

UNIVERSITÀ DEGLI STUDI DELLA TUSCIA DI VITERBO

DIPARTIMENTO DI SCIENZE ECOLOGICHE E BIOLOGICHE (DEB)

**CORSO DI DOTTORATO DI RICERCA IN ECOLOGIA E GESTIONE
DELLE RISORSE BIOLOGICHE (XXIV CICLO)**

**On the Ecology and Conservation of Marine Endangered Species,
Pinna nobilis (Linnaeus, 1758) and *Patella ferruginea* (Gmelin,
1791), in the Marine Protected Area of Penisola del Sinis - Isola di
Mal di Ventre (western Sardinia, Italy)**

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TITOLO DELLA TESI:

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1791), in the Marine Protected Area of Penisola del Sinis - Isola di
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To the strange world of Cabras...

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ABSTRACT/RIASSUNTO

Marine Protected Areas (MPAs) have been established to protect vulnerable species and habitats, to conserve biodiversity, to restore ecosystem integrity, to avoid user conflicts and to enhance the productivity of fish and invertebrate populations in the neighbouring zones. Often the lack of information on the species ecology can limit or make void the existing management measures. One of such cases is the Penisola del Sinis - Isola di Mal di Ventre MPA (hereafter Sinis MPA) which was instituted in 1997 in order to protect the local marine resources. This thesis, carried out in the Sinis MPA, was aimed at increasing the knowledge on the distribution, abundance and ecological traits of two endangered species, the molluscs *Pinna nobilis* and *Patella ferruginea*, as fundamental prerequisites for their management and conservation. These species were considered both for their conservation priority and because molluscs are commonly considered good indicators of MPA effectiveness.

The fan mussel *P. nobilis* is the largest bivalve of the Mediterranean Sea (it can exceed one meter in length). *P. nobilis* is a sessile suspension feeder endemic of this basin which lives in coastal areas, mainly on soft sediments colonized by seagrass meadows. In the last few decades, *P. nobilis* populations have declined drastically due to increasing anthropogenic pressure. Thus, *P. nobilis* has been declared a protected species since 1992. This study considered a population living within a *Posidonia oceanica* meadow in the Gulf of Oristano, a partially protected Zone of the Sinis MPA. Based on field surveys conducted in the summer of 2007 and 2009, differences in density, percentage of dead individuals, population structure, shell burial level and orientation of *P. nobilis* were investigated in relation to sites features (e.g. seabed compactness) and habitat type (*P. oceanica*, dead *matte*, sand, *P. oceanica* edge). Moreover, it was tested if spatial differences in density and orientation of *P. nobilis* were related to hydrological features such as wave height, wave direction, bottom current direction and speed. Although fairly widespread in the whole Sinis MPA, this species showed a remarkable density only within the Gulf of Oristano with mean value of 1 ind.100 m⁻², in line

with the majority of the Mediterranean populations. The substrate type resulted a considerable factor in defining *P. nobilis* distribution with significant higher density values on the meadow edge which was considered the most suitable habitat for *P. nobilis*. The substrate type and particularly its compactness influences also the shell stability; in fact, the burial level of individuals decreases with increasing fine sediments. A specimen not sufficiently deeply buried can be more susceptible to predation and hydrodynamics. In addition, it was demonstrated that bottom current direction and speed are the main factors influencing shell orientation. In contrast, the effect of wave action was found to be negligible. Where bottom current speed exceeded 0.07 m/s specimens resulted in line with the prevalent current direction with the ventral side exposed to incoming flow. This feature suggests that feeding strategy is an important factor in determining shell orientation, possibly in addition to drag. Moreover, it was assumed that the lagoon waters (Cabras and Mistras lagoon) and the sedimentation areas (e.g. gyre located in the site of Tharros) play a key role in determining high density of *P. nobilis*. Indeed, the food availability seem to be a driving force in determining the patchiness distribution of *P. nobilis* population with higher values in those sites close to the mouth of the lagoons, in sedimentation area or in the meadow channels where the water flow is conveyed.

The second endangered species considered in this thesis is *P. ferruginea*, an endemic limpet of the Mediterranean Sea. *P. ferruginea*, the biggest limpet of the Mediterranean Sea (up to 10 cm), is mostly affected by human exploitation. Moreover, considering that *P. ferruginea* is a protandrous species, human predation directly affects the sex ratio and, consequently, the reproductive population success. Due to the severe contraction of its geographic range and to the high vulnerability of the remaining populations, this invertebrate is actually considered the most threatened with extinction of the Mediterranean basin. Sardinia is one of the few areas where specimens of *Patella ferruginea* can still be observed. Despite the importance of this region for its protection, the available information on the distribution and conservation of Sardinian populations was substantially absent prior to this study. To fill this lack of information the population of *P. ferruginea* living within the Sinis

MPA was examined. In particular, during the summer 2009, a systematic census of *P. ferruginea* was carried out along a 8114 m georeferenced perimeter of coast in the no take-no entry zone of the Mal di Ventre Island. The aim was to provide a detailed map of the distribution of *P. ferruginea* (and of its morphotypes i.e. Rouxi and Lamarki form) and to investigate the effects of accessibility, exposure and slope of the coast on its occurrence. The studied population showed the lowest average density (0.02 ind/m) ever reported for this species, confirming the highly endangered status of *P. ferruginea* on Sardinia rocky shores. Moreover the lack of largest females (>80 mm) indicate a low reproductive potential supported by the limited presence of small specimens (<30 mm). These results constitute the first evidence of the extinction threat of *P. ferruginea* in the Sinis MPA and, probably, along the whole western Sardinian coast where no other large breeding population has been described. This study also demonstrated that the main constraint affecting the population success in the Mal di Ventre Island is human exploitation in relation to the accessibility of the coast. Illegal catches cause the low population density and the absence of largest individuals (>80 mm) which constitute the common fishing target, even though the study area is a no take-no entry zone. Also the exposure of the coast resulted a noteworthy factor in influencing the density and mean size of *P. ferruginea* as well as the distribution of its two morphotypes. Higher density and mean size were recorded on north-western exposed coast where Lamarki form is significantly confined. These findings suggest that a strong wave motion can support a higher percentage of the existing population including the less adaptable morphotype (Lamarki). However, this pattern could be largely influenced by the human exploitation which is lower at the exposed site.

Overall, this thesis contributed to increase the knowledge of population ecology of *P. nobilis* and *P. ferruginea*. In particular, new information on the *P. nobilis* - *P. oceanica* association and the conservation status of the local population of *P. ferruginea* was described, providing useful advices for the management and conservation of these species and their habitat. This work also showed how an inadequate surveillance, combined with a lack of environmental awareness, can lead to limit the effectiveness of a MPA as a flagship tool of

marine conservation. This issue is particularly noticeable for *P. ferruginea* whereas *P. nobilis* population appears to be less vulnerable to the local anthropogenic impact. Our results are in line with previous studies conducted in other MPAs showing that a large proportion of Italian Marine Protected Areas receive ineffective enforcement. A new strategy for the national MPAs, where major efforts are invested in public information and participation promotion on the decision making process, is needed. Thus, Italy should concentrate more on enforcing regulations within the MPAs equipping them with surveillance staff than in establishing of new marine reserves. Finally, the findings of this study provided scientific-based recommendations to the local authorities in order to reinforce the management of biological resources in the Sinis MPA. This has prompted the issue of a ministerial decree (M.D. 20/07/2011) for the enlargement of the Sinis MPA in the area colonized by *P. nobilis*. Specific awareness campaigns have also been conducted on the conservation of the studied species and a regional network, aimed at defining a common conservation strategy for both species, has been established.

*Le Aree Marine Protette (AMP) sono state istituite principalmente per proteggere le specie e gli habitat vulnerabili, conservare la biodiversità, ripristinare l'integrità degli ecosistemi, evitare conflitti fra portatori d'interesse ed aumentare la produttività delle risorse alieutiche nelle aree limitrofe. Spesso la mancanza di informazioni sull'ecologia delle specie può limitare o inficiare le norme di protezione esistenti. Un esempio è rappresentato dall'AMP "Penisola del Sinis- Isola di Mal di Ventre" (di seguito "AMP del Sinis"), istituita nel 1997 per proteggere le risorse marine locali. L'obiettivo principale di questa tesi, svolta presso l'AMP del Sinis, è lo studio di due molluschi protetti *Pinna nobilis* e *Patella ferruginea* volto ad aumentare le conoscenze riguardo la loro biologia ed ecologia, prerequisiti fondamentali per ideare misure di gestione ad hoc. Queste specie sono state scelte, oltre che per la loro priorità di conservazione, anche perché i molluschi sessili o scarsamente mobili sono considerati buoni indicatori dell'efficacia di un'AMP.*

Pinna nobilis è il più grande mollusco bivalve del Mediterraneo (può superare il metro d'altezza). È un filtratore sessile endemico di questo bacino che vive in aree costiere ed in particolar modo su sedimenti mobili colonizzati da praterie di fanerogame marine. Negli ultimi decenni le popolazioni di *P. nobilis* hanno subito un notevole declino a causa della crescente pressione antropica. Per questo, nel 1992, *P. nobilis* è stata dichiarata specie protetta. Questo studio ha esaminato la popolazione di *P. nobilis* presente in una prateria di *Posidonia oceanica* nel Golfo di Oristano, area inclusa nella Zona C dell'AMP del Sinis. Grazie all'attività di campo condotta nell'estate del 2007 e del 2009, è stata studiata l'influenza delle caratteristiche del sito (es. compattezza del substrato) e del tipo di habitat (*P. oceanica*, matte morta, sabbia, margine della prateria) sulla densità, percentuale di individui morti, struttura di popolazione, sul livello di infossamento e sull'orientamento di *P. nobilis*. Inoltre, i valori di densità e l'orientamento di *P. nobilis* dei sei siti indagati sono stati correlati alle caratteristiche idrologiche locali quali la direzione e l'altezza d'onda, la direzione e la velocità della corrente di fondo al fine di spiegare le differenze spaziali osservate. *P. nobilis*, nonostante sia una specie comune in tutta l'AMP, ha mostrato una densità rilevante (mediamente 1 ind 100 m⁻²) solo nella parte nord del Golfo di Oristano. Il tipo di substrato d'impianto è risultato un fattore importante nel definire la distribuzione spaziale della popolazione studiata; gli esemplari, infatti, colonizzano preferenzialmente il margine della prateria. Inoltre, è stato dimostrato come la compattezza del substrato influisca sulla stabilità della conchiglia con individui maggiormente infossati al diminuire della percentuale di sedimento fine. Un esemplare non sufficientemente infossato può essere più suscettibile alla predazione e all'idrodinamica. Inoltre, è stato dimostrato che la direzione e la velocità della corrente di fondo sono i principali fattori che influenzano l'orientamento delle conchiglie. Al contrario, l'effetto dell'azione delle onde è risultato trascurabile. Quando la velocità della corrente di fondo supera 0,07 m/s gli esemplari si dispongono in linea con la direzione prevalente della corrente con il lato ventrale esposto al flusso. Questa caratteristica suggerisce che la strategia alimentare sia un fattore importante nel determinare l'orientamento di *P. nobilis*, oltre alla minimizzazione della resistenza al flusso. Inoltre, si è

ipotizzato che l'input delle lagune (Cabras e Mistras) e le aree di sedimentazione (es. gyre localizzato nel sito di Tharros) svolgano un ruolo chiave nel determinare elevate densità di P. nobilis. Infatti, la disponibilità di cibo sembra essere fondamentale nel definire la distribuzione aggregata della popolazione di P. nobilis con picchi di densità nei siti in prossimità della foce del lagune, nelle zone di sedimentazione, nei margini o nei canali della prateria dove il flusso della corrente è maggiore.

La seconda specie considerata in questa tesi è P. ferruginea, una patella endemica del Mediterraneo. Essendo, fra le patelle, quella che raggiunge le dimensioni maggiori (fino a 10 cm) risulta la specie più interessata dallo sfruttamento umano. Considerando inoltre che è una specie proterandrica, risulta evidente come il prelievo influisca direttamente sulla sex ratio e, di conseguenza, sul successo riproduttivo di una popolazione. A causa della forte contrazione del suo areale di distribuzione geografico e dell'elevata vulnerabilità delle popolazioni rimaste, P. ferruginea è considerata l'invertebrato marino più a rischio di estinzione del Mediterraneo. La Sardegna è una delle poche aree in cui esemplari di P. ferruginea possono ancora essere osservati. Nonostante l'importanza di questa regione per la sua protezione, le informazioni disponibili sulla distribuzione e sullo stato di conservazione delle popolazioni locali era sostanzialmente assente prima di questo studio. Anche per contribuire a colmare questa lacuna è stata studiata la popolazione di P. ferruginea presente nell'AMP del Sinis. In particolare, durante l'estate 2009, è stato effettuato un censimento sistematico di P. ferruginea lungo un tratto di costa georeferenziato di 8114 m nella Zona A dell'Isola di Mal di Ventre. L'obiettivo era quello di fornire una mappa dettagliata della distribuzione di P. ferruginea (e dei suoi morfotipi: Rouxi e Lamarki) e di studiare gli effetti dell'accessibilità, dell'esposizione e dell'inclinazione della costa sulla sua presenza. La popolazione indagata ha mostrato la densità media più bassa (0.02 ind/m) mai riportata per questa specie, confermando l'elevato rischio di estinzione di P. ferruginea sulle coste della Sardegna. Inoltre, la mancanza di grosse femmine (>80 mm) è indice di un basso potenziale riproduttivo, indicazione supportata anche dalla presenza limitata di esemplari di piccole dimensioni (<30 mm). Questi risultati costituiscono la prima prova del forte rischio di

estinzione di P. ferruginea nell'AMP Sinis e, probabilmente, lungo l'intera costa occidentale della Sardegna, dove non sono state descritte altre grandi popolazioni riproduttive. Questo studio ha anche dimostrato come il limite principale del successo della popolazione dell'Isola di Mal di Ventre sia lo sfruttamento umano in relazione all'accessibilità alla costa. Quindi, nonostante l'area di studio sia una Zona A, le catture illegali sono risultate la causa della bassa densità di popolazione e dell'assenza degli esemplari più grandi (>80 mm) che costituiscono il target principale della pesca. Anche l'esposizione della costa è risultato un fattore determinante nell'influenzare la densità, la taglia media e la distribuzione dei morfotipi di P. ferruginea. Maggiori densità e taglie medie sono state osservate sulla costa esposta a nord-ovest dove la forma Lamarki è significativamente confinata. Questi risultati suggeriscono che i tratti di costa maggiormente esposti al moto ondoso possano essere in grado di supportare una più alta percentuale della popolazione, incluso il morfotipo meno adattabile (Lamarki). Tuttavia, questo pattern può essere influenzato dal prelievo umano che è di gran lunga inferiore nei siti esposti.

Nel complesso questa tesi ha contribuito ad aumentare la conoscenza dell'ecologia di popolazione di P. nobilis e P. ferruginea. In particolare, sono stati descritti nuovi aspetti riguardanti l'associazione P. nobilis - P. oceanica e lo stato di conservazione della popolazione locale di P. ferruginea fornendo utili consigli per l'effettiva protezione di queste specie e del loro habitat. Questo lavoro ha anche evidenziato come una sorveglianza inadeguata, unita ad una scarsa consapevolezza ambientale, possa limitare l'efficacia di un'AMP come strumento di conservazione degli ecosistemi marini. Ciò è particolarmente vero per P. ferruginea, mentre la popolazione di P. nobilis sembra essere meno vulnerabile all'impatto antropico locale. I risultati ottenuti sono in linea con studi precedenti condotti in diverse AMP nazionali che dimostrano come molte di esse non abbiano ancora raggiunto un livello di efficacia apprezzabile. È quindi necessaria l'adozione di una nuova strategia per il buon funzionamento delle AMP nazionali che preveda maggiori sforzi nell'ambito dell'informazione locale e della promozione alla partecipazione ai processi decisionali. L'Italia dovrebbe anche concentrarsi più sull'applicazione delle normative, dotando le AMP

di personale specificatamente addetto alla sorveglianza, che sulla costituzione di nuove riserve marine. Infine, i risultati di questa tesi hanno fornito numerose indicazioni supportate da dati scientifici utili alle autorità locali per migliorare la gestione delle risorse biologiche nell'AMP del Sinis. Ad esempio, questa tesi ha contribuito all'approvazione del decreto ministeriale (DM 20/07/2011) utile all'ampliamento dell'AMP del Sinis nella area del Golfo di Oristano colonizzata da P. nobilis. Sono state inoltre condotte specifiche campagne di sensibilizzazione sulla conservazione delle specie studiate ed è stata costituita una rete regionale finalizzata alla definizione di una comune strategia gestionale per entrambe le specie.

GENERAL INTRODUCTION

MARINE ECOSYSTEMS HEALTH AND MEDITERRANEAN MARINE PROTECTED AREAS

Nowadays the health of marine ecosystems is in serious decline worldwide due to multiple anthropogenic impacts including overfishing, pollution, invasive species introduction, coastal development and climate change (Foley *et al.*, 2010) that can alter ocean ecosystems beyond their natural range of variability (Fig. 1; Halpern *et al.*, 2008).

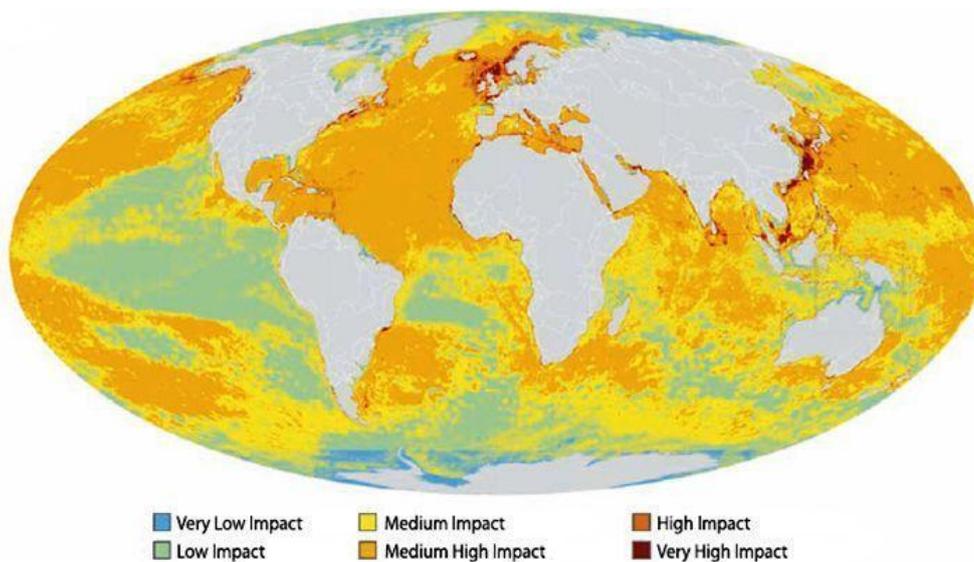


Fig. 1. Global map of cumulative human impact across on marine ecosystems (Halpern *et al.*, 2008).

Human influence on marine ecosystems has accelerated the loss of biodiversity (Fig. 2) directly by exploitation, pollution, and habitat damage, or indirectly through climate change and related effects on ocean biogeochemistry (Dulvy *et al.*, 2003), with largely unknown consequences (Worm *et al.*, 2006; Fig. 2). Although at the global scale marine extinctions are gradually known (Dulvy *et al.*, 2003), regional ecosystems such as estuaries (Lotze *et al.*, 2006), coral reefs (Pandolfi *et al.*, 2003), and coastal (Jackson *et al.*, 2001) and oceanic fish communities (Worm *et al.*, 2005) are rapidly losing populations, species, or functional groups.

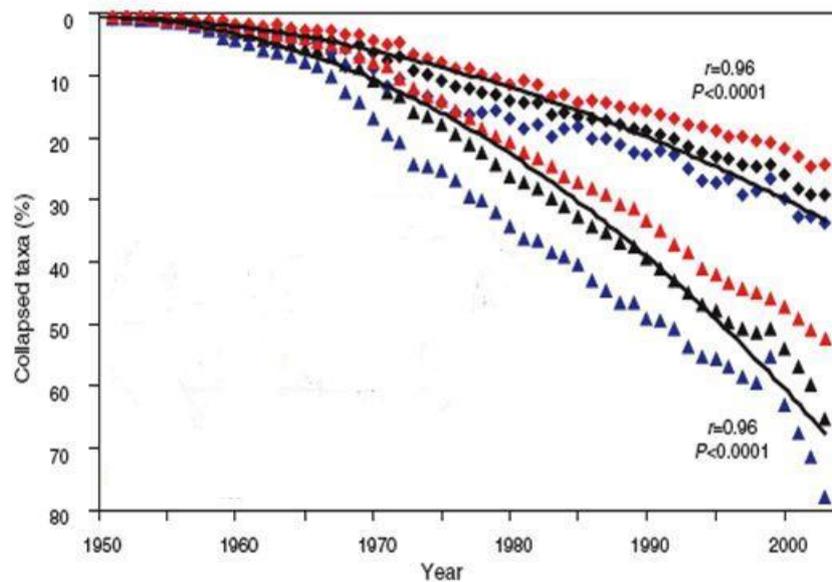


Fig. 2. Global loss of species in marine ecosystem. Diamonds = collapses by year; triangles = cumulative collapses. Data are shown for all (black), species-poor (<500 species, blue) and species-rich (>500 species, red) (Worm *et al.*, 2006).

The Mediterranean Sea is considered a fragile oasis of biodiversity, whose conservation is of primary importance from both an economic and ecological point of view. The Mediterranean Sea shows a great number of marine species in a relatively small water volume: between 4 and 18% of the world species can be found in only a volume about 0.3% of total marine waters (Bianchi & Morri, 2000; Abdul Malak *et al.*, 2011). However, considering its water volume, the Mediterranean Sea also faces enormous human pressure, originated mainly from the industrial revolution and increased by the development of modern technologies and globalization, which could exceed the capacity of natural recovery of this basin. Many calls for attention about the progressive degradation of the Mediterranean Sea have been done during the last decades, but only the development of active protection policies could have practical positive results to reverse this trend (Comeau *et al.*, 2005).

The institution of Marine Protected Areas (MPAs) represents an important management tool for ecosystem conservation as well as fisheries resource restoration. A MPA is defined by the International Union for the Conservation of Nature (IUCN) as “*any area of intertidal or subtidal terrain, together with its overlying water and associated flora, fauna,*

historical, and cultural features, which has been reserved by law or other effective means to protect part or all of the enclosed environment". Currently, MPAs comprise only 3.8% of the Mediterranean Sea. The majority of these MPAs are multiple-use marine areas, with several degrees of protection where certain human activities are still permitted (Harmelin, 2000; Badalamenti *et al.*, 2000; Francour *et al.*, 2001). Fully protected zones (called also "integral zones", "marine reserves", or "no take-no entry areas") cover only 202 km², corresponding to 0.01% of the total surface area of the Mediterranean Sea and 2.2% of the total surface area of the existing MPAs. Marine Protected Areas without integral zones typically provide more limited benefits. For instance, Di Franco *et al.* (2009) demonstrated that fish assemblages in Tavolara MPA (Sardinia, Italy) benefited only from the no take areas, being partial protected zones, where fishing was allowed, not effective. It has also been shown that, inside the marine reserves, the abundance, diversity, biomass and size of fishes, invertebrates and seaweeds increase significantly (e.g. Lester *et al.*, 2009). Commercially target species show the biggest changes, sometimes increasing their density by 10 or 20 times in marine reserves (e.g. Guidetti, 2007; Micheli *et al.*, 2004). These outcomes are consistent across different habitats and may spillover to nearby areas receiving less protection (Micheli *et al.*, 2004). Some species and habitats take many years, even decades, to respond, and the benefits can be removed in 1 to 2 years if the area is re-opened to fishing.

Although MPAs can be an effective tool to protect the marine health, they cannot protect against all types of anthropogenic stress such as pollution, climate change or widespread overfishing (Boersma & Parrish, 1999). Other complementary management strategies are needed along with the creation of MPAs, such as marine spatial planning, gear restrictions and biodiversity monitoring for the sustainable management and conservation of the marine ecosystems.

