community after the action of hydraulic dredge gear can therefore be equated to the recovery that takes place following natural disturbances in high energy costal sandy sediments.

All these restrictive management measures adopted over years, now supported by deepen and updated biological information on the species and on the interaction gear-species, explain how hydraulic dredging fisheries in Italy have been able to sustain a high fishing effort in the last 40 years. Within this time interval, this is the only fishing sector which has been able to keep the number of vessels and operators unchanged. However, the technical restrictions adopted allowed to keep this sector alive as well as to preserve the conservation of the species. For this reason, such management measures must be strictly followed by fishermen and Consortia for the protection of the species and the continued productivity of the sector. Last but not least, it is still strongly required trying to adopt mitigation measures to further reduce the impact exerted by dredging with a view to fishing based on the ecosystem integrity maintenance.

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