ENDANGERED SPECIES

An endangered species is a plant or animal species which is near extinction (Maczulak, 2010). Endangered status may lead to extinction, and extinction results in biodiversity loss. So in a sense, endangered species also symbolize the success or failure of today's scientific attempts to save biodiversity. The Red List published by the International Union for Conservation of Nature (IUCN) is widely recognised as the most comprehensive, scientifically-based source of information on the global conservation status of plant and animal species. It is subdivided in categories based on species extinction risk that can change as some populations become more threatened or recover (Fig. 3). IUCN Red List considers also taxa whose extinction risk cannot be evaluated due to insufficient knowledge, and which have therefore been classified as Data Deficient (DD) (Fig. 3). Until 2009, marine organisms have been poorly represented on the IUCN Red List, with less than 5% of the 45000 species on the IUCN Red List. To address this gap, the Global Marine Species Assessment (GMSA), a joint initiative of IUCN and Conservation International, has been formed to complete Red List assessments for approximately 20000 marine species by the year 2012. The Mediterranean regional assessment is one of the first considered and the categories definition for marine fishes is just completed under the GMSA (Fig. 4) (Abdul Malak *et al.*, 2011).

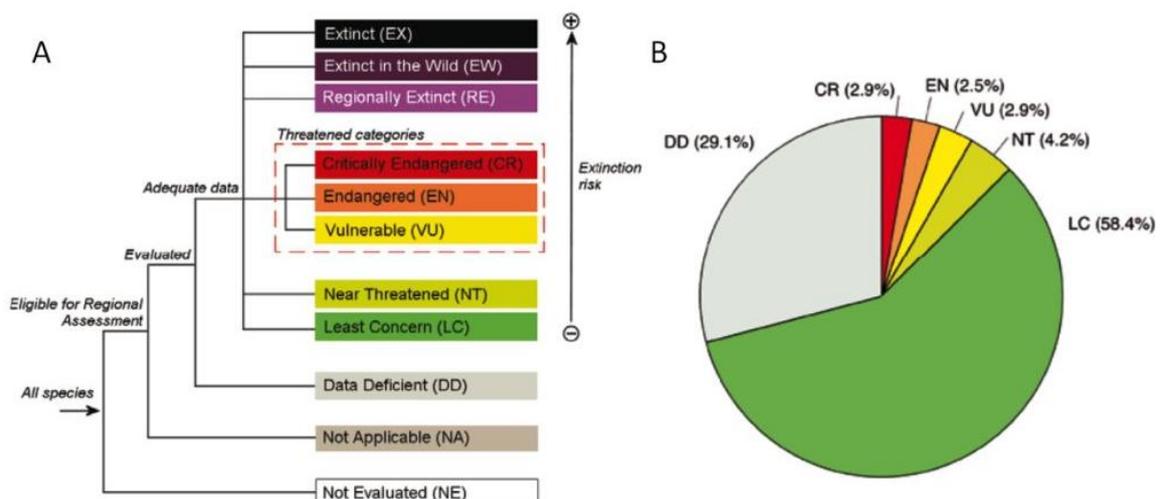


Fig 3. IUCN Red List categories (A) and Regional Red List status of all the marine fish species of Mediterranean Sea (B).

Despite the protection of endangered species has become an important issue in global conservation activities, through national and international legislation, few effective protection measures are currently in place, either for species or ecosystems, in the Mediterranean region (Bianchi & Morri, 2000). National protection status of a species varies according to country. However, widespread concerns are emerging over declines in abundance of species, especially in the northern Mediterranean (Abdul Malak *et al.*, 2011). There are four Conventions relevant to the conservation and management of the Mediterranean marine endangered species under various regional and international Conventions:

1. the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES);
2. the Bern Convention on the Conservation of European Wildlife and Natural Habitats;
3. the Barcelona Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean;
4. the Convention on the Conservation of Migratory Species of Wild Animals (CMS or Bonn Convention).

Marine Protected Areas have been set up to protect vulnerable species and ecosystems, to conserve biodiversity and minimize extinction risk, to re-establish ecosystem integrity, to avoid user conflicts and to enhance the productivity of fish and marine invertebrate populations around a reserve (Pauly *et al.*, 2002; Hooker & Gerber, 2004). In particular, protection of populations is generally recognized to be an essential part of endangered species protection and recovery. A focal question that marine researchers attempt to answer is what processes allow populations to recover after overexploitation. It was long assumed that marine populations are able to recover once the main mortality cause (e.g. fishing) is removed. There is now evidence for low recovery rates following severe depletion of marine fish species with varying life histories (Hutchings, 2001; Hutchings & Reynolds, 2004; Micheli *et al.*, 2008). Processes that affect population recovery include: (1) failure in reducing exploitation; (2) allee effects; (3) habitat modification; (4) species interactions and (5) loss of genetic diversity and increased susceptibility to disturbance and disease (Micheli *et al.*, 2008).

Therefore, studies on populations of threatened species within MPAs may show evidences of recovery, including increased abundance, changes in size/age structure, and increased recruitment or, conversely, a lack of recovery data due to some of the factors previously described.

Pinna nobilis

The fan mussel *Pinna nobilis* (Linnaeus, 1758) is the largest bivalve mollusc of the Mediterranean Sea exceeding one meter in length (Fig. 4). *Pinna nobilis* is a suspension feeder endemic of this basin which lives in coastal areas, between 0.5 and 60 m depth, mainly on soft sediments colonized by seagrass meadows, such as *Posidonia oceanica* (Richardson *et al.*, 1999). The triangular shape allows the partially burial of the shell in the sediment and the byssus threads, attached to solid structures in the substrate, ensure the shell stability (Zavodnik, 1967).



Fig. 4. *Pinna nobilis* individuals: juvenile (sx) and adults (dx).

P. nobilis is a long-lived hermaphrodite species (about 30 years); sexual maturity is reached between the first and second years, therefore organisms smaller than 20 cm are considered juveniles (Šiletić & Peharda, 2003; Galinou-Mitsoudi *et al.*, 2006).

The occurrence of *P. nobilis* in the Mediterranean Sea has been drastically reduced during the last decades as a consequence of illegal trade and incidental killing by trawling,

anchoring (Richardson *et al.*, 2004; Rabaoui *et al.*, 2007) and gillnets (Ayaz *et al.*, 2006; Katsanevakis and Thessalou-Legaki, 2009). In addition, this species is also considered vulnerable because of the widespread decline of its common habitat (i.e. seagrass beds), caused by the growing human impact on the Mediterranean basin (Richardson *et al.*, 1999, 2004). Consequently, *P. nobilis* has been recognised as an endangered species by the European Council Directive (92/43/EEC: Habitats Directive, Annex IV) and the Barcelona Convention (10/06/95, Annex II). Despite the need for specific conservation strategies, knowledge on the ecology and biology of this species and the monitoring of the main population is surprisingly scarce (Rabaoui *et al.*, 2007).

Patella ferruginea

Patella ferruginea (Gmelin 1791) is an endemic limpet of the Mediterranean Sea (Fig. 5). It is a grazer which generally lives in the high mesolittoral, but can occur also in the supralittoral (Paracuellos *et al.*, 2003; Guerra-García *et al.*, 2004; Casu *et al.*, 2006) and in the low mesolittoral (Casu *et al.*, 2004).



Fig. 5. *P. ferruginea* specimen.

This species is characterized by a very slow growth and reproduction rate and reaches sexual maturity between the second and third years at a shell length of 25–30 mm (Guerra-García *et al.*, 2004). *P. ferruginea* is a protandrous species (Espinosa *et al.*, 2008) with a

reversal size from male to female of about 60 mm in shell length (Espinosa *et al.*, 2006). The oocytes are relatively large (142–170 μm) suggesting the presence of a short planktonic phase estimated in about 10 days (Laborel-Deguen & Laborel, 1991; Guerra-García *et al.*, 2004; Espinosa, 2009).

Although originally *P. ferruginea* was commonly distributed through the whole Mediterranean, nowadays its presence is limited to few and restricted coastal areas in the western basin (Cretella *et al.*, 1994; Guerra-García *et al.*, 2004; Espinosa & Ozawa, 2006). The extreme reduction of its occurrence is due mainly to human exploitation and habitat degradation and favoured by low fertility and dispersive capability (Paracuellos *et al.*, 2003). It is important to note that *P. ferruginea*, as the biggest limpet of the Mediterranean (up to 10 cm), is the most affected by exploitation due to an unintentional monospecific collection. Moreover, considering that larger individuals are females, human predation directly affects the *sex ratio* and, consequently, the reproductive populations success (Guerra-García *et al.*, 2004). Due to the contraction of *P. ferruginea* geographic range and the high vulnerability of the remaining populations, this species is actually considered the most threatened with extinction in the Mediterranean Sea (Guerra-García *et al.*, 2004; Espinosa, 2009) and, hence, protected by the European legislation (Annex II of Bern and Barcelona Conventions, Annex IV of Habitat Directive). Sardinia is one of the few Mediterranean areas where specimens of *P. ferruginea* can still be observed. Despite the importance of this region for the protection of this species, the available information on the distribution and conservation of Sardinian populations is substantially absent.

STUDY AREA

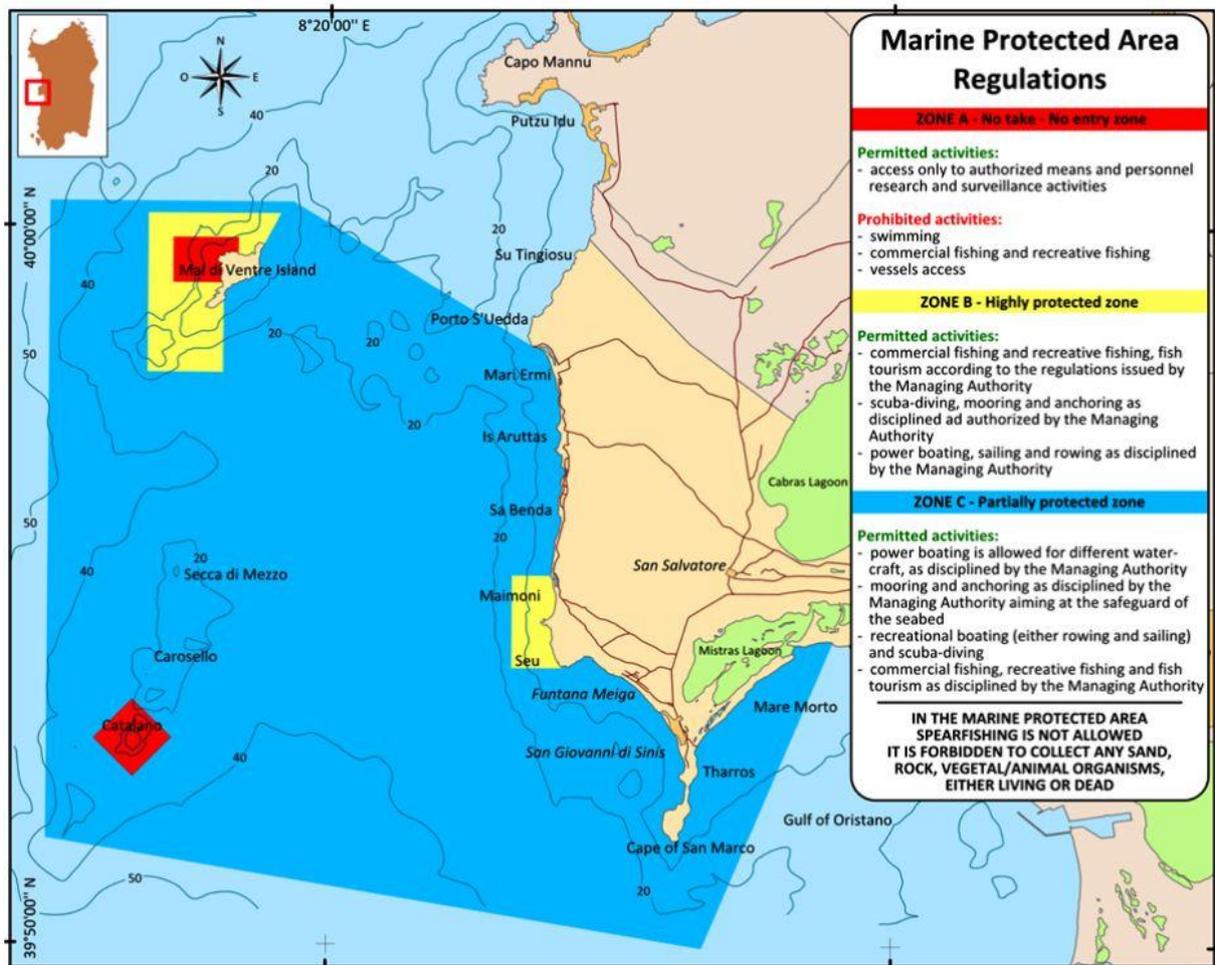


Fig. 6. Penisola del Sinis - Isola di Mal di Ventre MPA: location, zoning and regulation.

This thesis was carried out in the Marine Protected Area of Penisola del Sinis – Isola di Mal di Ventre (hereafter Sinis MPA) located on the western coast of Sardinia (Fig. 6). The coastal and aquatic environments within the MPA are extremely heterogeneous. They include the coastal zone between the cliffs of Su Tingiosu on the north to the Gulf of Oristano on the south together with Mal di Ventre and Catalano Islands. The Sinis Peninsula is characterized by high basaltic or calcareous cliffs altering by wide beaches of which the most famous, located on the area extending from Maimoni to Mari Ermi, are quartz grained composed (Fig. 6). Catalano is a small basaltic outcrop whereas Mal di Ventre is a granitic island of about 5 nautical miles away from the coast, with a surface lower than 1 Km². Generally the seabed

profile is slightly tilted and only in few points falls below 40 meters depth. Extensive *P. oceanica* meadows cover most of the seabed surface growing both on hard and soft substrates.

Established in 1997, the Sinis MPA has recently undergone two changes in size and zoning (Fig. 7). In particular, no take-no entry zones and highly protected areas were drastically reduced in number and surface. Currently, this MPA covers a surface of about 25000 ha, 1.5% of which is a no take-no entry area. These changes were taken especially under the pressure of the local population, primarily fishermen.

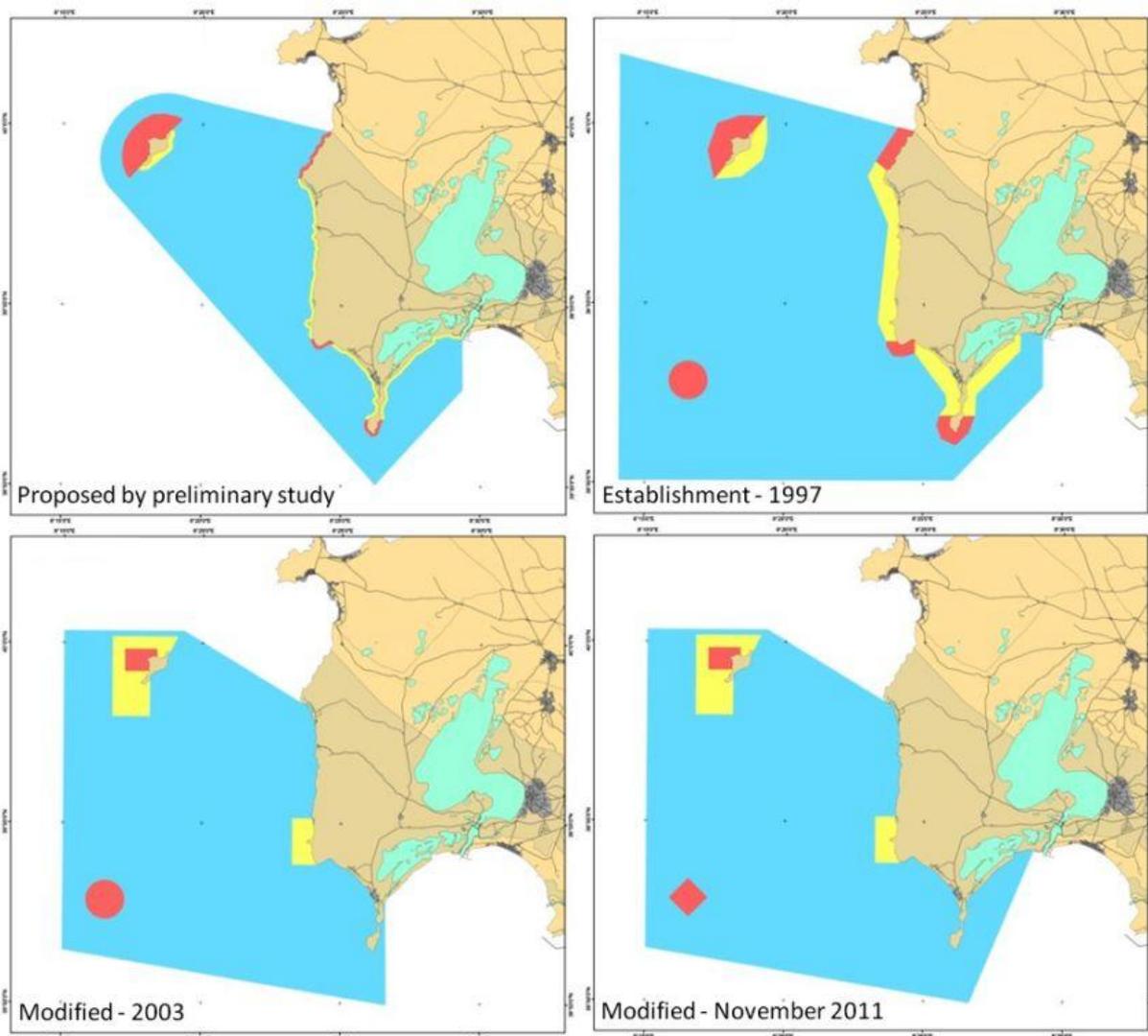


Fig. 7. Changes in size and zoning of Penisola del Sinis - Isola di Mal di Ventre MPA.

The Sinis MPA is managed by the Municipality of Cabras whose territory covers a surface of 102.18 sq km: 73% is devoted to farming, 13.5% is occupied by wetlands (e.g the

Cabras and Mistras lagoons), about 10% by woodlands and natural areas, while only 2.5% consists of urbanized areas with a resident population of about 9000 inhabitants. The Sinis MPA is a destination with a low tourist density mostly assembled during the summer months, especially in August. The lack of infrastructures and awareness on the use of environmental and cultural resources as a key factor for the local development are the main braking elements of tourism development in the Sinis territory. Thus, since the substantially lack of pollution sources and the low demographic density, the main threats to marine resources in the Sinis territory are represented by recreational and professional harvesting. Indeed the local fishing fleet, composed of more than 100 units, is one of the major in Italy (Casola *et al.*, 2008).

OBJECTIVE OF THE THESIS

The preservation of biodiversity, particularly at the populations and habitats level, is the main goal of a MPA. However, a central role in promoting the sustainable use of resources and forms of recreation and tourism compatible with the environment (e.g. pleasure boating, diving) is also recognised. All these functions, considered as a whole and properly activated, can facilitate the achievement of many benefits related to all dimensions of sustainable development (environmental, economic and social dimension). Often the lack of information on the species ecology can limit or make void the existing management measures.

This work is aimed at increasing the knowledge on the distribution, biological characteristics and ecological traits of two endangered species, *Pinna nobilis* and *Patella ferruginea*, as fundamental prerequisites in defining specific protection measures in the Sinis MPA. These species were considered both for their priority for conservation and because molluscs are considered good indicators of MPA effectiveness (Gladstone, 2002). Thus, the ecology and distribution of *P. nobilis* and *P. ferruginea* populations were investigated in order to answer three main questions:

- 1) Which is the current health of the two species within the Sinis MPA?
- 2) Which are the main biotic or abiotic factors that can affect the populations success?

3) Currently, can the Sinis MPA be considered an effective tool for their conservation?

This thesis is arranged in three different chapters, followed by an overall discussion:

Chapter 1 - It focuses on density and distribution patterns of *P. nobilis* population living within a *P. oceanica* meadow in the northern part of the Gulf of Oristano (Western Sardinia, Italy). The influence of habitat type, meadow coverage, shoot density and *matte* compactness on the biological features (i.e. population density and structure, spatial distribution, shell burial and orientation, and percentage of dead individuals) is also highlighted. This chapter provides additional information valuable for the effective management and conservation of this species.

Chapter 2 - Here the effect of hydrodynamics on the spatial distribution of *Pinna nobilis* population was investigated. Density and orientation of *P. nobilis* were related to wave height, wave direction, bottom current direction and speed by means of a numerical modelling approach with the aim of improve the understanding of the role of hydrodynamics on the occurrence of this species. Six different sites in the Gulf of Oristano were considered in order to correlate the spatial distribution patterns to the site-specific hydrodynamics.

Chapter 3 - This section was aimed to gather basic information regarding the population structure and distribution of *P. ferruginea* in the Mal di Ventre Island where no information on its status was available. The effects of accessibility, exposure and slope of the coast on the occurrence of *P. ferruginea* was investigated as a tool for the effective management and conservation of this species in the Sinis MPA. The obtained outcomes could be extremely useful also to set up a regional conservation strategy based on the central role of the Sardinian MPAs network.

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CHAPTER 1. - DENSITY AND DISTRIBUTION PATTERNS OF THE ENDANGERED SPECIES *PINNA NOBILIS* WITHIN A *POSIDONIA OCEANICA* MEADOW IN THE GULF OF ORISTANO (ITALY)

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ABSTRACT

Pinna nobilis is an endemic bivalve of the Mediterranean Sea. In the last decades *P. nobilis* populations have declined drastically due to increasing anthropogenic pressure and it has been declared a protected species since 1992. Despite the need for conservation, knowledge of the ecology and monitoring of the main populations of *P. nobilis* are limited. This study considered a population living within a *Posidonia oceanica* meadow in the Gulf of Oristano (western Mediterranean, Italy). The study area, about 150 hectares, part of which is included within a Marine Protected Area (MPA) and a Site of Community Importance (SCI), was subdivided in 3 sub-areas. The percentage cover of different habitat types (*P. oceanica*, dead matte and sand) in each sub-area was measured and meadow features (substrate coverage, matte compactness and shoot density) characterised. The hypotheses of differences in density, percentage of dead individuals, population structure, shell burial level and orientation of *P. nobilis*, were investigated according to sub-areas and to habitat type. The spatial distribution was patchy, and the habitat type resulted a key factor in determining both density and distribution. A strong edge effect was demonstrated: more than half of the observed individuals colonized the *P. oceanica* border. Matte compactness and shoot density were found to affect the density and distribution of *P. nobilis*. Shell burial level and percentage of dead individuals varied with sub-areas and habitat types. Size distribution was bimodal and common shell orientation was observed in two sub-areas. These results contribute to increase the knowledge of population ecology of this species and to provide useful information for implementing conservation policies.

Keywords: *Pinna nobilis*, population density, spatial distribution, Gulf of Oristano, western Mediterranean, edge effect, *Posidonia oceanica* meadow, Marine Protected Areas.

INTRODUCTION

The endemic species *Pinna nobilis* (Linnaeus, 1758) is the largest bivalve of the Mediterranean Sea; the shell exceeds 1 m in length (Richardson *et al.*, 1999; García-March *et al.*, 2002). Fan mussels have a triangular shape (García-March *et al.*, 2007b) and live partially buried in the substrate (García-March *et al.*, 2002; Richardson *et al.*, 2004). Generally, the sediment covers between one-third (Zavodnik, 1967; García-March *et al.*, 2007b) and one-fifth (Richardson *et al.*, 1999) of the shell, depending on substrate type, its compactness (Zavodnik, 1967) and shell size (Richardson *et al.*, 1999). Fan mussels anchor themselves in the sediment attaching byssus threads to solid structures buried in the substrate (García-March *et al.*, 2007b).

Pinna nobilis is a long-lived species; Butler *et al.*, (1993) suggested a maximum age of 20 years, although a 27-year-old specimen was found (Galinou-Mitsoudi *et al.*, 2006). The species shows one of the fastest shell growth rates (up to 1 mm d⁻¹) recorded for bivalves (Katsanevakis, 2007b). In spite of the high variability of growth curves in the studied Mediterranean populations (Vicente *et al.*, 1980; Richardson *et al.*, 1999; Šiletić & Peharda, 2003; Galinou-Mitsoudi *et al.*, 2006; Rabaoui *et al.*, 2007), *Pinna nobilis* is fast-growing during the first three years, thereafter it shows a much slower growth rate (Katsanevakis, 2005 and literature therein). *Pinna nobilis* has a highly variable recruitment, both on spatial and temporal scale (Katsanevakis, 2007a). Its larval stage lasts 5–10 days (De Gaulejac & Vicente, 1990) and settlement occurs mostly during late summer and early autumn (Richardson *et al.*, 1999; Katsanevakis, 2007b). Specimens with a shell height up to 20 cm are considered juveniles (Šiletić & Peharda, 2003). Knowledge of mortality rates during various ontogenetic phases is scarce: for instance, no data is available on the survival rate of the planktonic stages, although information does exist on the decrease of post larval mortality rate with age, due to the lower vulnerability of large individuals to predation (Fiorito & Gherardi, 1999; García-March *et al.*, 2007b). Higher mortality rates occur during the first year; indeed Katsanevakis (2007b) suggested a ‘refuge size’ for shell width of about 8 cm.

Pinna nobilis occurs in coastal areas, between 0.5 and 60 m depth, principally on soft sediments colonized by seagrass meadows, such as *Posidonia oceanica* (Richardson *et al.*, 1999; García-March *et al.*, 2007a), *Cymodocea nodosa* (Richardson *et al.*, 2004; Centoducati *et al.*, 2007), *Zostera marina* and *Nanozostera noltii* (Katsanevakis, 2005), but also on bare sand (Richardson *et al.*, 1999; Katsanevakis, 2007b; Rabaoui *et al.*, 2007), mud (Richardson *et al.*, 1999), maërl beds (García-March *et al.*, 2008), pebbly bottoms (Zavodnik, 1967; Richardson *et al.*, 1999) or among boulders (García-March *et al.*, 2008).

According to the literature, the density of *P. nobilis* ranges between 0.001 (Centoducati *et al.*, 2007) and 600 ind. 100 m⁻² (De Gaulejac & Vicente, 1990; Catsiki & Catsikieri, 1992), but typical values are quite low (García-March *et al.*, 2007a), and 1 ind. 100 m⁻² is the most widespread value found in the Mediterranean populations (Šiletić & Peharda, 2003 and literature therein).

General knowledge of the main constraints which control the population structure of *P. nobilis* is still incomplete, although previous papers have highlighted specific features concerning spatial distribution. Usually fan mussels have a patchy distribution (Richardson *et al.*, 2004; Katsanevakis, 2005), a common orientation of the valves is evident in some locations and a depth-related size segregation has often been observed (Zavodnik, 1967; García-March *et al.*, 2007a and literature therein). The patchiness in spatial distribution is associated with optimal habitat availability (e.g. the presence of seagrass meadows); the common orientation of the valves is generally attributed to the increased mortality rate of inadequately oriented individuals in relation to environmental factors (i.e. deep currents or bottom slope) (García-March *et al.*, 2007a and literature therein). Depth seems to be one of the most significant factors for explaining population density and size distribution (Katsanevakis, 2005). Higher densities have been recorded at an intermediate depth, within pinnids depth range, probably due to the decrease of hydrodynamic stress and of illegal catching (García-March *et al.*, 2007a,b; Katsanevakis, 2007b). Size distribution is correlated to depth: small specimens are characteristic of shallow areas, whereas the larger ones are more abundant in deep sites. This pattern was initially explained by an hypothetical

dislodgement capability of these bivalves (Zavodnik, 1967) that would make them capable of moving towards deep sites as they grew older. Nowadays the more supported hypotheses are related to higher mortality of large individuals in shallower waters due to amateur collectors and/or increased hydrodynamic stress compared to deeper sites (García-March *et al.*, 2007a, b; Katsanevakis, 2007b).

The total abundance of *P. nobilis* in the Mediterranean Sea has been significantly reduced during the last decades as a consequence of recreational and commercial fishing, and incidental killing by trawling and anchoring (Richardson *et al.*, 2004; Katsanevakis, 2005; Katsanevakis, 2007a,b; Rabaoui *et al.*, 2007). Moreover, this species is also considered vulnerable because of the widespread decline of its common habitat (e.g. *P. oceanica* meadows), caused by the increasing anthropogenic pressure in the Mediterranean basin (Richardson *et al.*, 1999; García-March *et al.*, 2007b; Richardson *et al.*, 2004). Consequently, *P. nobilis* has been listed as an endangered species according to the European Council Directive (92/43/EEC: Habitats Directive, Annex IV) and the Barcelona Convention (10/06/95, Annex II). Despite the presence of these protective measures, knowledge of population structure, spatial distribution and abundance is surprisingly scarce (García-March *et al.*, 2007b; Katsanevakis, 2007b; Rabaoui *et al.*, 2007). In order to effectively protect this endangered species, more information about distribution and ecology of all conspicuous *P. nobilis* populations is needed (Katsanevakis, 2005; Katsanevakis, 2007a,b). Currently, this lack of information is severe for Italian coasts and particularly in Sardinia where, despite the presence of several conspicuous populations, only few studies about their ecological features have been published (i.e. Porcheddu *et al.*, 1998; Caronni *et al.*, 2007; Addis *et al.*, 2009).

The present study investigates a *P. nobilis* population in the northern part of the Gulf of Oristano (Western Sardinia, Italy). It focuses on population density and structure, spatial distribution, shell burial and orientation, and percentage of dead individuals. The influence of some environmental factors (i.e. habitat type, meadow coverage, shoot density and *matte* compactness) on the biological features cited above is also highlighted. This paper provides

additional information valuable for the effective management and conservation of this species.

MATERIALS AND METHODS

Study area

The Gulf of Oristano is located on the western coast of Sardinia (Italy) (Fig. 1a). The gulf, which extends over 150 km² and is 15 m deep in the middle, is characterized by a flat bottom, 70% of which is colonized by *Posidonia oceanica* (De Falco *et al.*, 2008). The study area, located in the north-western part of the gulf, is partially included within the *Penisola del Sinis - Isola di Mal di Ventre* MPA and the *Mistras* SCI (ITB000034) (Fig. 1b). The *P. oceanica* meadow colonizes the sub-horizontal sandy substrate at a minimum depth of 2 meters. The meadow is not continuous due to the occurrence of sandy patches, *intermatte* channels and wide zones of dead *matte*. The study area extends for about 150 hectares along the coast from *Torre Vecchia* (39° 51.960'N; 008° 26.412'E) to *Mar Morto* (39° 53.300'N; 008° 27.210'E) (Fig. 1c). Despite protection, this area is subjected to an increasing human pressure due to recreational boating and fishing activities.

Study area characterization

In order to investigate the main characteristics of the seabed, a survey was carried out during summer of 2007. The whole area was divided into 3 sub-areas, Torre Vecchia, Tharros and Mar Morto (Tv, Th and Mm respectively) (Fig. 1); the percentage cover of different habitat types (*P. oceanica*, dead *matte* and sand) was measured at each sub-area. The percentage cover of habitat types was estimated by means of the Point Intercept Transect (PIT) method (Bianchi *et al.*, 2004) using three random transects within each sub-area. For each transect, a 400 m line, marked every 5 m, was rolled down from the coast (2 m depth) toward the open sea. The habitat type was recorded at each fixed point along the transect and the respective percentages were calculated.

For each sub-area, the meadow density was evaluated at depths of 2 m and 5 m. The number of *P. oceanica* shoots was counted in 10 replicate quadrats (50 x 50 cm) at each sampling station, and the results were averaged and converted to the square metre.

The compactness of live *matte* was assessed by means of 10 replicate strokes of a penetrometer at each sampling station. The penetrometer is composed of a tripod base and a 2 m long rod, 8 mm in diameter. The rod was placed inside the base perpendicular to the bottom, and a 5 kg weight was dropped 50 cm onto a stop in the middle of the rod. The impact of the weight hitting the stop provided a constant force in order to drive the rod into the rhizome *matte*. Results were averaged and compactness was defined according to Francour *et al.*, (1999): strong (penetration <50 cm), medium (penetration between 50–100 cm) and weak (penetration >100 cm).

Two-way analysis of variance (ANOVA) on shoot density and *matte* compactness was carried out in order to test for any significant differences between sub-areas and depths. Cochran's test was used to test for the homogeneity of variances and data were transformed when necessary (Underwood, 1997). Post hoc comparisons, by mean of the Student-Newman-Keuls test (SNK), were carried out in case of significant differences from ANOVA.

***Pinna nobilis* population analysis**

The survey was performed by means of SCUBA diving between June and July 2007. Twenty, 400 m long, strip transects perpendicular to the coastline and spaced at about 150 m from each other were explored from the 2 m bathymetric line. A pair of divers recorded and measured the individuals of *P. nobilis* encountered within a 2 m corridor for both sides of each 400 m line; thus the surveyed area was about 1600 m² per transect. For each *P. nobilis* specimen observed, minimum width (w) and unburied length (UL) were measured by means of a multi-calliper (García-March *et al.*, 2002); shell orientation (Or) was determined using an underwater compass and considering the magnitude of the angle α formed by the vector Or and the magnetic North (Fig. 2). The specimen status (dead or alive), the exact depth (by using the electronic depth meter of a diving computer), and the type of substrate (*P. oceanica*, dead *matte*, sand or *P. oceanica* edge) were recorded. In addition, the geographical coordinates were marked for each individual along the transect; to do this a positively buoyant

messenger was sent up to the surface in order to record the exact position from the boat by means of a Global Positioning System (GPS).

Total shell height (HT) was estimated according to García-March and Ferrer Ferrer (1995): $HT = (1.79 w + 0.5 \pm 0.2) + UL$. This formula was considered suitable for populations of different areas (Šiletić & Peharda, 2003).

The shell orientation measurements were grouped in the following four sectors: NE (1°–90°), SE (91°–180°), SW (181°–270°), NW (271°–360°). In order to test the occurrence of preferential valve directions, orientation distributions were compared to an uniform distribution by means of a *chi*-square test considering subareas and habitat types. The sand areas were excluded from this analysis because of the low sample size (only five *P. nobilis* were recorded on sand).

The Kruskal-Wallis test was applied to test whether density, shell size, percentage of dead individuals and burial level of the shell significantly varied between sub-areas. The burial level of the shell was estimated as percentage of the difference between the estimated HT and the measured UL. Another Kruskal-Wallis test was performed in order to investigate if this burial level significantly varied depending on habitat types. Moreover, for each subarea a *chi*-square test was applied in order to analyse if percentage of dead individuals was homogeneous between habitat types. Again, because of the small sample size, sand areas were excluded from the analysis. In addition, the *chi*-square test was applied in order to investigate whether *P. nobilis* colonizes preferentially the edge of the *P. oceanica* meadow. In order to perform this analysis, we assumed, using the most conservative approach, that the surface of the meadow was equal to the area delimited by its edge.

The spatial distribution of individuals in the surveyed area was investigated using the GPS data on individuals' position and the ArcGIS 9.2 ESRI program. Every transect was subdivided in 8 cells (50 x 4 m) and the Morisita's Index (MI) was calculated for each transect and subarea (excluding transect 7 and 14, considered as boundaries between adjacent sub-areas). In addition, the Standardized Morisita's Index (MIS) was used with the whole data set (Bakus, 2007).

In order to detect potential significant differences between sub-areas based on some features of pinnids, a canonical discriminant analysis (Canonical Analysis of Principal Coordinates or CAP) was performed by subdividing each transect in 4 cells (100 x 4 m) (Anderson & Willis, 2003; Anderson & Robinson, 2003). For each cell, the number of individuals, substrate type, main orientation, mean width, mean total length and mean depth were the variables under consideration.

RESULTS

Study area features

The study site is characterized by the dominance of a *Posidonia oceanica* meadow which in Mar Morto occupied more than 80% of the area (Fig. 3). A general trend of increase in *P. oceanica* cover was evident from Torre Vecchia to Mar Morto, whereas the presence of dead *matte* had the opposite effect. The sand extension was comparable for all sub-areas and corresponded to about the 20% of the total cover (Fig. 3).

The mean shoot density of the meadow ranged between 244 ± 16 (mean \pm SE) and 391 ± 21 shoots m^{-2} (in Mm 2 m depth and Tv 5 m depth, respectively) (Fig. 4); these values are very low compared to those recorded in other Mediterranean areas at the same depths and, according to Pergent *et al.*, (1995), they correspond to a meadow of abnormal density. The analysis of variance showed significant differences in the number of shoots for both sub-area and depth (Table 1). Post hoc comparisons (SNK) highlighted that Tv had a higher shoot density in comparison with Th and Mm; moreover a significantly higher number of shoots was recorded, at 5 m depth than at 2 m depth (Table 1, Fig. 4).

The mean compactness of *matte* ranged between 29 ± 4 cm (Th, 5 m depth) and 55 ± 6 cm (Mm, 2 m depth) (Fig. 5). Based on Francour *et al.*, (1999) scale, all sites were characterized by strong compactness, except for Mm at 2 m depth that presented a medium level of compactness. *Matte* compactness varied significantly between sub-areas and depths (Table 1). In particular, Mm showed higher penetrability values in comparison with the other sub-areas, and was less compact at 2 m depth than at 5 m depth (Table 1, Fig. 5).

***Pinna nobilis* population**

A total of 310 live *Pinna nobilis* was recorded, corresponding to 1 ± 0.2 ind. $100 m^{-2}$ (mean \pm SE) population density. Population density varied significantly between sub-areas: Th showed higher mean values of abundance than Tv and Mm (Fig. 6). Moreover, *P. nobilis* density of individuals differed among substrates: 182 specimens were located on the *P.*

oceanica edge, and this marginal zone was the site more inhabited by this species (Table 2), as demonstrated by the result of the *chi*-square test ($\chi^2 = 4.99$, $p < 0.0001$, $df = 1$). Dead *matte* substrate supported a higher population density (3.8 ind. 100 m⁻²) than *P. oceanica* (0.3 ind. 100 m⁻²) or sand (0.1 ind. 100 m⁻²).

Moreover, 50 dead *P. nobilis* individuals were recorded along the transects; this number corresponded to 14% of the total sampled (Table 2). The Kruskal-Wallis test showed significant differences in percentage of dead individuals between sub-areas: Mm had significantly higher values than Th and Tv (Fig. 7). Th showed the highest variability and, although no significant differences were detected when compared with Tv, a general growing trend was evident from Tv to Mm (Fig. 7). The *chi*-square test highlighted that although the number of dead individuals did not differ between habitat types in Tv, there was higher value than expected within the meadow in Th, and a high number dead specimens was recorded within the meadow, but mainly on the edge of the meadow in Mm (Table 3).

The population structure showed a bimodal distribution (Fig. 8): the first mode corresponded to the 20–25 cm size class, the second one (individuals 60–65 cm high) was more marked. In order to assess whether depth could have had a relevant role in influencing population structure, the study area was divided into 2 depth zones (1–3.5 m and 3.6–6 m). The corresponding size distributions are shown in figure 8a: despite the higher abundance in the deeper zone, no significant difference in population structure was found. On the other hand, different patterns were observed when considering the 3 sub-areas (Fig. 8b): Tv was characterized by small-size individuals (10–30 cm) and large pinnids were substantially absent. This size distribution was significantly different (Kruskal-Wallis test, $p < 0.01$) when compared to Th and Mm (Fig. 8b). Th showed the same trend described for the global population distribution, with two peaks clearly represented. In Mm small sizes were lacking and individuals with a size range between 55 and 70 cm prevailed in this sub-area.

In addition, the burial level of the shell contributed to differentiate the 3 sub-areas, showing a significant decreasing trend from Tv to Mm (Kruskal-Wallis Test; $p < 0.001$). This result pointed out a marked spatial pattern in shell burial capability of *P. nobilis*, with Mm

specimens less-deeply buried in the seabed (Fig. 9). Regarding substrate type, the Kruskal-Wallis test showed more-deeply buried individuals into the dead *matte* than in live *P. oceanica* (inside the meadow or on the edge; Fig. 10).

The results of *chi*-square test showed that it was possible to discard random shell orientation in two subareas only (Table 4). Significant differences were recorded in Tv ($p < 0.05$) and in Th ($p < 0.001$): in Tv, specimens were preferentially oriented toward the third quadrant, whereas in Th pinnids were oriented toward the first and fourth quadrant (Table 4).

The data on *P. nobilis* dispersion highlighted an aggregated distribution of individuals. The values of Morisita's Index (MI) calculated for each transect showed a clumped dispersion in 14 cases (Table 5). The same index, when considering the sub-areas, always showed an aggregated distribution of pinnids (Table 5). Moreover, this spatial pattern was confirmed by Morisita's Standardized Index ($MIS = 0.505$) independent from sample size.

The result of the CAP test (Canonical Analysis of Principal coordinates) indicated a significant effect ($p = 0.0001$) of pinnid features on the degree of separation among sub-areas with a squared canonical correlation of $\delta^2 = 0.62$ (Table 6). Mm showed a percentage of allocation success (94%) higher than Th (60%) and Tv (19%), resulting in a better characterized sub-area as a group. In other words, the fan mussels recorded in Mm exhibited more homogeneous features, which further contributed to the peculiarity of this sub-area in relation to the other ones. This separation among sub-areas may be also observed in the corresponding CAP scatter plot (Fig. 11). Here the samples are well parted, with Mm individuals mostly on the left side, Tv on the right side, and Th, exhibiting intermediate features, in the middle part of the plot.

DISCUSSION

The population density of *Pinna nobilis* in the northern part of the Gulf of Oristano, corresponded to 1 ± 0.2 ind. 100 m^{-2} , which is in line with the most common value found in the Mediterranean populations (Šiletić & Peharda, 2003 and literature therein). However our results are more than 6 times lower than those obtained by Addis *et al.*, (2009) in the same gulf (i.e. 6.3 ind. 100 m^{-2}). In the literature concerning other areas, the recorded densities range from 0.001 (Centoducati *et al.*, 2007) to 600 ind. 100 m^{-2} (De Gaulejac & Vicente, 1990; Catsiki & Catsikieri, 1992). This high variability could depend on the aggregated distribution of this species, but sampling bias could be also responsible. Density may be overestimated when the sampling areas with no specimens are excluded from the mean estimate or when the investigated area coincides with the patches with the highest population densities (e.g. Galinou-Mitsoudi *et al.*, 2006; Centoducati *et al.*, 2007). When the study species is distributed in clumps, the selection of both a properly surveyed area size and a suitable sampling method is essential for an accurate knowledge of abundance and distribution of individuals. In our case, a preliminary survey was crucial in designing the areas the divers had to monitor. In order to facilitate the detection of individuals it seemed appropriate to choose for each transect an area only two meters wide and covered by rare *P. oceanica*.

The results of population density showed significant differences between substrates and sub-areas. Specimens were mostly located on the *P. oceanica* edge. Density was higher within dead *matte* than inside the meadow, whereas bare sand showed the lowest values. This typical pattern could be due to the specific features of substrates, which cause different survival rates and therefore, highly variable densities. For example, *P. oceanica* allows a solid anchorage and an effective protection of pinnids (García-March, 2003), unlike bare sand. Marginal zones, however, could be a good compromise between the need for a certain level of protection and the need for efficiency in the filtering activity. As it is reported in literature (Bologna & Heck, 1999 and literature therein), suspension feeding organisms living along

habitat edges may experience higher food fluxes compared with those in the inner part of the meadows. The same pattern observed for *P. nobilis* was found by Bologna and Heck (1999) in their research on the density of *Argopecten irradians* on meadow scale in Florida. Scallops were significantly more abundant at edges of *Thalassia testudinum* beds than in their interior or in nearby unvegetated sediment. These authors suggest that scallops living along the edges of grass beds have an advantage in obtaining food resources but that they are confronted by a trade off between increased growth rates and increased predation mortality. Understanding how the landscape mosaic of a *P. oceanica* meadow, and particular its edge, influences the pattern of survivor and distribution of *P. nobilis* is a fundamental issue for the conservation of this species.

The assumption on the greater survival of *P. nobilis* on the edge of *P. oceanica* agrees with the results of *P. nobilis* distribution analysis and shell orientation. The aggregated distribution suggests that despite the presence of an extensive meadow, the seabed is sufficiently heterogeneous to create patches which are more or less advantageous for the growth of *P. nobilis*. These favourable microhabitats match the sites where specimens are clumped. Shell orientation was random in Mar Morto in agreement with the results of Addis *et al.*, (2009), but common shell orientations were recorded in Tharros and Torre Vecchia. A common orientation should be expected when hydrodynamism is a key factor (García-March *et al.*, 2007b). In the northern part of the Gulf of Oristano no selective pressure on the population could possibly be present due to a slow water flow. Indeed, this area is one of the few locations sheltered from the Mistral, the prevailing wind which blows along the Western Sardinian coasts (Cucco *et al.*, 2006). The different distribution of shell orientations detected at sub-area scale is probably due to local seabed profiles and small-scale water circulation, but oceanographic data would be necessary in order to confirm this hypothesis.

Significant differences in population density were evident among sub-areas: the highest value was recorded in Tharros. Tharros had intermediate values of percentages cover of *P. oceanica* and dead *matte* in comparison with the other sub-areas. Moreover, Tharros had a *matte* compactness similar to that of Torre Vecchia (the second sub-area per density of

pinnids), but a lower meadow density. Since *P. nobilis* does not suffer hydrodynamic stress in the study area, the water speed attenuation due to *P. oceanica* leaves is probably not relevant. Ultimately, the results indicate that, within the meadow, substrate type, seabed compactness and shoot density are the variables that mainly contribute in determining the actual densities and distribution patterns of *P. nobilis*. Other factors not considered in this study could be of great relevance (e.g. predation, and water circulation at different spatial scales).

Size population structure showed a bimodal distribution with the first modal class lower than the second one. This pattern had never been found in the Mediterranean Sea, where unimodal distributions (e.g. Šiletić & Peharda, 2003; García-March *et al.*, 2007a) or plurimodal curves with decreasing peaks (Katsanevakis *et al.*, 2007) had been previously recorded. The investigated area was characterized by a mature population mainly composed of large size specimens (>60 cm), that could be from 9, according to Vicente *et al.*, (1980) and Richardson *et al.*, (1999), to 23 years old and more, according to Galinou-Mitsoudi *et al.*, (2006). The first mode (20–25 cm) could correspond to specimens aged between 1 and 6 years old (Vicente *et al.*, 1980; Richardson *et al.*, 1999; Šiletić & Peharda, 2003; Galinou-Mitsoudi *et al.*, 2006; Rabaoui *et al.*, 2007). Moreover, the dimension class that was almost completely lacking in the Gulf of Oristano (30–45 cm) could include 2-12-year-old individuals, according to Richardson *et al.*, (1999) and to Galinou-Mitsoudi *et al.*, (2006). Despite the high variability of *P. nobilis* growth rate among Mediterranean populations, these observations suggest that, for a time period ranging from 1 (Richardson *et al.*, 1999) to 6 years (Galinou-Mitsoudi *et al.*, 2006), recruitment might not have been successful in the Gulf of Oristano, due to lack of larvae or high post-settlement mortality. Although long-term studies of other Mediterranean populations demonstrated the failure of juvenile supply for a single year (Šiletić & Peharda, 2003; Katsanevakis *et al.*, 2007), the scarcity of a wide range of sizes observed in our study suggests the possibility of subsequent years of difficulty in population recovery. In order to understand the degree of variability in recruitment success of *P. nobilis*, pluriannual studies on recruitment and population dynamic are necessary. The population size structure of Torre Vecchia was significantly different in comparison with the

other sub-areas. Torre Vecchia had a larger percentage of small specimens, suggesting a higher resilience of *P. nobilis* in this sub-area. This pattern observed in Torre Vecchia was comparable to the population structure detected by Addis *et al.*, (2009) for the estuarine area of the Gulf of Oristano indicating the presence of more than one area potentially suitable for the success of the species.

In the study area, the population structure evidenced no differences with depth. Actually, due to gulf morphology and sampling methodology, the study did not consider depths over 6 meters. Therefore, the lack of a substantial depth gradient might have prevented the detection of any differences, as highlighted in other Mediterranean locations (García-March *et al.*, 2007a; Katsanevakis *et al.*, 2007).

Data on the percentage of dead individuals indicated that 14% of recorded specimens were dead: this value is low when compared to other locations, though few studies focused on this topic. In Mar Grande (Ionian Sea) Centoducati *et al.*, (2007) found a mortality of 78.46%, mostly imputable to pollution and anthropogenic pressure. The value of mortality recorded in Moraira (Spain, western Mediterranean) peaked to 44.4% (dead ind. year⁻¹) and hydrodynamic stress was considered the main cause (García-March *et al.*, 2007a). Differences in percentage of dead individuals recorded among subareas could be explained by substrate type. This result agrees with the hypothesis that specific features of substrates may cause different survival rates. However, more information is necessary in order to evaluate the possible additive or synergistic effects of other ecological factors affecting the survival of individuals. The significant higher values recorded in Mar Morto may be related to the closeness of the Mistras and Cabras lagoons. The input of fine sediment from neighbouring lagoons into the gulf (Como *et al.*, 2007) should account for the lower *matte* compactness recorded in Mar Morto. High levels of silt content and suspended sediment could have contributed to the high number of dead specimens and the recruitment failure in this sub-area. The excess of sediment loading may damage the cilia of fan mussels reducing their respiration and feeding activities (Katsanevakis, 2005; García-March *et al.*, 2008); similar detrimental effects have been reported for *Atrina zelandica* (García-March *et al.*, 2008 and literature

therein). The burial level of the shells seems to confirm this hypothesis. The significant decreasing trend in burial level observed from Torre Vecchia to Mar Morto pinnids is a likely response to high fine sediment content. This compensatory response for avoiding detrimental effects on gills could lead to shell instability and could account for the highest number of dead individuals detected in Mar Morto. A high vulnerability of pinnids which are not deeply burrowed in sediment was reported by Richardson *et al.*, (1999). The pattern previously described from Tv to Mm could be strengthened by the decreasing percentage cover of dead *matte* that determines a significant increase of burial level of the shell even if the dead *matte* did not exceed 10% of the total surface in all subareas. However our hypothesis about the importance of suspended sediment on pinnids survival contrasted with the findings of Addis *et al.*, (2009), hence more data are necessary in order to define the influence of lagoons on the success of the *P. nobilis* population.

The CAP analysis shows that, despite the fact that the three sub-areas represent a geographical continuum, differences in the features of pinnids determined a significant separation among groups, even though squared canonical correlation is not very high. In particular, Torre Vecchia had a moderate *P. nobilis* density and few dead individuals, specimens were small, deeply buried and directionally oriented. Tharros presented the highest density with the prevalence of large sizes, although small specimens were present, pinnids were deeply buried and directionally oriented; data on dead individuals were variable, although low values were prevalent. At Mar Morto, the lowest population density and the highest percentage of dead specimens were recorded; individuals were large, randomly oriented and less deeply buried. These findings also suggested that Mar Morto is probably no longer a suitable area for survival and growth of a long-lasting population of *P. nobilis*. This hypothesis is also confirmed by the present scarcity in pinnid recruitment in this sub-area.

This study contributes to increase the knowledge of the *P. nobilis* population in the Gulf of Oristano. It provides useful information for the management and conservation of this species and its habitat. Since the specimens are mostly distributed on the edge between *P. oceanica* and sand patches, the common practice of anchoring on sandy patches within the

meadow in order to reduce the impact on *P. oceanica* has proven to be effective for the preservation of the plant, but not for *P. nobilis*. Hence, the compulsory use of buoy fields, already present along the coast, is recommended. The common use of gillnets and pots in the Gulf of Oristano must be strictly regulated and controlled in order to minimize their mechanic impact on *P. nobilis* as observed by Ayaz *et al.*, (2006) along the Turkish coast. Moreover, the prohibition of these fishing gears must be taken into account where the population is abundant, at least within the MPA and SCI limits. Furthermore, it is essential to promote an awareness campaign and steady patrolling of the area in order to prevent illegal catching of the largest individuals and the use of forbidden fishing methods, like trawl-nets, which have the potential to remove a conspicuous fraction of population (Hall-Spencer *et al.*, 1999).

A deeper scientific knowledge of the ecological traits of *P. nobilis* and a long-term monitoring study are necessary in order to improve management measures for the preservation of this species in the Gulf of Oristano.

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TABLES AND FIGURES

Table 1. Results of ANOVAs and SNK tests on shoot density and *matte* compactness.

Source of variation	df	Shoot density			<i>Matte</i> compactness		
		MS	F	p	MS	F	p
Sub-area	2	46301.600	15.23	0.0000	6.988	7.68	0.0012
Depth	1	50228.267	16.53	0.0002	4.580	5.03	0.0290
Sub-area X Depth	2	8059.467	2.65	0.0797	1.698	1.87	0.1646
RES	54	3039.407			0.910		
TOT	59						
Cochran's test		C = 0.2381 (ns)			C = 0.3524 (ns)		
Transformation		no			sqrt(x+1)		
SNK		Tv>(Th;Mm); 2m<5m			Mm>(Th;Tv); 2m>5m		

Table 2. Number of recorded *P. nobilis* per substrate (P = *P. oceanica*; S = sand; M = dead *matte*; Edge = *P. oceanica* edge: boundary between *P. oceanica* and sand or dead *matte*). The surface area of each substrate was calculated from the mean values obtained from the Point intercept transects.

	P	S	M	Edge	Tot	%
Nr. of dead <i>P. nobilis</i>	19	2	3	26	50	14
Nr. of live <i>P. nobilis</i>	72	5	51	182	310	86
Surface area (100 m²)	247	59	14		320	

Table 3. Result of *chi*-square test (O = observed frequencies; E = expected frequencies) to test *P. nobilis* dead individuals according to habitat types (P = *P. oceanica*; M = dead *matte*; Edge = *P. oceanica* edge) and sub-areas.

			M		P		Edge	
			O	E	O	E	O	E
Torre Vecchia								
$\chi^2=4.64$ df=2 p<0.0981	Dead		1.00	2.80	3.00	1.82	2.00	5.32
	Alive		19.00	17.20	10.00	11.18	36.00	32.68
Tharros								
$\chi^2=6.79$ df=2 p<0.0335	Dead		1.00	3.08	10.00	6.58	9.00	15.40
	Alive		21.00	18.92	37.00	40.42	101.00	94.60
Mar Morto								
$\chi^2=16.79$ df=2 p<0.0002	Dead		1.00	1.26	4.00	2.52	12.00	4.34
	Alive		8.00	7.74	14.00	15.48	19.00	26.66

Table 4. Result of *chi*-square test for the analysis of differences in shell orientation within sub-areas (O = observed frequencies; E = expected frequencies).

		Quadrant	O	E
Torre Vecchia				
$\chi^2=8.78$ df=3 p<0.034	1-90°		13	16.25
	91-180°		18	16.25
	181-270°		25	16.25
	271-360°		9	16.25
Tharros				
$\chi^2=17.01$ df=3 p<0.0007	1-90°		52	40.5
	91-180°		21	40.5
	181-270°		36	40.5
	271-360°		53	40.5
Mar Morto				
$\chi^2=2.41$ df=3 p<0.4918	1-90°		8	10.25
	91-180°		8	10.25
	181-270°		14	10.25
	271-360°		11	10.25

Table 5. Results of Morisita’s Index (MI). Note that transects 7 and 14 were considered as the limits between sub-areas (Tv = Torre Vecchia; Th = Tharros; Mm = Mar Morto).

	Transect		Sub-area		
	MI	Distribution		MI	Distribution
1	-	-			
2	1.52	clumped			
3	0.86	uniform	Tv	2.47	clumped
4	1.07	random			
5	1.33	clumped			
6	1.24	clumped			
7	1.00	random	-	-	-
8	1.31	clumped			
9	1.21	clumped			
10	2.65	clumped	Th	2.23	clumped
11	1.97	clumped			
12	2.55	clumped			
13	2.04	clumped			
14	1.43	clumped	-	-	-
15	0.80	uniform			
16	1.96	clumped			
17	0.86	uniform	Mm	1.53	clumped
18	3.20	clumped			
19	1.60	clumped			
20	2.00	clumped			

Table 6. Results of canonical analysis of principal coordinates (CAP). m = number of principal coordinate axes used in the CAP procedure. %Var = percentage of the total variation explained by the first m principal coordinate axes. Allocation success = percentage of points correctly allocated in each group. δ^2 = squared canonical correlation. Tv = Torre Vecchia. Th = Tharros;. Mm = Mar Morto.

m	% Var	Allocation success (%)				δ^2	p
		Tv	Th	Mm	Total		
6	100	18.75	60.00	94.44	59.26	0.619962	0.0001

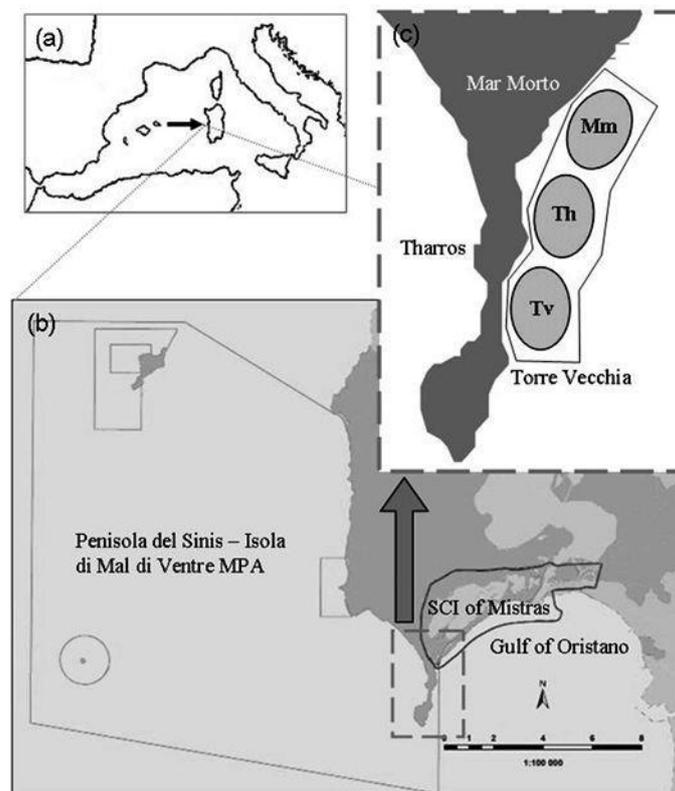


Fig. 1. Location map of the study area. (a) Western Mediterranean Sea, the arrow indicates the Gulf of Oristano; (b) northern part of the Gulf of Oristano and limits of the *Penisola del Sinis - Isola di Mal di Ventre* MPA and *Mistras* SCI; (c) study area with included sub-areas (Tv = Torre Vecchia, Th = Tharros, Mm = Mar Morto).

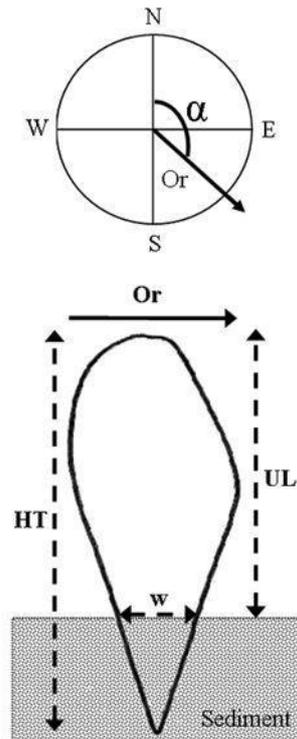


Fig. 2. Biometry of *P. nobilis* (w = minimum width; UL = unburied length; HT = total length; Or = orientation direction).

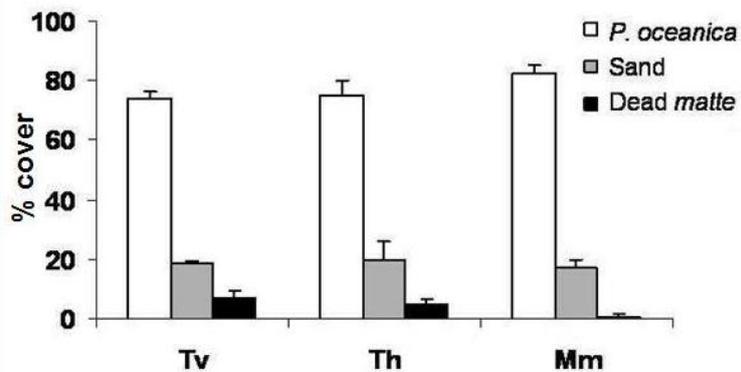


Fig. 3. Percentage cover of different substrates (i.e. *P. oceanica*, sand, dead *matte*) in the sub-areas considered (Tv = Torre Vecchia, Th = Tharros, Mm = Mar Morto).

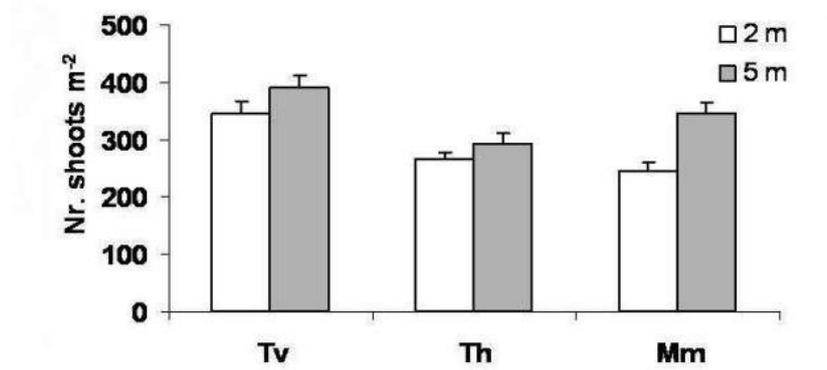


Fig. 4. Meadow density per sub-areas (Tv = Torre Vecchia, Th = Tharros, Mm = Mar Morto) and depths.

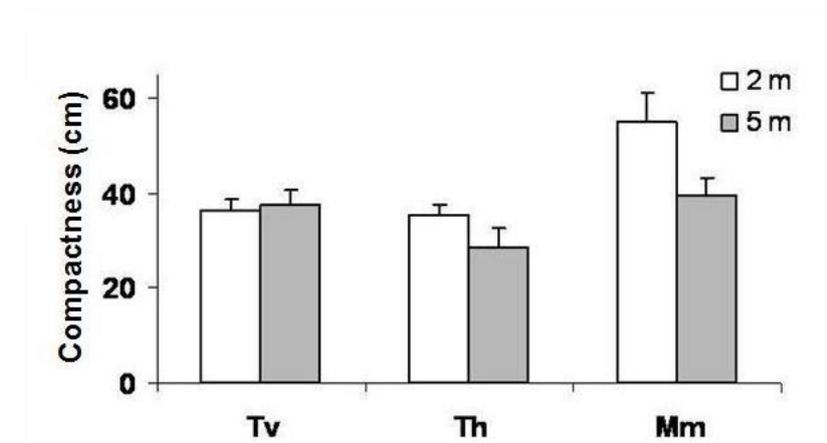


Fig. 5. Matte compactness per sub-areas (Tv = Torre Vecchia, Th = Tharros, Mm = Mar Morto) and depths.

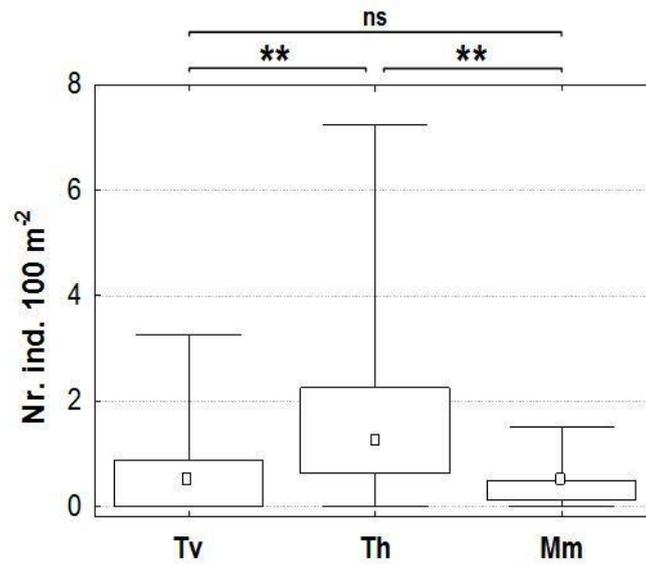


Fig. 6. Density of *P. nobilis* (\square = 25%-75%; \blacksquare = median; Ξ = min-max) per sub-areas (Tv = Torre Vecchia, Th = Tharros, Mm = Mar Morto). Statistical differences among sub-areas are reported (** = $P < 0.01$; ns = not significant).

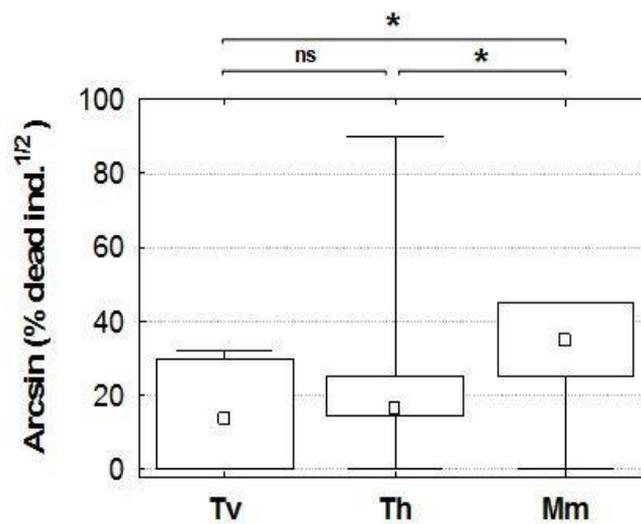


Fig. 7. Percentage of dead individuals of *P. nobilis* (\square = 25%-75%; \blacksquare = median; Ξ = min-max) per sub-areas (Tv = Torre Vecchia, Th = Tharros, Mm = Mar Morto). Data were arc-sin transformed (Zar, 1999). Statistical differences among sub-areas are reported (* = $P < 0.05$; ns = not significant).

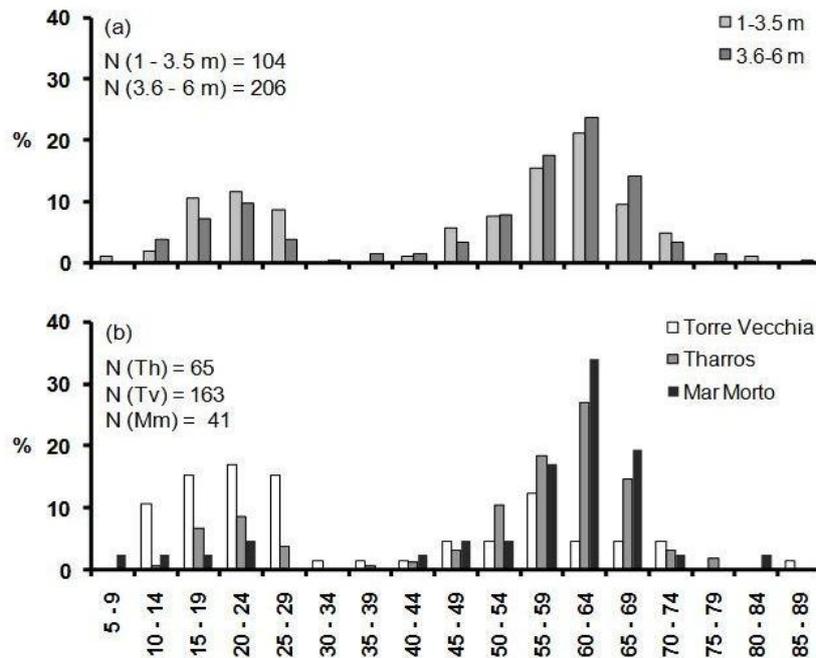


Fig. 8. Percentage frequencies of size classes (5 cm) in populations of *P. nobilis* according to depth levels (a) and sub-areas (b).

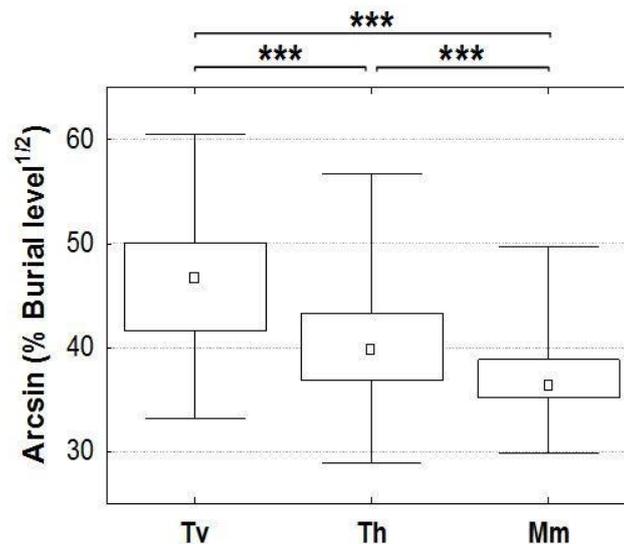


Fig. 9. Burial level (%) of the shells of *P. nobilis* (\square = 25%-75%; \square = median; Ξ = min-max) per sub-areas (Tv = Torre Vecchia, Th = Tharros, Mm = Mar Morto). Data were arc-sin transformed (Zar, 1999). Statistical differences among sub-areas are reported (***) = $P < 0.001$).

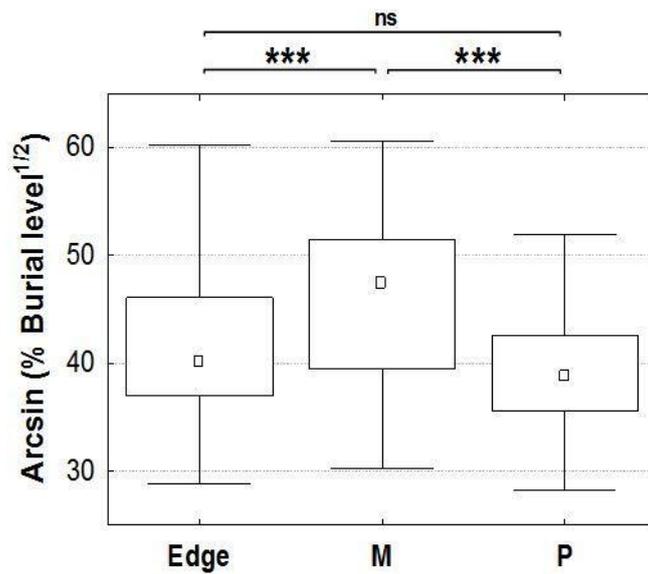


Fig. 10. Burial level (%) of the shells of *P. nobilis* (\square = 25%-75%; \square = median; Ξ = min-max) per habitat types (P = *P. oceanica*, M = dead *matte*, Edge = *P. oceanica* edge). Data were arcsin transformed (Zar, 1999). Statistical differences among sub-areas are reported (***) = $P < 0.001$; ns = not significant).

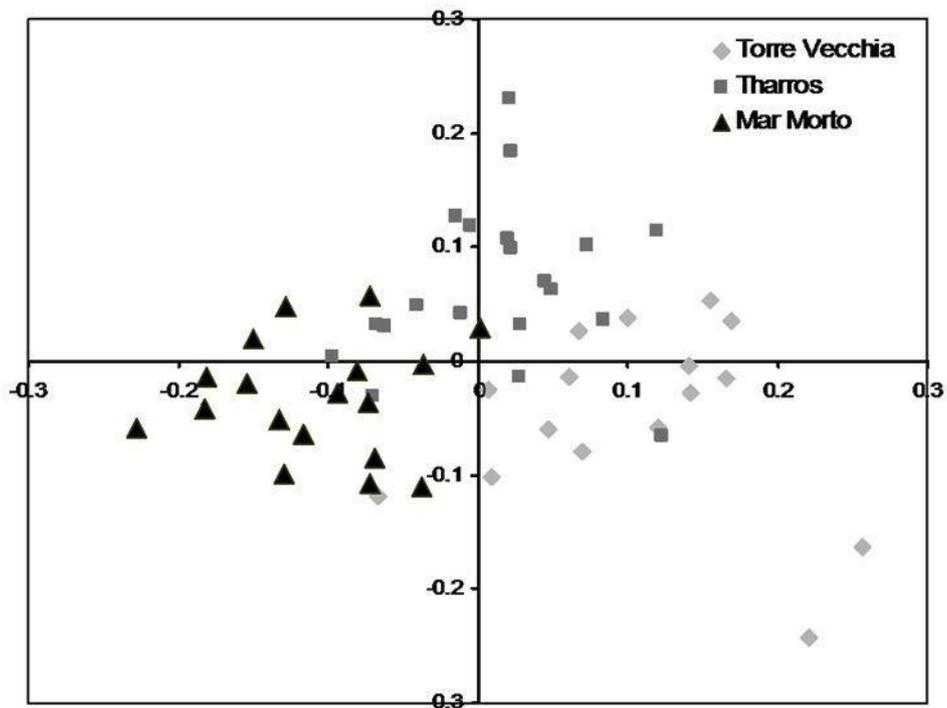


Fig. 11. Scatter plot of the canonical axes for the CAP discriminant analysis

**CHAPTER 2. – THE EFFECT OF HYDRODYNAMICS ON SHELL ORIENTATION AND
POPULATION DENSITY OF *PINNA NOBILIS* IN THE GULF OF ORISTANO
(SARDINIA, ITALY)**

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ABSTRACT

Pinna nobilis is the largest endemic bivalve of the Mediterranean Sea, declared protected since 1992. Although hydrodynamic stress induced by waves is known to influence density, size and orientation of *P. nobilis*, the effect of other hydrological features is unknown. This paper considers a *P. nobilis* population living within a *Posidonia oceanica* meadow in the Gulf of Oristano (Sardinia, Italy). We hypothesize that spatial differences in density and orientation of *P. nobilis* may be related to significant wave height (H_s), wave direction (D_w), bottom current direction (D_{BC}) and speed (S_{BC}). A population of *P. nobilis* was investigated at different sites and its distribution was correlated to hydrodynamics by means of a numerical modelling approach. The spatial distribution was patchy with mean densities ranging from 0.06 to 6.7 ind. 100 m^{-2} . A non uniform distribution of shell orientations (O_s) was demonstrated in 4 sites out of 6. D_{BC} and S_{BC} were the main factors affecting O_s , while waves had little influence. A S_{BC} of 0.07 m s^{-1} appears to be the threshold for inducing specimen directionality with shells aligned to the current and the ventral side exposed to the flow, suggesting that feeding strategy is a key factor in determining O_s in addition to drag minimization. The role of adjacent lagoons in supporting high densities due to the increase of food availability was also highlighted. These findings demonstrate the usefulness of modeling techniques in explaining spatial distribution pattern of *P. nobilis* and contribute to increase the knowledge of its ecological needs.

Key words: *Pinna nobilis*, shell orientation, hydrodynamics, finite elements, western Mediterranean.

INTRODUCTION

The fan mussel *Pinna nobilis* (Linnaeus, 1758) is a Mediterranean endemic bivalve which can exceed 1 m in length (García-March *et al.*, 2007a). Since 1992, *P. nobilis* is under strict protection as an endangered species (92/43/EEC: Habitats Directive, Annex IV), in steady decline due to anthropogenic pressure. *P. nobilis* principally occurs in subtidal soft sediment covered by seagrass meadows between 0.5 and 60 m depth (Butler *et al.*, 1993; Hendriks *et al.*, 2011). *P. nobilis* lives partially buried in the substrate with up to one-third of the shell and anchoring by byssus threads (Zavodnik 1967; Richardson *et al.*, 1999). *P. nobilis* populations usually show aggregated distributions and high variable densities according to habitat availability (Richardson *et al.*, 2004; Katsanevakis 2005), ranging between 0.001 (Centoducati *et al.*, 2007) and 600 ind. 100 m⁻² (De Gaulejac & Vicente 1990; Catsiki & Catsikieri 1992).

It has long been demonstrated that hydrodynamics affect all aspects of the life history of benthic species, such as the rate of fertilization of gametes, dispersion, settlement, growth and mortality (e.g. Carrington Bell & Denny 1994 and literature therein; Loo *et al.*, 1996; Abelson & Denny 1997; Genovese & Witman 1999; Orlov 2001; Powers 2009; McQuaid & Mostert 2010). Several authors highlighted the importance of water flow and food supply in affecting the spatial distribution of sessile suspension feeders (Guichard & Bouget 1998; Manriquez & Castilla 2007; Arkema 2009; Eléaume *et al.*, 2011; Tagliapietra *et al.*, 2012). However, little is known about the influence of hydrological features on *P. nobilis* density and orientation. Indeed, few studies were conducted on this topic, mainly focusing on the stress induced by waves (i.e. García-March *et al.*, 2007b; Hendriks *et al.*, 2011). García-March *et al.*, (2007a,b) demonstrated that waves action can determine depth-related size segregation with a lack of large individuals at shallow sites, decreasing mortality rate for large specimens with depth and common shell orientations. Therefore, in shallow exposed shore, the association between *P. nobilis* and *Posidonia oceanica* (L. Delile, 1813) meadow is fundamental for water speed attenuation due to *P. oceanica* leaves. García-March *et al.*,

(2007b) found that, for *P. nobilis* individuals living in *P. oceanica* meadow, the minimum and the dislodgment drag force corresponded to 9 and 45 N respectively; these values are in agreement with the experimental data of Hendriks *et al.* (2011). However, no information is available on the potential role of other hydrological features, in defining the spatial distribution of individuals. Yet, bottom current flow is considered one of the main food source for sessile species (Eléaume *et al.*, 2011 and literature therein). *P. nobilis* is a siphon-lacking suspension feeder (Fishelson 2000): the water flow enters from the ventral side of the shell along the lower pallial cavity following the profile of the gills, and comes out on the dorsal side. The analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes of *P. nobilis* individuals living within a seagrass meadow showed that detached seagrass epiphytes are the most important food source, followed by particulate organic matter, sediment organic matter and seagrass fragments (Cabanellas-Reboredo *et al.*, 2009). Subsequent stomach contents analysis also highlighted the importance of detritus fraction in the diet of *P. nobilis*, particularly for small specimens (Davenport *et al.*, 2011).

Here, we assessed the distribution pattern of *P. nobilis* population at different sites in the northern sector of the Gulf of Oristano characterized by different hydrodynamics and distance from the adjacent lagoons. We aimed to test the relationship between shell orientation (O_S) and local hydrological features – significant wave height (H_S), wave direction (D_W), bottom current direction (D_{BC}) and speed (S_{BC}) – investigated using a numerical modeling technique. The potential role of the local currents and the outflow of lagoons water on *P. nobilis* density were also considered.

MATERIALS AND METHODS

Study area

The Gulf of Oristano is a semi-enclosed basin of about 150 km² on the western coast of Sardinia (Italy) (Fig. 1). It is a shallow water basin (max depth of 15 m) connected to the Sardinian Sea through an opening 9 km long between two rocky capes. The surrounding shoreline of the Gulf is mostly sandy and characterized by the presence of several lagoons systems, including the Cabras and Mistras lagoon in the northern sector. The Gulf is characterized by a soft bottom, 70% of which is colonized by *Posidonia oceanica* meadows (De Falco *et al.*, 2008).

The hydrodynamic regime in the Gulf is mainly subjected to winds action, while baroclinic forcings have no influence on the water circulation (Cucco *et al.*, 2006). The dominant wind in the western Sardinia is Mistral, which blows from the northwest sector, between 300 and 315 degrees, and can be considered the main meteorological forcing (Cucco *et al.*, 2006).

The study area, partially included within the Marine Protected Area of the “Penisola del Sinis - Isola di Mal di Ventre”, is located in an extensive *P. oceanica* meadow in the north-western sector of the Gulf. It is about 6 Km in length from the coast of Torre Vecchia (*Tv*; 39°51.960’N, 008° 26.412’E) to Sa Mardini (*Sm*; 39°53.698’N; 008° 29.113’E) (Fig. 1). The seabed of the study area, mainly composed by biogenic sediment, is relatively homogeneous (De Falco *et al.*, 2008). The mean coverage of the meadow is about 80% with a slight increase of fragmentation, due to the presence of *intermatte* channels and sandy patches, from *Sm* to *Tv* (see also Coppa *et al.*, 2010).

Numerical models

Water circulation and wave propagation inside the Gulf of Oristano were investigated by means of numerical modeling techniques. A hydrodynamic numerical model (SHYFEM3D-WWM) based on the finite element method was applied to the area including

the Gulf and the adjacent coastal sea. The model adopted has been used previously to investigate the hydrodynamics of the Gulf of Oristano and lagoon system (Ferrarin & Umgiesser 2005; Cucco *et al.*, 2006) and in other shallow water coastal areas (Cucco & Umgiesser 2006; Bajo *et al.*, 2007; Bellafiore *et al.*, 2008).

The numerical tool consists of: a hydrodynamic module, the SHYFEM3D, (Umgiesser *et al.*, 2004; Cucco *et al.*, 2009) which resolves the shallow water equations in their formulations with water levels and transports and, a phase averaging wind wave model (WWM; Hsu *et al.*, 2005a,b; Ou *et al.*, 2006; Zanke *et al.*, 2006) which solves the Wave Action Equations. Both modules use finite elements for horizontal spatial integration and a semi-implicit algorithm for integration in time. A full description of the equations systems and of the numerical techniques adopted is found in Umgiesser *et al.* (2004) and Roland *et al.* (2009).

The coupling of wave and current modules was accomplished through the wave induced surface stresses, computed using the radiation stress theory of Longuet-Higgins and Stewart (1964), as formulated by Xia *et al.* (2004) accounting for 3D wave-current interaction. This numerical tool is able to reproduce the changes in time and space of the main wind wave parameters (i.e. the significant wave height, the mean wave direction and the mean wave period) and of the 3D water circulation.

SHYFEM3D-WWM uses finite element unstructured mesh for representing the model domain. The investigated area was reproduced by means of a finite element grid composed by X nodes and Y triangular elements with a spatial resolution varying between 500 m for open sea areas to 20 m for the area of interest inside the Gulf. The vertical direction was reproduced by 32 zeta layers, with layers depth varying logarithmically between 2 to 50 meters.

The simulation set-up

The hypotheses to be tested with the model application were to verify if the orientations of *Pinna nobilis* individuals and their spatial distribution within the 6 sampling

sites were depending on the local hydrological features. With this aim, a 3 years simulation run was performed, forcing the numerical model with wind, atmospheric pressure and astronomic tides. Baroclinic forcings were not taken into account, because the wind and the tide are the main factors influencing water circulation in such coastal area. The wind and tidal data used as surface and open boundary conditions were provided by meteorological numerical model (SKIRON, Kallos *et al.*, 1997, 2005) and a global tidal model for the years 2007–2009.

From the simulation results and for each sampling site, H_S , D_W , D_{BC} and S_{BC} were computed with a 3-hour temporal frequency. For each time series, the mean D_W , the mean D_{BC} and the frequency distribution (0° = North; 90° = East; 180° = South; 270° = West) were computed using a circular statistic program (*Oriana 4*).

A further analysis was carried out in order to define, within the investigated sea domain, the area of influence of the exiting lagoons waters nearby the study area (Mistras lagoon and Cabras lagoon, Fig. 1). This was achieved by simulating the fate of a passive tracer released at the lagoon inlets within the 3-year hydrodynamic run. An advection diffusion numerical module included into the WWM-SHYFEM3D was used to reproduce the tracer transport. Results were compared with the field data on the spatial variability of the population density of *P. nobilis*.

Fieldwork and data analysis

Field surveys were performed by means of SCUBA diving. Six sites were considered in summer 2009: Torrecchia (*Tv*), Tharros (*Th*); Mar Morto (*Mm*) which refer to Area A and Mistras shallow (*Mis*), Mistras deep (*Mid*), Sa Mardini (*Sm*) representing Area B (Fig. 1). Sites *Tv*, *Th*, *Mm* of Area A were also monitored in summer 2007.

Strip transects (400 x 4 m) perpendicular to the coastline were adopted for a total of 24 replicates (4 transects per 6 sites) and 18 replicates (6 transects per 3 sites) in 2009 and 2007, respectively. All transects started from the 2 m bathymetric line with the exception of the deeper site (*Mid*) in which the surveys were performed at a depth of 7–10 m. For each

individual, minimum shell width and unburied length were measured using a multi-calliper (García-March *et al.*, 2002); total shell height was estimated according to the equation defined by García-March and Ferrer Ferrer (1995) considered suitable for populations of different areas (Šiletić & Peharda, 2003). Shell orientation was determined using an underwater compass and considering the magnitude of the angle formed by the magnetic north and the dorsal side of the shell (hence, 0° indicate that the dorsal side is oriented to North, 90° to East, 180° to South and 270° to West). This measure, correlated to the hydrological features, allow us to consider if individuals were disposed in order to minimize the drag force only, to maximize feeding or both. Finally, depth, geographical coordinates and the substrate type (*Posidonia oceanica*, dead *matte*, sand or *P. oceanica* edge) were recorded for each individual.

Two way ANOVA was used to detect any difference according to the interaction of time per site and one way ANOVA was applied to investigate the variability in population density among sites and depths in 2009. Times, sites and depths were fixed factors with transects as replicates. Cochran's test was used to test for the homogeneity of variances and post hoc comparisons, by mean of the Student-Newman-Keuls (SNK) test, were carried out in case of significant differences in the ANOVA. The size/frequency distributions were compared between depths by means of Kolmogorov-Smirnov test. Statistical tests on data of O_S were performed excluding individuals located in the meadow channels since the O_S could be affected by specific water circulation due to channel occurrence (Folkard 2005; Kendrick *et al.*, 2005). The data of O_S did not show significant differences between years (Mardia-Watson-Wheeler test, $p < 0.05$ for all considered sites) therefore, since *Pinna nobilis* specimens do not change their orientation throughout development (García-March *et al.*, 2007a), the samples were pooled. Circular histograms were plotted for each site and mean O_S and concentration parameter (k) as value of angles dispersion were calculated (Batschelet, 1981). In order to evaluate if the O_S pattern of the sites was significantly different from a uniform distribution, a Watson's U^2 test was applied. Data of O_S were compared with the D_{BC} and D_W for each sampling sites using a circular-circular correlation (Batschelet, 1981). This analysis

was performed in order to test whether the observed values of O_S have a tendency to cluster around the mean D_W and D_{BC} . In case of significant correlation, the relationship between the values of water speed and O_S concentration parameter was tested using a linear regression.

RESULTS

Waves

In table 1 maximum and mean values of H_s are reported for the 6 time series extracted for areas corresponding to the *Pinna nobilis* sampling sites. Both maximum and mean values of H_s , reveal that the areas of interest are sheltered from the wind wave action. In fact, the main wave regime is induced by the Mistral wind, a northwesterly wind which generates waves that do not propagate inside the northern part of the Gulf, because this area is protected by the Cape of San Marco (see Fig. 1). As an example, during an intense Mistral wind storm able to produce off-shore wave fields with H_s greater than 3 meters, the waves propagating behind the Cape of San Marco toward the northern Gulf coastline, rarely exceed the 0.5 m in height (Fig. 2). The induced orbital velocities at the bottom for the interested areas are always lower than 0.6 m s^{-1} . Based on García-March *et al.*, (2007b) this current gives rise to a drag force of about 5 N depending on shell size and orientation.

Bottom currents

In figure 3, the six circular histograms describing the distribution of the D_{BC} are reported. D_{BC} shows a non uniform distribution (Watson's U^2 test), suggesting a preferred D_{BC} in all cases (Table 1).

As for the analysis of the waves field, we considered the Mistral wind as the main physical forcing influencing the distribution of D_{BC} and S_{BC} inside the Gulf. In figure 4, the simulated bottom current field generated by a typical Mistral wind event, lasted 4 days with an average wind speed of 12 m s^{-1} , is reported for the area A and B. This can be considered as the main circulation pattern in this area and therefore can be taken as the reference scenario for discussing the results obtained from the circular statistical analysis.

In *Tv*, *Th* and *Mm*, the mean D_{BC} (\pm circular SD) are $241^\circ \pm 52^\circ$, $34^\circ \pm 91^\circ$, $313^\circ \pm 110^\circ$ with average speed values of 0.15, 0.09 and 0.04 m s^{-1} (Table 1). In such area (Fig. 4), the D_{BC} shapes as a small anti-cyclonic gyre with currents speeds that are higher, up to 0.2 m s^{-1} ,

along the San Marco peninsula and reduce their intensity to $<0.1 \text{ m s}^{-1}$, off-shore. The *Tv* sampling site is located in correspondence of the branch of the eddy which carries the coastal waters toward the open sea out of the Gulf. The direction of this coastal flow varies between south-east to southeast-east depending on the intensity of the wind event, similar to the mean D_{BC} obtained by the statistical analysis for the *Tv* sampling site. The *Th* sampling site is extending over the area where a northwestward off-shore flow bends to generate the southeasterly coastal flow. Most parts of the transects are located in correspondence of the external northwestward flow. The mean D_{BC} of *Th* is in line with this current field pattern. The *Mm* sampling site is located in the nearby of an area where the flow field diverges. The position of such divergence zone is varying with the time depending on the strength of the wind event. Therefore, the *Mm* site can be found in correspondence either of a coastal southwestward or of a coastal northwestward flow as confirmed by the biaxial distribution of the corresponding D_{BC} (Fig. 3).

The mean D_{BC} (\pm circular SD) calculated for sampling site *Mis*, *Mid* and *Sm* was $22^\circ \pm 87^\circ$, $188^\circ \pm 95^\circ$, $148^\circ \pm 65^\circ$ with average S_{BC} of 0.08, 0.06 and 0.16 m s^{-1} (Table 1). The typical bottom current pattern in this area (Fig. 4) is characterized by the presence of a main northeastward stream with low values of current speed (S_{BC} around 0.08 m s^{-1}) in the outer area and by a more intense southeastward stream (S_{BC} up to 0.2 m s^{-1}), along the coastline. The *Sm* sampling site is located in the nearby of the inlets where the lagoons waters outflow occurs and its mean D_{BC} (188°) is compatible with the flow pattern described in figure 4. The *Mis* sampling site is located in correspondence of the area where the flow switch from north-northwest to southeast, with most of the transects affected by the main north-northwestward stream, as confirmed by the mean D_{BC} (22°). Finally, the *Mid* sampling station is extending over the edge between the mean outer stream and the bottom current pattern generated by the lagoon water outflow. In this site, the D_{BC} is quite variable as showed by the related circular histogram (Fig. 3).

For all six sampling sites, the computed average directions were in agreement with the bottom current patterns generated by the action of the Mistral wind, confirming that the long-term variability of the water circulation in the Gulf is dominated by this forcing.

Tracer analysis

In figure 5 the results obtained by the simulation of the advection and diffusion of the lagoons waters tracer are reported. The tracer concentration computed at each numerical time-step and for each element of the numerical grid had been averaged for the duration of the entire run. All the sampling sites located in area A are not affected by the influence of the Mistras and Cabras lagoon exiting waters. On the other hand, for sampling sites *Mis* and *Sm*, located in area B the average tracer concentration varies between values greater than 30% to values around 7%, indicating a potential influence of the lagoon waters on this area. For the sampling station *Mid*, the concentration values are <0.5%, similar to values found for stations located in area A, denoting therefore a negligible influence of the lagoon waters.

***Pinna nobilis* population and hydrodynamics**

During our surveys a total of 1285 live *Pinna nobilis* was recorded over a surface area of 67200 m². The majority of specimens was found within the meadow (52%), 2% on sand, 10% on dead *matte* and 36% on the *P. oceanica* edge of which 178 individuals were located on the *intermatte* channels border.

Data on O_S, displayed by circular histograms, showed different distribution patterns in relation to sites (Fig. 6; Table 1). Watson's U² test revealed a non uniform distribution for all sites with the exception of *Mm* and *Mid* (Table 1). In *Tv*, *P. nobilis* individuals were oriented towards southwest with a mean O_S (\pm circular SD) of 239° \pm 106° (k=0.37) while the northeastern and southeastern directions prevailed in *Th* (mean O_S=13° \pm 105°; k=0.38) and *Sm* (mean O_S=132° \pm 98°; k=0.48) respectively. Despite a higher dispersion value (k=0.23), *Mis* was characterized by specimens mainly oriented towards north with a mean O_S of 6° \pm 119°.

The sites that showed a non uniform O_S distribution were compared to D_W and D_{BC} in order to investigate possible correlations. Circular-circular correlations were performed calculating the correspondent jackknifed probabilities (Quenouille, 1956; Batschelet, 1981). Shell orientation resulted completely independent from D_W ($r = 0.117$; $p > 0.05$) confirming that the values of H_S (Tab.1) and the induced water speeds (max 0.6 m s^{-1}) obtained by means of the SHYFEM3D-WWM numerical model, were too low to determine any directionality. Thus, in the Gulf of Oristano, wave action can be excluded as a factor affecting *P. nobilis* O_S . Conversely, O_S was significantly linked to D_{BC} ($r=0.985$; $p<0.05$). Concentration parameter of O_S and S_{BC} had a significant positive linear correlation ($p<0.05$) showing the importance both of D_{BC} and S_{BC} in determining a preferred O_S (Fig. 7).

Density did not vary significantly between years (Time X Site: $F=1.79$, $p=0.19$), ranging between 0.06 and 6.69 ind. 100 m^{-2} . The analysis of variance highlighted significant differences in population density according to site ($F=44.29$, $p<0.0001$). Furthermore, the SNK test found two significant peaks of population density ($p<0.01$): the first correspond to the *Th* site and the second, significant more pronounced ($p<0.01$), in the *Mis-Sm* zone. The minimum was represented by *Tv* and *Mm* sites with no significant difference in density values between them (Fig. 8). The analysis of variance, comparing different depths in the same area, found a significantly higher density in the shallow site ($F=60.22$, $p<0.001$), conversely the frequency distribution of the size classes resulted homogeneous (Kolmogorov-Smirnov test; $D=0.104$, $p>0.05$).

Comparing population density of *Pinna nobilis* with the outflow dispersion of lagoons water obtained by the analysis of tracer concentration it is possible to observe that the highest density value was recorded in those sites closer to the mouths of the lagoons and that the abundance value progressively diminishing away from the lagoons to the open sea. The only exception that does not seem respect this gradient is *Th* where high density values occurred. Nevertheless, this site is interested by peculiar hydrodynamics. In fact, the typical water circulation pattern in such area is characterized by the presence of an anticyclonic gyre. In figure 9, the vertical averaged current velocity field generated by the Mistral wind is reported.

Considering the barotropic flow field, the *Th* sampling site is located in correspondence to the centre of the gyre. In such area, the current speed is low and the water residence times are high (Cucco *et al.*, 2006). This generally gives rise to sedimentation phenomena which held a greater amount of organic matter for benthic organisms.

DISCUSSION

For sessile marine species, hydrodynamics can affect respiration, nutrient transport and waste removal, but also can be detrimental e.g. by sweeping specimens away diminishing their survival likelihood. The shape and the position of an individual, affecting the interaction between its body and fluid, can have a relevant impact on the survival chances in a given habitat (Nakamura, 1994; Gaylord *et al.*, 2001; Denny *et al.*, 2003; Denny, 2006; García-March *et al.*, 2007a,b).

This study suggests that, in the Gulf of Oristano, waves action is not a significant factor influencing orientation of *Pinna nobilis* due to its low energetic features. Work on the effect of wave action on *P. nobilis* considers a range of drag forces between 9.2 N (mean minimum drag force) and ≈ 45 N (maximum dislodgment force) (García-March *et al.*, 2007b). The wave drag force estimated for our areas are much lower than this range (5 N), and therefore wave action is unlikely to have an effect on shell orientation. This is confirmed by the lack of a significant relationship between D_W and O_S . Moreover, a further indication of the low influence of the wave energy is the lack of size segregation between deep and shallow sites of Mistras. Indeed, in water swept environments, large individuals prevail in deep sites as a consequence of a lower water flow energy than in the shallow ones (García-March *et al.*, 2007a,b).

Nevertheless, a non-random distribution of O_S was demonstrated in four sites out of six, i.e. their mean O_S was significantly related to the mean D_{BC} . Excluding the wave action, drag minimization in relation to current and feeding strategy may be the factors affecting O_S . In this study, not only do specimens result in line with the D_{BC} but also the ventral side of their shells is preferentially oriented to the incoming flow. This suggests that feeding is an important factor in determining O_S , possibly in addition to drag. If drag was the only factor in determining O_S , orientation of the ventral and dorsal side of the shell towards the flow would have been equally likely. It has long been known that suspension feeders are strongly dependent on current flow and that their orientation tends to significantly match current

direction as demonstrated for barnacles (Vogel, 1983) deep sea foraminifera (Linke & Lutze, 1993), crinoids (Eléaume *et al.*, 2011) or sand dollars (Francisco & Herzka, 2010). A similar shell orientation pattern, aimed at optimizing food uptake, was also observed in mussel which oriented their inhalant apertures towards the incoming flow (Pascoe *et al.*, 2009). The mechanism and relevant life stages that allow shell orientation towards the flow need further investigation. Although individuals bigger than 10 cm are known to maintain their orientation during growth, some degree of movement and reorientation could be possible during a short period after settlement (García-March *et al.*, 2007a).

The advantage of a particular orientation in either maintaining stability or capturing particles depends on the consistency of the flow direction (Nakamura, 1994). Indeed, filtration rates can depend quite strongly on the rate of ambient flow, resulting one of the main factors in determining specimens growth (Vogel, 1983). There is a wealth of literature concerning the effects of flow speed on bivalve feeding (mostly for mussels, scallops and oysters), however only a few studies have been conducted in the field (e.g. Dolmer, 2000; Sarà & Mazzola, 2004). Generally filtration rates at low current speeds (e.g. 0.01–0.05 m s⁻¹ for mussels) are indirectly flow-limited due to the effects of reductions in ambient particle concentrations (Dolmer, 2000). On one hand, increasing current speeds (e.g. >0.06 m s⁻¹ for mussels) were shown to compensate the effects of particle depletion, resulting in density-dependent growth in bivalve stocks by replacing the food-depleted water with a new source of available seston (Dolmer, 2000; Sarà & Mazzola, 2004). On the other hand, at higher velocities (e.g. >0.08 m s⁻¹ for mussels) filtration rates (Widdows *et al.*, 2002), with siphons facing into the flow, are directly inhibited by water speed effects on the system of the bivalve pump due to pressure differentials between inhalant and exhalant openings (Wildish & Saulnier, 1993; Newell *et al.*, 2001). No information is available on upper and lower limits of tolerance to current velocities of *P. nobilis*. Our findings, showing a strong bottom current directionality for all considered sites, suggests that *P. nobilis* shows a non random orientation only where average water speed is beyond 0.07 m s⁻¹. The significant correlation between S_{BC}

and concentration value of O_S confirms the fundamental role of this variable in inducing preferred O_S .

Previous studies demonstrate that mussels filter water only when they are supplied with sufficient amounts of food (Dolmer, 2000; Newell *et al.*, 2001). *Mytilus edulis* (Linnaeus, 1758) responds to increasing ambient food concentrations by increasing their pumping rates with a minimum threshold of seston to activate filtering activity of about 4×10^3 particles ml^{-1} (Newell *et al.*, 2001). Thus, growth of bivalve populations is not a simple function of ambient food concentration or water flow velocity but rather a function of the combined effects of these two factors (Muschenheim & Newell, 1992; Sarà & Mazzola, 2004; Tagliapietra *et al.*, 2012). Although this study does not provide any quantification of the potential amount of available food for the local population of *P. nobilis*, some inferences can be reported to explain its patchiness distribution. Lagoon waters are generally richer in nutrients, phytoplankton and organic matter than coastal waters (Magni *et al.*, 2008; Padedda *et al.*, 2010). They can be an important source of food for the benthic species in the Gulf of Oristano via particle transport (Como *et al.*, 2007), possibly influencing their distribution in relation to the distance from the inlets (Magni *et al.*, 2008). The results obtained from the analysis of tracer dispersion show that the only sites potentially affected by the positively nutrimental output from Mistras and Cabras lagoons are *Mis* and *Sm* where the highest density value of *P. nobilis* occurred. Moreover, the sedimentation zone shown by the numerical model in correspondence to the site of *Th* is likely to be characterized by the greatest amount of available suspended matter within the Area A, explaining the second peak of population density. Thus we suggest that both food availability and bottom current features (i.e. D_{BC} and S_{BC}) contribute to explain the spatial density pattern of *P. nobilis* in the Gulf of Oristano. These factors could be responsible of the patchiness distribution of *P. nobilis* population in the study area with higher density values in those sites close to the mouth of the lagoons, in sedimentation area due to gyre formation (i.e. eddy located in Tharros) or in the *intermatte* channels where the water flow is conveyed. These results are in agreement with the previous hypothesis of a high efficiency in the filtering activity on the meadow borders explaining the

specimens aggregation on the edges (Coppa *et al.*, 2010; Coppa *et al.*, 2011) and contribute to increase the knowledge on the *P. nobilis*-*P. oceanica* association mediated by hydrodynamics. However, to support our model results, field data on spatial and temporal variation in food quantity and quality are necessary. Some understanding of interactions between neighboring individuals could also be useful to evaluate the intraspecific competition for food and to explain the feeding dynamics in high density patches.

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TABLES AND FIGURES

Table 1. *Pinna nobilis* shell orientation (O_S), wave height (H_S), wave direction (D_W), bottom current direction (D_{BC}) and speed (S_{BC}) in the six considered sites (Tv , Th , Mm , Mis , Mid , Sm). The value of concentration parameter (k) and Watson's U^2 test are reported.

		<i>Tv</i>	<i>Th</i>	<i>Mm</i>	<i>Mis</i>	<i>Mid</i>	<i>Sm</i>
<i>Pinna nobilis</i>	Mean O_S	239°	13°	256°	6°	10°	132°
	K	0.37	0.38	0.17	0.23	0.33	0.48
	U^2 test	0.225	0.455	0.061	0.308	0.157	0.919
	p-level	<0.025	<0.005	ns	<0.005	ns	<0.005
Bottom current	Mean D_{BC}	241°	34°	313°	22°	188°	148°
	K	1.8	0.58	0.32	0.66	0.53	1.23
	U^2 test	43.873	8.518	8.277	10.546	7.805	25.522
	p-level	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
	Max S_{BC} ($m\ s^{-1}$)	0.25	0.16	0.14	0.13	0.11	0.18
	Mean S_{BC} ($m\ s^{-1}$)	0.15	0.09	0.04	0.08	0.06	0.16
Waves	Mean D_W	350°	345°	358°	12°	25°	8°
	Max H_s (m)	0.4	0.5	0.4	0.6	0.7	0.9
	Mean H_s (m)	0.2	0.2	0.3	0.4	0.4	0.5

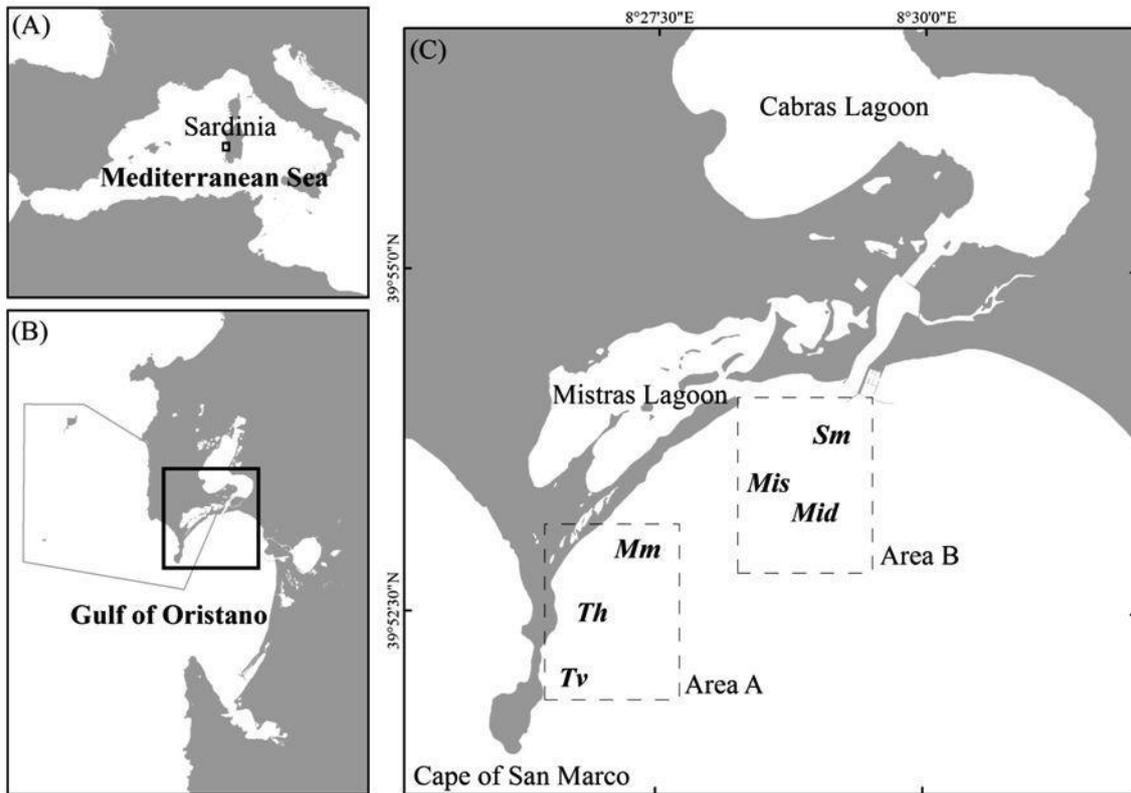


Fig. 1. Location map of the study area. (A) Western Mediterranean Sea; (B) Gulf of Oristano with the limit of the Sinis Marine Protected Area (grey perimeter) and the study area (black perimeter); (C) northern part of the Gulf with the limits of Area A and Area B (dashed grey perimeters) and the sites location (*Tv*, Torre Vecchia; *Th*, Tharros; *Mm*, Mar Morto; *Mis*, Mistras shallow; *Mid*, Mistras deep; *Sm*, Sa Mardini).

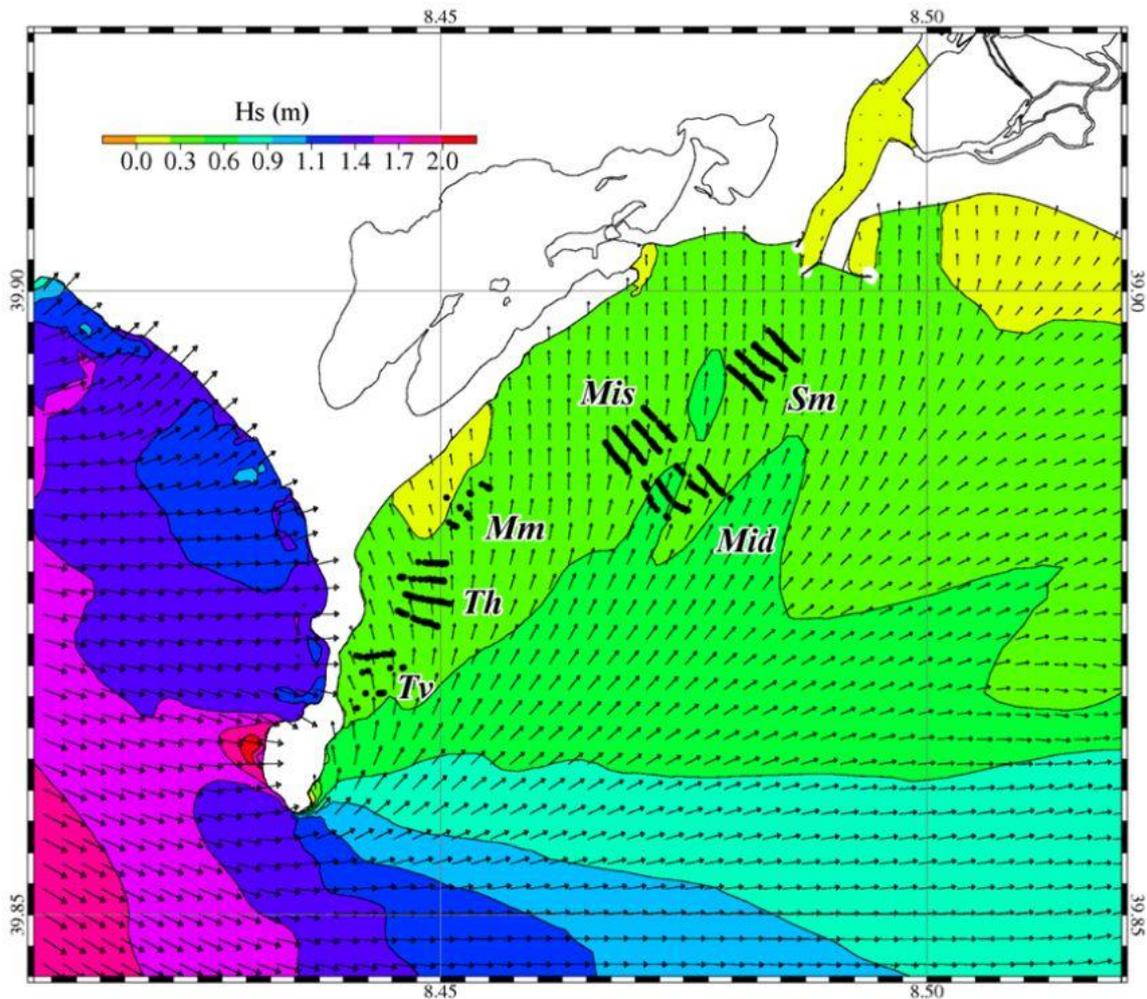


Fig. 2. Spatial distribution of wave height (H_s) and direction (D_w) during a Mistral wind storm within the interested area. The location of sampling sites are evidenced by the black dotes indicating the presence of alive *P. nobilis* individuals.

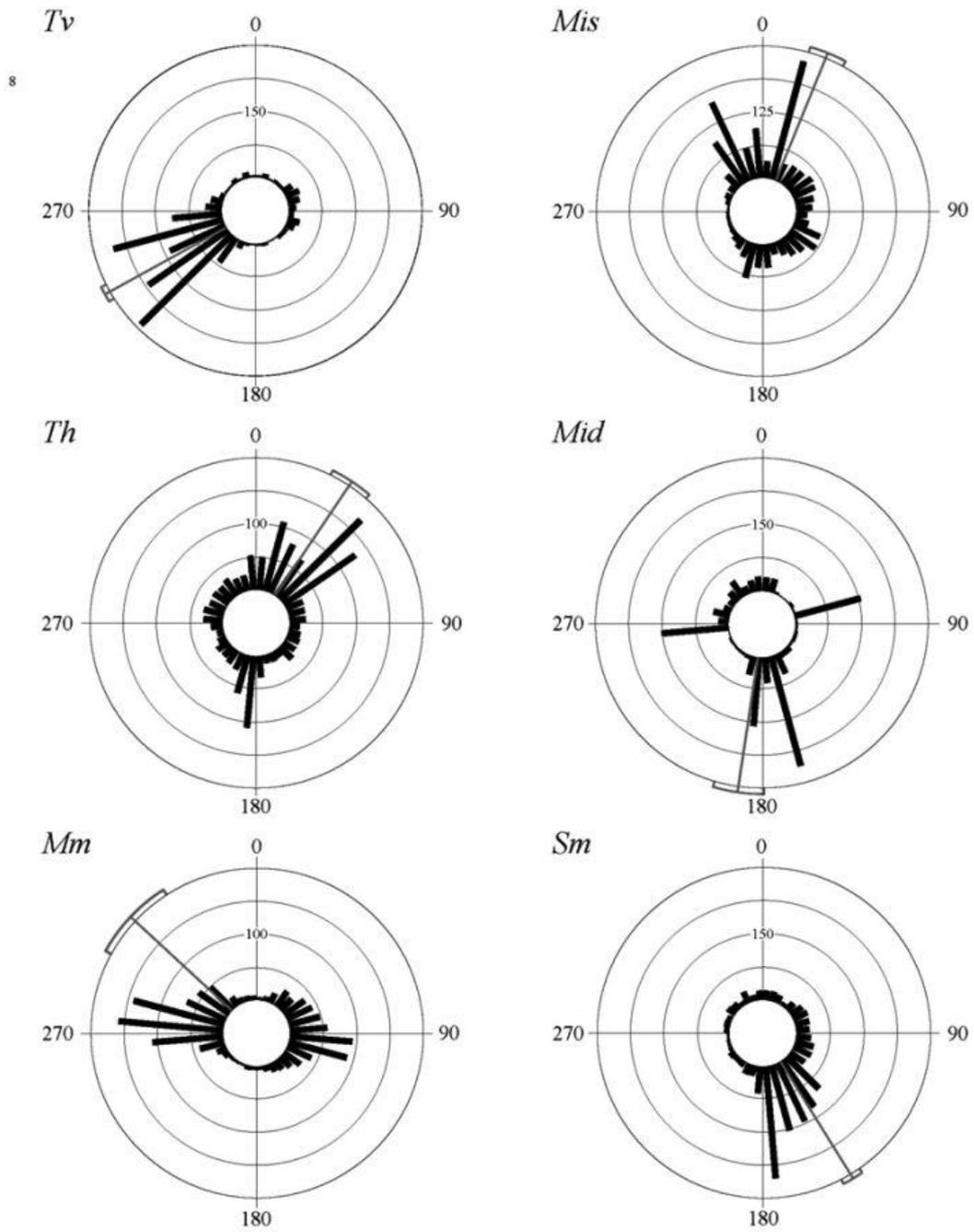


Fig. 3. Bottom current directions (D_{BC}) per sites (*Tv*, *Th*, *Mm*, *Mis*, *Sm*). Mean D_{BC} and 95% confidence intervals are reported.

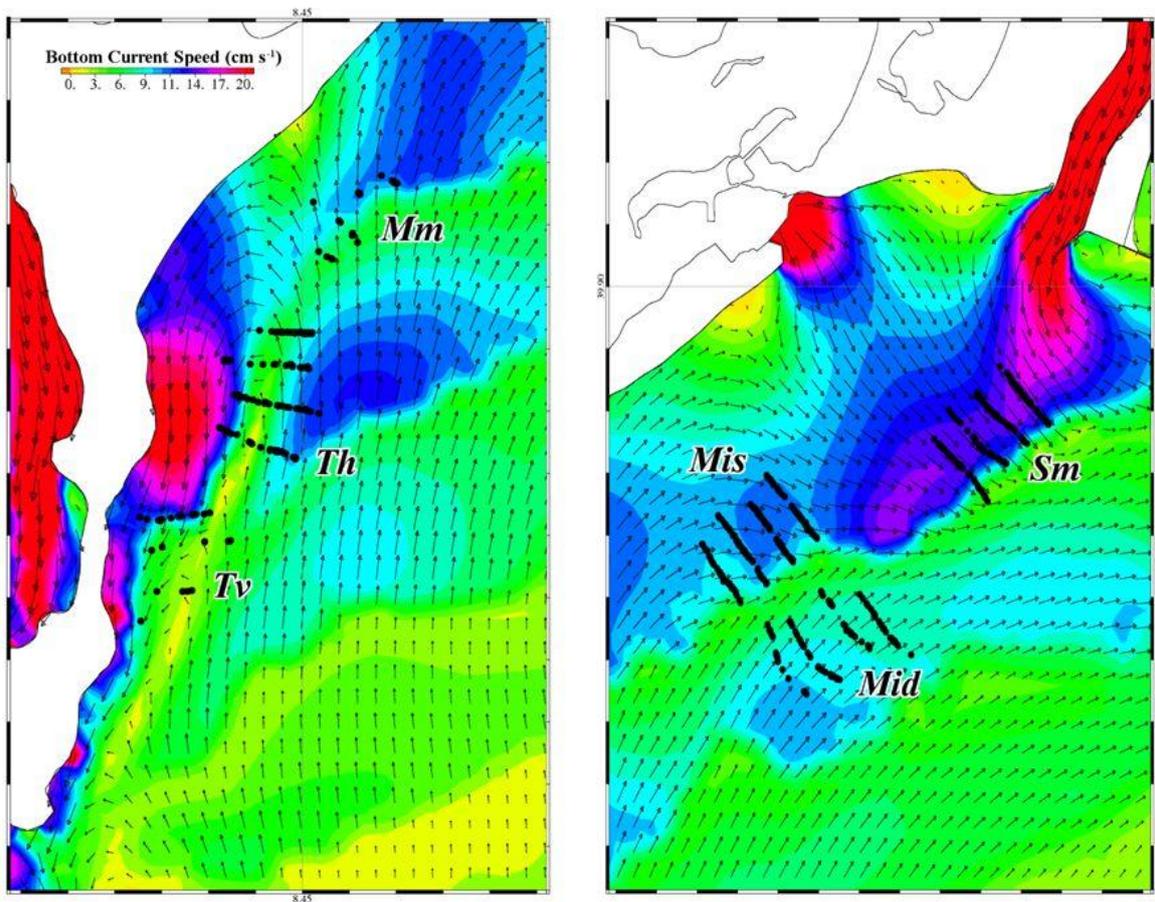


Fig. 4. Spatial distribution of the bottom currents direction (D_{BC}) and speed (S_{BC}) during a Mistral wind storm. Zoom for the Area A (left panel) and for the Area B (right panel).

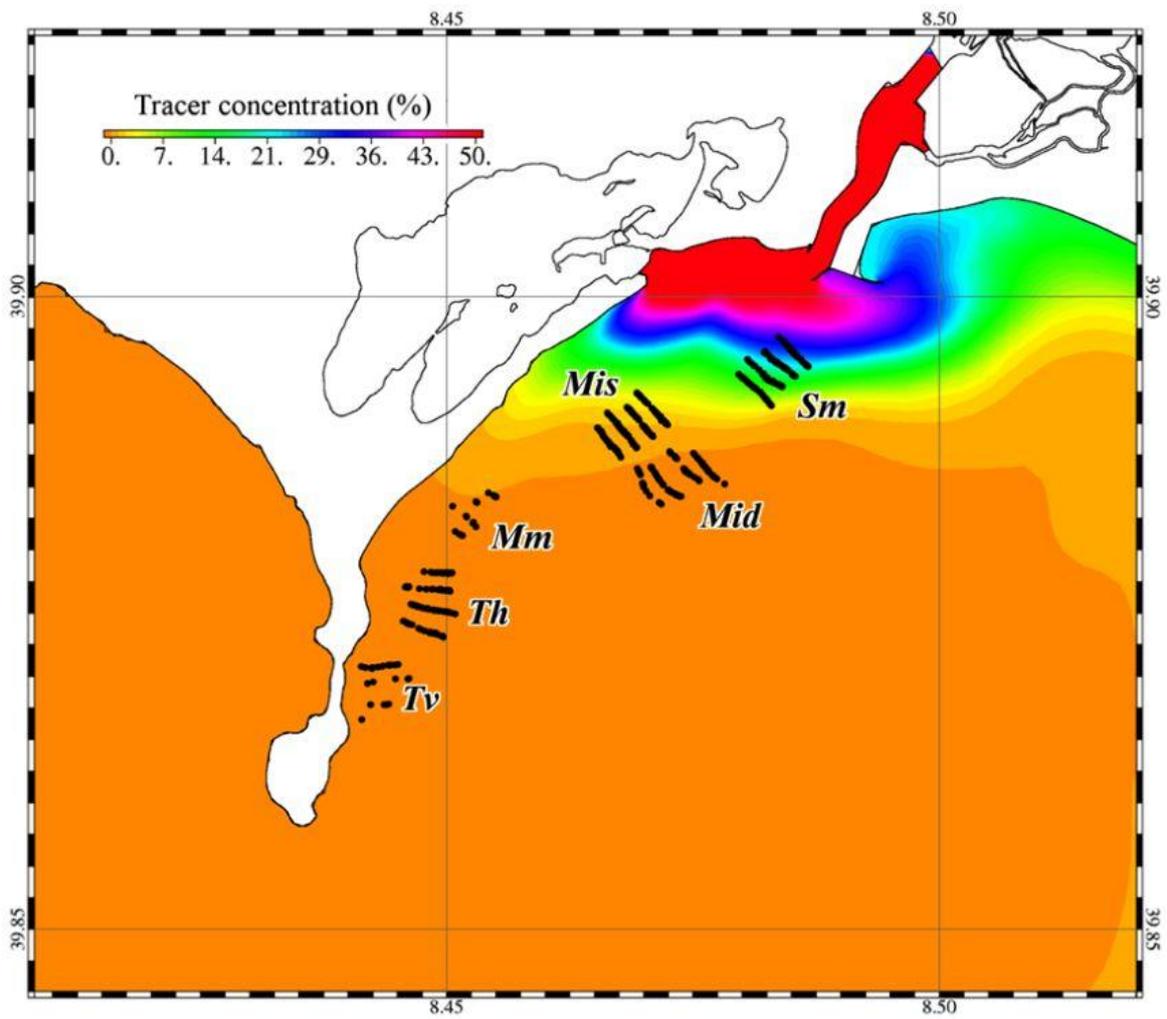


Fig. 5. Distribution of the average tracer concentration computed during a 3-year simulation.

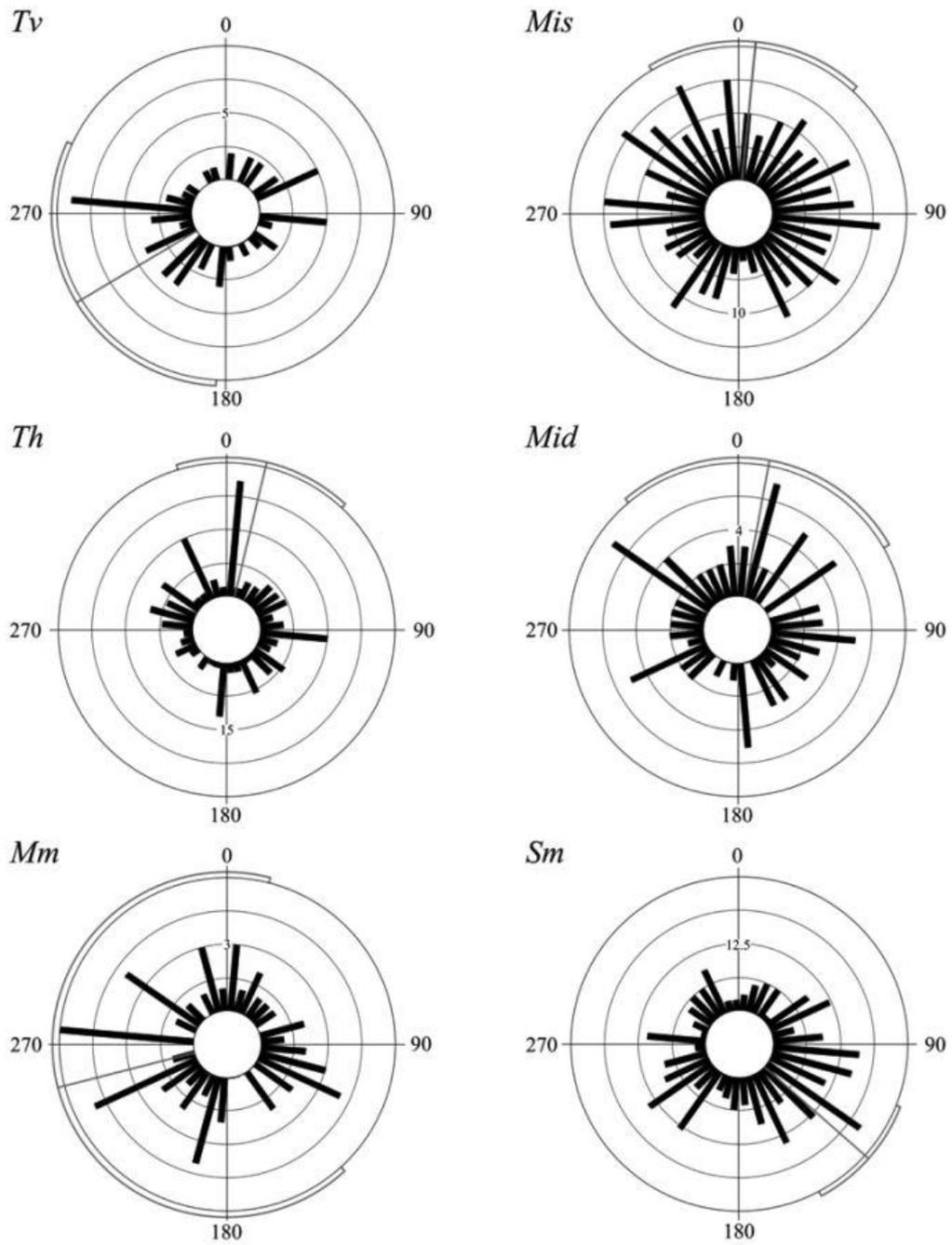


Fig. 6. Shell orientations (O_S) of *Pinna nobilis* per sites (*Tv*, *Th*, *Mm*, *Mis*, *Sm*). Mean O_S and 95% confidence intervals are reported.

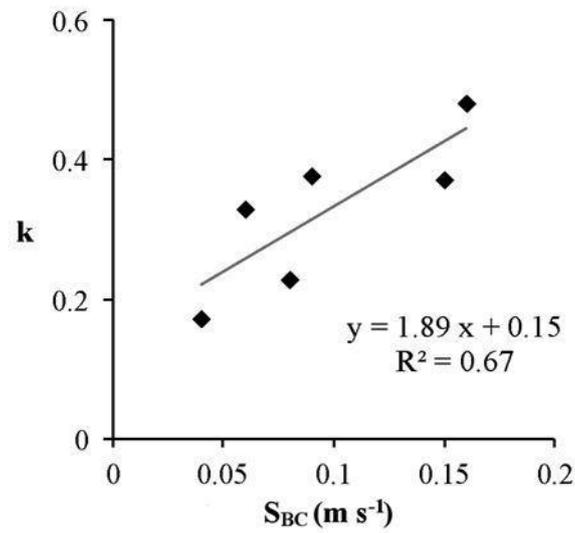


Fig. 7. Correlation between bottom current speed (S_{BC}) and concentration parameter (k) of *Pinna nobilis* orientation per site ($p < 0.05$).

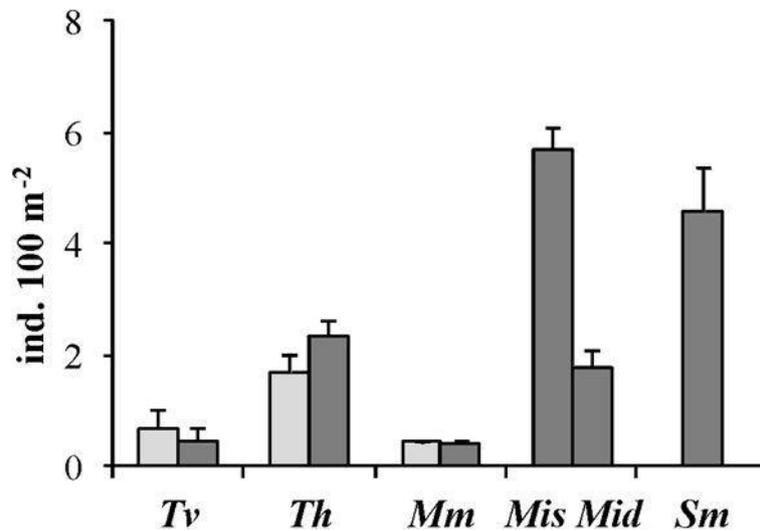


Fig. 8. Density of *Pinna nobilis* per site (*Tv*, *Th*, *Mm*, *Mis*, *Mid*, *Sm*), years (2007, light grey; 2009, dark grey) and depth (*Mis*: 2–5 m; *Mid*: 7–10 m).

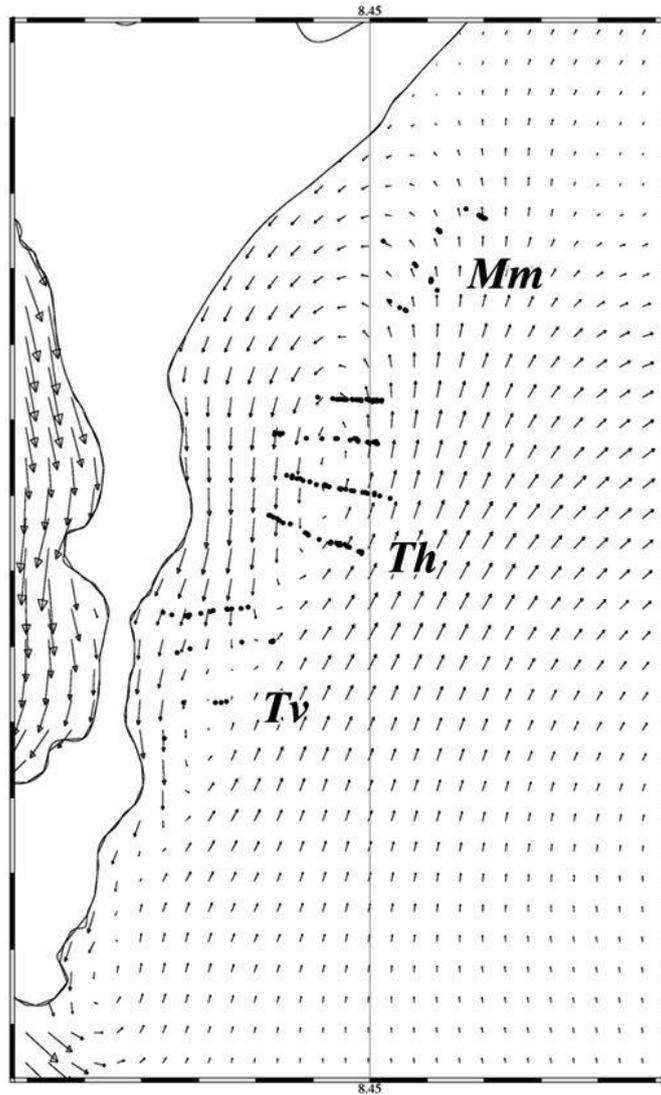


Fig. 9. Depth averaged current field computed for the Area A as generated by a Mistral wind storm.

**CHAPTER 3. - DENSITY AND DISTRIBUTION OF *PATELLA FERRUGINEA* IN A
MARINE PROTECTED AREA (WESTERN SARDINIA, ITALY): CONSTRAINT
ANALYSIS FOR POPULATION CONSERVATION**

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ABSTRACT

The endemic limpet Patella ferruginea is the most endangered invertebrate of the Mediterranean Sea. Our study examined a population of P. ferruginea in the Marine Protected Area of Penisola del Sinis-Isola di Mal di Ventre (Sardinia, Italy). During the summer 2009, we carried out a systematic census of P. ferruginea along a 8114 m georeferenced perimeter of coast in the no take - no entry zone to assess its density, spatial distribution, and morphometric characteristics. Our aim was to provide a detailed map of the distribution of P. ferruginea and to investigate the effects of accessibility, wave exposure and slope of the coast on its occurrence. Patella ferruginea showed the lowest mean density ever reported (0.02 ind/m) and a unimodal population structure characterized by fewer females and juveniles. Accessibility had a major negative effect on the occurrence of P. ferruginea. Exposure was also an important factor in influencing its density, size composition and specimen position within the mesolittoral, while the slope had little influence. Morphometric analysis showed the dominance of the Rouxi form, while the Lamarcki form was confined to exposed sites. Our results demonstrate a highly endangered population of P. ferruginea and suggest that human pressure represents the main risk factor.

Keywords: *Patella ferruginea*, Marine Protected Area, Sardinia, Human influence, Endangered species, GIS applications.

INTRODUCTION

Patella ferruginea (Gmelin, 1791) is an endemic limpet of the Mediterranean Sea, listed in the European Council Directive 92/43/EEC as the most endangered marine invertebrate on western Mediterranean rocky shores (Ramos, 1998; Espinosa, 2009). This species generally occurs in the high mesolittoral, but can also be found in the supralittoral (Paracuellos *et al.*, 2003; Guerra-García *et al.*, 2004; Casu *et al.*, 2006), often associated with the lichen *Verrucaria symbalana* Nyl., 1873 (Doneddu & Manunza, 1992), and in the low mesolittoral (Casu *et al.*, 2004). Usually *P. ferruginea* lives on rocky shores exposed to mid-high hydrodynamics with high oxygen concentrations and low pollution levels; however, high densities have been reported to occur in sheltered areas (Guerra-García *et al.*, 2004 and literature therein). According to the literature, the mean density of *P. ferruginea* populations ranges between 0.06 (Paracuellos *et al.*, 2003) and 6.86 (Espinosa *et al.*, 2009a) individuals per linear meter (ind/m), with peaks up to 15 ind/m and exceptionally >50 ind/m under optimal conditions (MMAMRM, 2008).

The variability of the shell shape has led to the subdivision of *P. ferruginea* into two morphotypes (Payraudeau, 1826): the Lamarcki form, with rare and large ribs and a flattened shell, and the Rouxi form, having more numerous and thin ribs on a conical shell. Espinosa & Ozawa (2006) demonstrated that the two forms are different ecotypes rather than different species or subspecies, with significant differences in their height (H)/length (L) ratio (H/L Lamarcki <0.37; H/L Rouxi > 0.37). The most supported hypothesis to explain these differences is related to the position of limpet on the shore and, consequently, to factors such as desiccation stress and water turbulence. Limpets located in the high mesolittoral normally require a greater water reserve and, therefore, present a higher internal volume (conic shell) than those colonizing the low mesolittoral, more exposed to hydrodynamics and with a flatter profile (Paracuellos *et al.*, 2003 and literature therein). It is also known that the space colonization on the shore is species determined and the grazing area of individuals can change

according to hydrodynamics (e.g. Hawkins *et al.*, 2000 and literature therein). However, different spatial patterns of the two forms of *P. ferruginea* have not been demonstrated yet.

The main predators of *P. ferruginea* are crabs (e.g. *Pachygrapsus marmoratus* Fabricius, 1787, *Carcinus mediterraneus* Czerniavsky, 1884, *Eriphia verrucosa* Forskal, 1775), gastropods (e.g. *Stramonita haemastoma* Linnaeus, 1758, *Lunatia poliana* Della Chiaje, 1826) and, occasionally, birds (Laborel-Deguen & Laborel, 1991a; Espinosa *et al.*, 2007a; Espinosa *et al.*, 2008a; Espinosa, 2009; Tlig-Zouari *et al.*, 2010). *Patella ferruginea* is a long-lived species; its longevity was estimated to range between 8 and 35 years depending on environmental conditions (Espinosa *et al.*, 2008b). *Patella ferruginea* has a short larval stage lasting 10 days maximum, and very slow growth and reproduction rates (Laborel-Deguen & Laborel, 1991b; Guerra-García *et al.*, 2004). It is a protandrous species (Espinosa *et al.*, 2008c), achieving sexual maturity as a male between the second and the third year at a shell length of 25-30 mm and changing to female when it exceeds 40 mm (Guerra-García *et al.*, 2004). However, Espinosa *et al.* (2006) suggest that most of the females are individuals of at least 60 mm. The maturation of the gonads begins in September and therefore the reproductive season occurs in autumn (MMAMRM, 2008). Settlement is positively influenced by chemical cues of adult conspecifics (Rivera-Ingraham *et al.*, 2011).

Originally, *P. ferruginea* was distributed through the whole western Mediterranean, but currently its presence is reduced to few coastal areas in the western basin (Cretella *et al.*, 1994; Paracuellos *et al.*, 2003; Guerra-García *et al.*, 2004; Espinosa & Ozawa, 2006; Espinosa *et al.*, 2007a). This is thought to be mainly caused by human collection and anthropogenic impact on coastal ecosystem (Paracuellos *et al.*, 2003; Espinosa *et al.*, 2006). Nowadays, populations of *P. ferruginea* can be observed along the coast of Morocco, Algeria, Tunisia, Spain, Corsica, Sardinia, Pantelleria, the Strait of Sicily and Tuscan Archipelago, although the populations of Sardinia and Corsica are in clear regression (Guerra-García *et al.*, 2004 and literature therein; Espinosa *et al.*, 2009b). Due to the contraction of *P. ferruginea* geographic range and populations, this species is now considered the most threatened with extinction in the Mediterranean Sea (Guerra-García *et al.*, 2004; Espinosa, 2009) and, hence, protected by

the European laws (Ann. II of Bern and Barcelona Conventions, Ann. IV of Habitat Directive). In spite of this, literature on the ecology and distribution of *P. ferruginea* is scarce and limited to a few areas.

In Sardinia, western Mediterranean, genetic population studies demonstrated the presence of *P. ferruginea* populations in three Marine Protected Areas (MPAs), including Tavolara - Punta Coda Cavallo, Asinara and Penisola del Sinis - Isola di Mal di Ventre (Casu *et al.*, 2004; Casu *et al.*, 2006; Lai *et al.*, 2009). Casu *et al.* (2004) and Lai *et al.* (2009) highlighted a remarkable genetic homogeneity among the Asinara samples, suggesting little genetic divergence at the small spatial scale. A comparison of the Sinis and Asinara populations confirmed a very low rate of gene flow (Casu *et al.*, 2006). Such poor connectivity between MPAs could be due to the overexploitation of the species in the surrounding unprotected areas which are easily accessible by humans (Lai *et al.*, 2009). Anthropogenic pressure affecting *P. ferruginea* populations was also demonstrated by Doneddu & Manunza (1992) in Chiscinagghiu Bay (north Sardinia), Cristo *et al.* (2007) and Cristo & Caronni (2008) in Capo Ceraso Bay (Olbia, north east Sardinia). Other sites where *P. ferruginea* has been reported in Sardinia include the Maddalena archipelago and the harbour of Arbatax (Doneddu & Manunza, 1992). Overall, the spatial distribution, abundance and status of *P. ferruginea* along the Sardinian coast remain largely unknown. This makes the planning and implementation of management strategies for the conservation of the species at local level difficult.

This study was carried out in the MPA of Penisola del Sinis - Isola di Mal di Ventre (western Sardinia, Italy) where no information on the status of *P. ferruginea* was available. We aimed to gather basic information regarding the population structure and distribution of *P. ferruginea* and to investigate the effects of accessibility, exposure and slope of the coast on its occurrence as a tool for the effective management and conservation of this species.

MATERIALS AND METHODS

Study area

This study was carried out in the MPA of Penisola del Sinis - Isola di Mal di Ventre, on the western coast of Sardinia (Fig. 1). This MPA, established in 1997 by National Decree of the Environment Ministry, covers a surface of more than 25000 ha and includes 30 km of coast. It consists of two ‘no take-no entry’ areas (defined as “Zones A” according to Italian law) which cover 1.5% of the total surface, two areas of ‘highly protection’ (Zones B), which cover 4% of the total surface, and a wide buffer area of ‘partially protection’ (Zone C). Although restrictions to human uses are generally higher in Zone B than in Zone C (Claudet *et al.*, 2006), in the Sinis MPA differences between the two categories are very limited. Indeed, the actual level of enforcement and the respect of the rules in the studied MPA are low, as demonstrated by Guidetti *et al.* (2008), who used fish assemblage as indicator of the MPA effectiveness.

Our research was conducted on the western side of the Mal di Ventre Island where a population of *Patella ferruginea* has been recently reported (Casu *et al.*, 2006). Mal di Ventre is a granitic island 5 nautical miles away from the Sardinian coast, with a surface lower than 1 km². The western side of the island, exposed to the prevailing wind (Mistral), is jagged with a lot of emerged rocks that constitutes a risk for landing and some high cliffs that prevent the coastal access by land. On the contrary, the eastern side of the island is flat and characterised by numerous small beaches.

In order to estimate as precisely as possible the size of the population of *P. ferruginea* we choose to perform a systematic census of the study area that include the whole Zone A of Mal di Ventre Island and a little portion of Zone B (<15% of the total) near to the limits of the no take-no entry area (Fig. 1). Field observations were carried out between July and September 2009 by a team of 2–3 snorkelers in order to optimise the detectability of individuals.

Perimeters analysis

The perimeter of the coastline was estimated using aerial georeferenced photos with high resolution and a specific GIS application *Arcgis 9.2 ESRI* program. This allowed us to subdivide the perimeter of the analyzed coastline in relation to the following factors: accessibility, exposure (i.e. exposed and sheltered surfaces with respect to the prevailing north-west Mistral wind, 225°–44° and 45°–224°, respectively) and slope (vertical substrate or not). Three levels were considered to assess the degree of accessibility of the coast as suggested by Paracuellos *et al.* (2003): (i) easily accessible - strips easily reachable by land and sea; (ii) semi accessible - sectors reachable only partially and with difficulty by land and sea; (iii) hardly accessible - isolated rocks or cliffs hardly reachable by swimming from the coast or by boat due to the presence of dangerous rocky outcrops.

***Patella ferruginea* population analysis**

The georeferenced mesolittoral strip was systematically examined for the presence of *P. ferruginea* (Fig. 1). For each observed specimen the following variables were considered: geographical coordinates, length (mm), width (mm), height (mm), distance from the lowest tide level (cm), accessibility, exposure and slope of the coast. Specimens smaller than 10 mm were excluded from the analyses because the external morphological characters of the shell still do not show clearly the specific features of this species (i.e. corrugated edge and marked ribs)(Casu *et al.*, 2010). However, their number was limited (about ten) possibly belonging to either *P. ferruginea* or the co-occurring *Patella rustica* Linnaeus, 1758, *Patella ulyssiponensis* Gmelin, 1791 and *Patella caerulea* Linnaeus, 1758. The length and width of each individual were measured to the nearest 0.1 mm using a calliper. The height was also measured using a modified calliper without removing the specimens from the substrate, thereby minimizing stress. Biometric data (i.e. length, width and height) were used to describe the structure of the population and to identify the presence and the abundance of the two morphotypes described for *P. ferruginea* (Espinosa & Ozawa, 2006).

In order to compare the “size” and/or the “height from minimum tide level” in relation to “accessibility”, “exposure”, “slope” of the coast and to the “morphotype”, nonparametric tests were applied using STATISTICA 8 software. If the considered factors had more than two levels (e.g. accessibility) a Kruskal-Wallis test was performed; otherwise Mann-Whitney U test or Kolmogorov-Smirnov test were used. For the slope estimation, a subdivision in sectors was considered: vertical (90° – 60°), inclined (60° – 30°), horizontal (30° – 0°), negative (-30° – 0°). With the aim to unravel whether *P. ferruginea* density changes in relation to the accessibility, exposure and slope of the coast a chi-square test was assessed. Only for the latter analysis (slope) a reduction into 2 levels (vertical cliff or not) was applied, due the impossibility of distinguishing the various degrees of coastal slope using aerial photos. A chi-square test was applied to assess whether the morphotype distribution depends on the exposure of the coast. Additionally, the occurrence of a preferred exposure in relation to quadrants [(NE (1° – 90°), SE (91° – 180°), SW (181° – 270°), NW (271° – 360°)] was tested for the individuals found on isolated rocks. The sample distribution was compared to a uniform distribution by means of a chi-square test.

RESULTS

Study area features

The total perimeter of the monitored coast was 8114 m (Table 1). The length of the easily and hardly accessible shore was predominant, accounting for 39% and 38% of the total coast, respectively (Table 1; Fig. 2a). More than half of the coast (4774 m) was exposed to the Mistral wind, while more than one third was represented by cliff (Table 1, Fig. 2b,c).

***Patella ferruginea* population**

A total of 196 individuals of *P. ferruginea* was found in the study area, corresponding to a mean density of 0.02 ind/m (Fig. 2d). The shell height/length ratio, used to distinguish the two morphotypes (Espinosa & Ozawa, 2006), highlighted the prevalence of the Rouxi form (N=133) over the Lamarcki form (N=63) (Fig. 3). The Mann-Whitney U test showed not significant spatial differences between the two morphotypes (U=4026; p=0.659), with an average position within the mesolittoral of 8.7 cm \pm 0.5 above the lowest tidal level. However, the Lamarcki form was significantly more abundant in the exposed sites ($\chi^2=4.13$; p=0.04); contrarily, the Rouxi form showed a homogeneous distribution according to hydrodynamics ($\chi^2=0.24$; p=0.62).

The population structure showed a unimodal distribution with a peak of individuals of 50–59 mm length and a maximum length of 78 mm (Fig. 4). The relatively small portion of individuals <30 mm and the lack of large-sized specimens (>80 mm, mainly females) was an indication of a threatened population (Fig. 4). The comparison of frequency distributions with healthier populations (e.g. Habibas, Algeria; Fig. 4) confirmed significant differences between the two populations (p<0.001, Kolmogorov-Smirnov test). The mean size of the three levels of accessibility did not differ significantly (H=0.445, p=0.8), while the respective variance values did (median-based Levene test, p<0.0001). Post-hoc comparison (Tukey test) highlighted a significantly lower (p<0.001) variance value of the easily accessible sites compared to the semi-accessible and hardly accessible sites. This was due to the absence of

both juveniles (<20 mm) and large adults (>70 mm), which instead were commonly found on the semi-accessible and hardly accessible coast (Fig. 5).

The density was largely dependent on the level of accessibility of the coast, with 148 specimens found along the hardly accessible perimeter (0.05 ind/m), 42 on semi-accessible sites (0.02 ind/m) and only 6 individuals in the easily reachable sites (0.002 ind/m) (Table 2). The chi-square test confirmed that the density of *P. ferruginea* was significantly higher in the hardly accessible sites (Table 2). Within these sites, this analysis also highlighted a homogeneous occurrence of individuals in relation to the slope and a significantly higher density along the exposed coast (south-west to north-east: 225°–44°). The analysis of *P. ferruginea* distribution in relation to directional quadrant discarded the hypothesis of a random distribution ($\chi^2=20.13$, $p<0.0002$). In particular, 40% of the individuals found on isolated rocks (N=173) were grouped on the coast oriented towards the fourth quadrant (Mistral wind direction). These results demonstrated a preference of *P. ferruginea* for sites with the highest level of hydrodynamics. Furthermore, the results of the Mann-Whitney U test showed that specimens in the exposed sites were significantly larger than those in the sheltered sites (U=3433.5; $p=0.0102$; $N_1=126$, $N_2=70$; Fig. 6), also occupying a significantly lower position within the mesolittoral (U=3282; $p=0.0029$; $N_1=126$, $N_2=70$; Fig. 7). Finally, no significant effect of inclination (vertical: N=106; inclined: N=72; horizontal: N=9; negative: N=9) was found in relation to the shell size (H=1315, $p=0.7256$), nor to the position of specimens within the mesolittoral (H=601; $p=0.2035$).

DISCUSSION

The population of *Patella ferruginea* in the Mal di Ventre Island showed the lowest average density (0.02 ind/m) ever reported for this species. In particular, it was three times lower than that found on the Alboran Islands similarly affected by human collection (Paracuellos *et al.*, 2003). This study thus confirms the highly endangered status of *P. ferruginea* on Sardinian rocky shores where it is reported at serious risk of extinction (Guerra-García *et al.*, 2004 and literature therein; Espinosa *et al.*, 2009b). Our results demonstrated that the level of accessibility of the coast still plays a major role in determining the occurrence of *P. ferruginea* (e.g. Paracuellos *et al.*, 2003; Guerra-García *et al.*, 2004; Espinosa *et al.*, 2009a), even though within the no take-no entry zone, with a density of 0.05 and 0.002 ind/m in the hardly and easily accessible sites, respectively. Indirect evidence of how Zone A is disregarded comes from our recurrent observations of leisure boats and/or snorkelers in the latter sites, in spite access to Zone A is banned except for research or surveillance purposes. In fact, the importance of no take areas for the conservation of the marine ecosystem is increasingly emphasized worldwide (e.g. Manríquez & Castilla, 2001; Claudet *et al.*, 2008; Chittaro *et al.*, 2009). However, we consider that the local protection measures of the Zone A are not effective and assume that illegal catches constitute the main constraint for the population success in Mal di Ventre Island.

The size distribution of *P. ferruginea*, with fewer individuals >70 mm, was a further indication of human exploitation, as larger individuals constitute the main target of illegal catches (Sagarin *et al.*, 2007; Espinosa, 2009). For instance, Espinosa *et al.* (2009a) on the Strait of Gibraltar reported individuals >100 mm in protected ('under custody') military areas, not found in adjacent 'without custody' areas. The human exploitation, and the consequent loss of larger-sized females, is the main cause of the vulnerability of the populations, especially in protandrous species (Hawkins *et al.*, 2000). The evidence of a tight link between size and reproductive output of *P. ferruginea* was demonstrated by Espinosa *et al.* (2006). They showed that an 8-cm female spawns a number of oocytes ten time greater than an

individual of 6 cm in length. Moreover, the decreased population density further reduces the success of external fertilization and recruitment as a consequence of a higher dispersal of individuals (Hawkins *et al.*, 2000 and literature therein). In our study area, such a population structure profile, with few individuals <30 mm and >70 mm, most apparent in the easily accessible sites, confirmed that *P. ferruginea* is threatened. Thus, the effective observance of Zone A has to be pursued in order to increase the mean size and hence the reproductive effort and viability of this population.

In our study area, the exposure of the coast to wave actions was also an important factor influencing the distribution of *P. ferruginea*, as previously found in studies focusing on the effect of hydrodynamics on limpets in the intertidal zone (e.g. Denny, 2000; Denny & Blanchette, 2000; Denny *et al.*, 2003). Density was significantly higher on the coast most exposed to the prevailing north-western Mistral wind, thus with the greater wave action. Other studies suggested that *P. ferruginea* is usually associated with rocky shores exposed to wave action (Porcheddu & Milella, 1991; Doneddu & Manunza, 1992; Tlig-Zouari *et al.* 2010). In contrast, Guerra-García *et al.* (2004) showed that high densities occur also in sheltered areas with little water exchange. Our results suggest that strong wave motion, in addition to possibly promoting the success of the population due to an increased grazing surface (Espinosa *et al.*, 2007b), makes the same (most exposed) sites the least accessible to human exploitation. This can also partly explain the higher density of larger individuals in the exposed sites (Fig. 6), while the lower position of *P. ferruginea* within the mesolittoral (Fig. 7) may be related to a different temporal and spatial use of the grazing area. However in order to explain the distribution of *P. ferruginea* according to hydrodynamics, experimental studies are needed in order to evaluate the possible additive or synergistic effects of other abiotic and biotic factors (e.g. insulation, abundance and distribution of predators and competitors, variability in algal assemblage) affecting these patterns.

Our results provided the first evidence of a spatial separation between the two morphotypes of *P. ferruginea* within the mesolittoral. In particular, while the distribution of the Rouxi form was unrelated to the exposure of the coast, the Lamarki form was significantly

confined to the exposed sites. This corroborates earlier hypothesis of a higher adaptability of the Rouxi form due to a larger internal volume which allows longer periods of respiratory autonomy (Paracuellos *et al.*, 2003 and literature therein). We infer that this can also be a reason for the greater success of the Rouxi form (N=133) over the Lamarki form (N=63) in our study area.

Finally, contrary to previous studies, we did not find any differences on *P. ferruginea* distribution according to the slope of the substrate. For instance, along the Spanish coast *P. ferruginea* generally colonizes inclined substrates with a lower preference for vertical cliff (MMAMRM, 2008), while Doneddu & Manunza (1992) recorded a higher colonization of *P. ferruginea* on horizontal substrates. These heterogeneous results suggest that the choice of a particular slope by *P. ferruginea* may be site-specific, i.e. related to the local characteristics. However, the extension of the surveyed area (>8 km) did not allow us to associate the main observed slopes with the respective surface, nor previous studies on *P. ferruginea* did. A specific study aimed at clarifying this aspect is necessary.

Overall, the results of this study highlighted the limited effectiveness of Zone A against human pressure. However, they also provided useful information on the distribution of *P. ferruginea* as the basis for its protection and conservation in the MPA of Penisola del Sinis - Isola di Mal di Ventre. We infer that the low frequency and the high predictability of local surveillance, as well as the modest fines provided by the Italian legislation to transgressors, are major drawbacks limiting the effectiveness of this MPA as a flagship tool of marine conservation (e.g. Agardy, 1994; Allison *et al.*, 1998; Branch & Odendaal, 2003; Mora *et al.*, 2006; Chittaro *et al.*, 2009, Espinoza-Tenorio *et al.*, 2010 and literature therein). In addition, according to the Italian legislation, the application of sanctions to transgressors (e.g. people catching protected species within Zone A) is sole responsibility of national enforcement bodies, with little power given to the local staff or collaborators of the MPA. Nowadays the lack of an effective and direct surveillance of MPAs is considered one of the greater handicaps (e.g. Rodríguez-Martínez 2008; Guidetti *et al.*, 2008; García-Gómez *et al.*, 2010). A partial solution to these problems would be a stricter control of the MPA, including the

presence of national enforcement bodies during the patrol activities of the MPA staff as proposed by Rodríguez-Martínez (2008), or the use of a video surveillance system as suggested by Espinosa *et al.* (2009a). In fact, experience has demonstrated that the main causes that lead to the failure of a MPA can be ascribed to socioeconomic factors such as the low participation of local communities (Rodríguez-Martínez, 2008; Ferse *et al.*, 2010). Studies performed in several countries demonstrated that the majority of the local users perceive themselves as the recipients of rules against their interests, thus negating any possible benefits (Ferse *et al.*, 2010). This also implies that the time needed to benefit from any conservation measures may exceed the time that the local communities are willing to wait for concrete results (Ferse *et al.*, 2010 and literature therein).

We may conclude that, due to the general regression of the geographic distribution of *P. ferruginea* (e.g. Ramos, 1998; Guerra-García *et al.*, 2004; Espinosa, 2009), the protection of the hot spots of this species should be considered a priority. In fact, MPAs should play a fundamental role as a refuge area for the remaining populations (Espinosa *et al.*, 2009a,b; Martins *et al.*, 2010; Van Hoey *et al.*, 2010). In order to prevent the extinction of *P. ferruginea* in Sardinia it will be important to combine the knowledge and experience of all stakeholders as the Spanish experience has shown (MMAMRM, 2008). Only the understanding of the causes of degradation and the consequent formulation of shared conservation and participatory strategies may prevent the extinction of this species.

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TABLES AND FIGURES

Table 1. Classification of the coast in relation to its accessibility, exposure and slope.

Accessibility	Exposure	Slope	Length (m)
1. Easily accessible (3140 m; 39%)	a. Exposed	i. Cliff	402 (5%)
		ii. No vertical rocks	1618 (20%)
	b. Sheltered	i. Cliff	180 (2%)
		ii. No vertical rocks	940 (12%)
2. Semi accessible (1863 m; 23%)	a. Exposed	i. Cliff	504 (6%)
		ii. No vertical rocks	596 (7%)
	b. Sheltered	i. Cliff	379 (5%)
		ii. No vertical rocks	384 (5%)
3. Hardly accessible (3111 m; 38%)	a. Exposed	i. Cliff	912 (11%)
		ii. No vertical rocks	742 (9%)
	b. Sheltered	i. Cliff	650 (8%)
		ii. No vertical rocks	807 (10%)
Total perimeter monitored			8114 m

Table 2. Result of chi-square test (Obs, observed frequencies; Exp, expected frequencies) to test the density of *Patella ferruginea* in relation to the accessibility, exposure and slope of the coast.

Accessibility	Exposure	Cliff		No vertical rocks	
		Obs	Exp	Obs	Exp
1. Easily accessible $\chi^2=64.63$ df=3 p<0.00001	a. Exposed	2.00	9.72	2.00	39.09
	b. Sheltered	1.00	4.33	1.00	22.69
2. Semi accessible $\chi^2=4.54$ df=3 p<0.2088	a. Exposed	13.00	12.18	15.00	14.39
	b. Sheltered	3.00	9.16	11.00	9.27
3. Hardly accessible $\chi^2=87.90$ df=3 p<0.00001	a. Exposed	45.00	22.02	48.00	17.93
	b. Sheltered	20.00	15.71	35.00	19.50

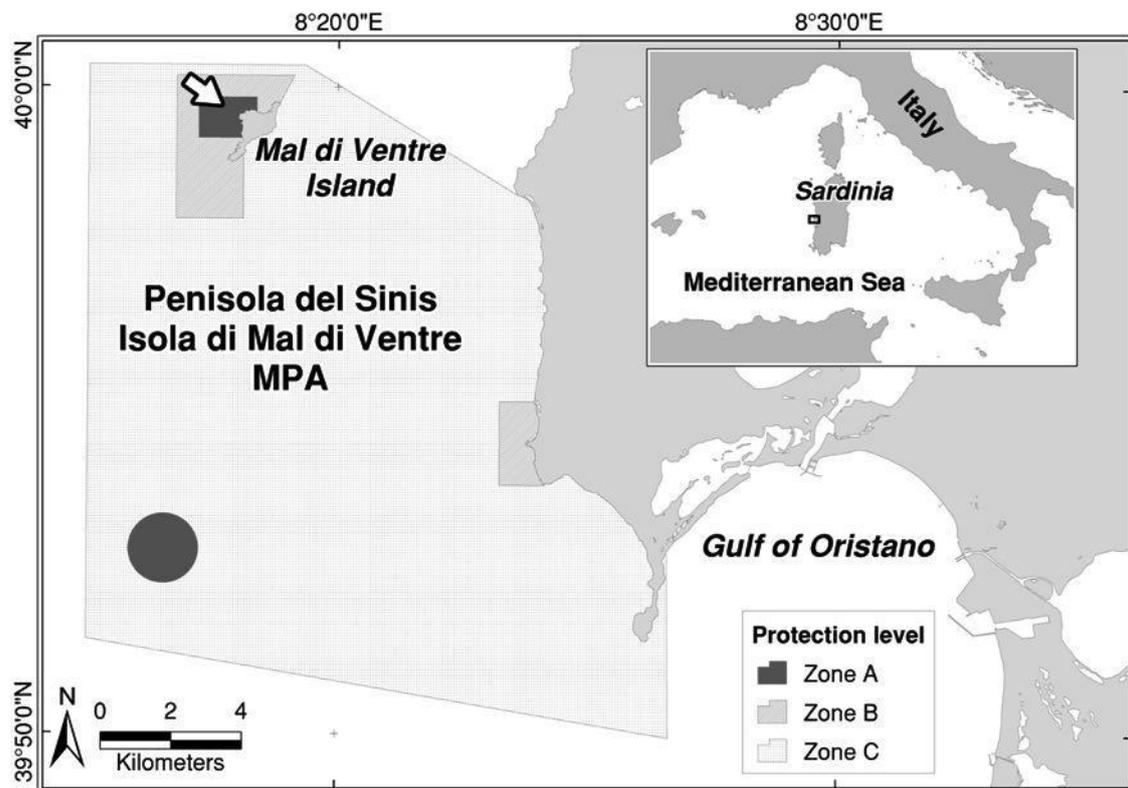


Fig. 1. Location of the Marine Protected Area (MPA) of Penisola del Sinis - Isola di Mal di Ventre (western Sardinia, Italy), and the study area in the northwestern sector of the Mal di Ventre Island (Zone A; white arrow).

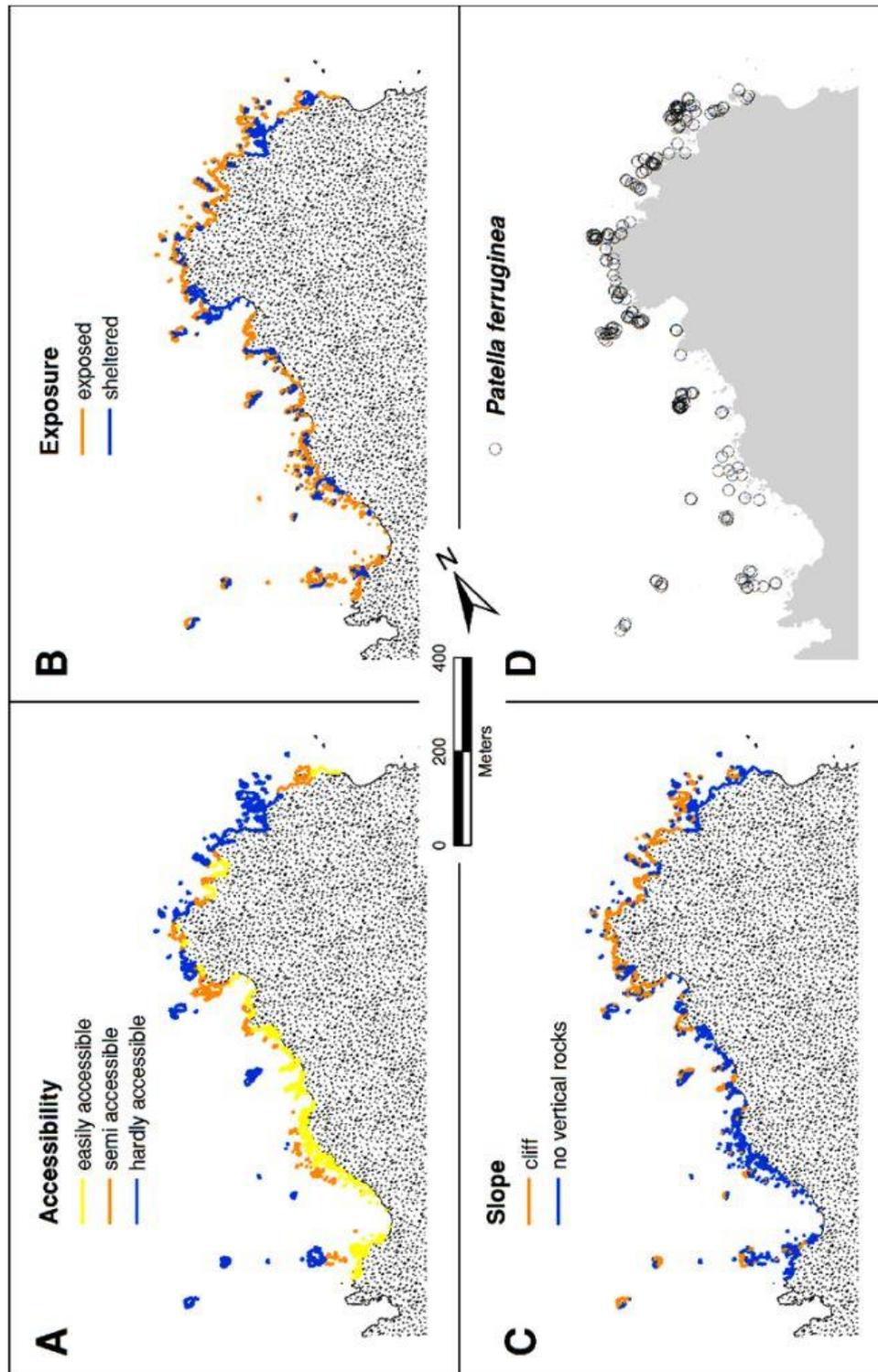


Fig. 2. Analyzed perimeter of the Mal di Ventre Island classified in relation to the accessibility (A), exposure (B) and slope (C) of the coast, and the georeferenced distribution *Patella ferruginea* individuals (D).

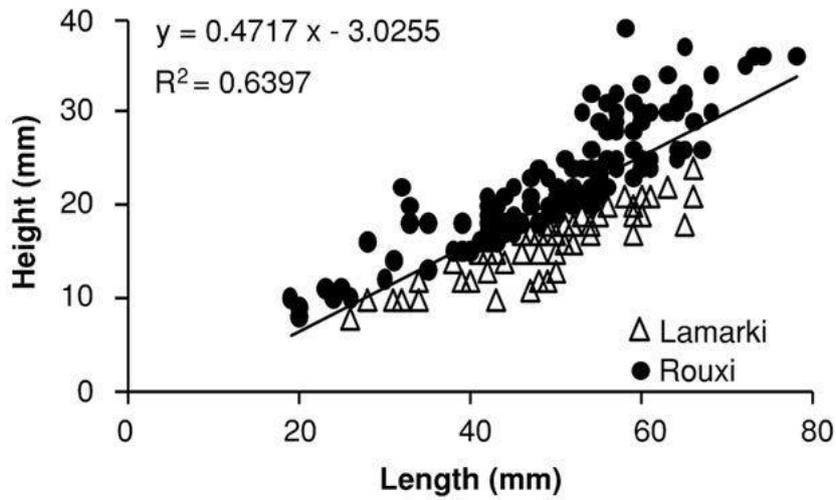


Fig. 3. Relationship between height vs. length of *Patella ferruginea* ($p < 0.001$). The height (H)/length (L) ratio was used to distinguish the Lamarki ($H/L < 0.37$) and the Rouxi ($H/L > 0.37$) morphotypes.

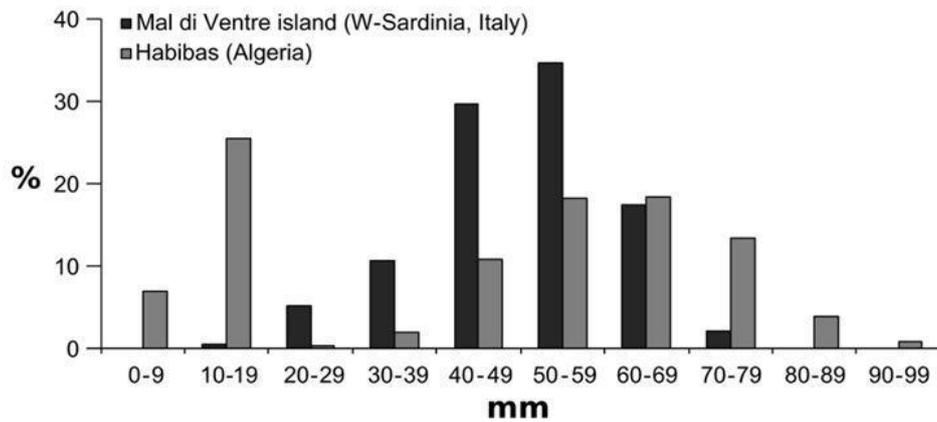


Fig. 4. Size-class frequency of *Patella ferruginea* population in the Mal di Ventre Island (present study, black columns) and Habibas, Algeria (Espinosa, 2009; grey columns).

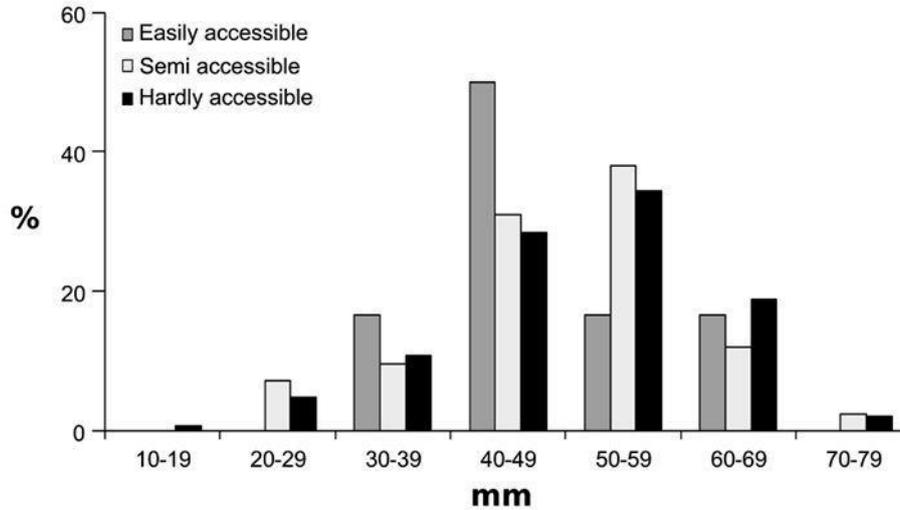


Fig. 5. Size-class distribution of *Patella ferruginea* in relation to the accessibility of the coast.

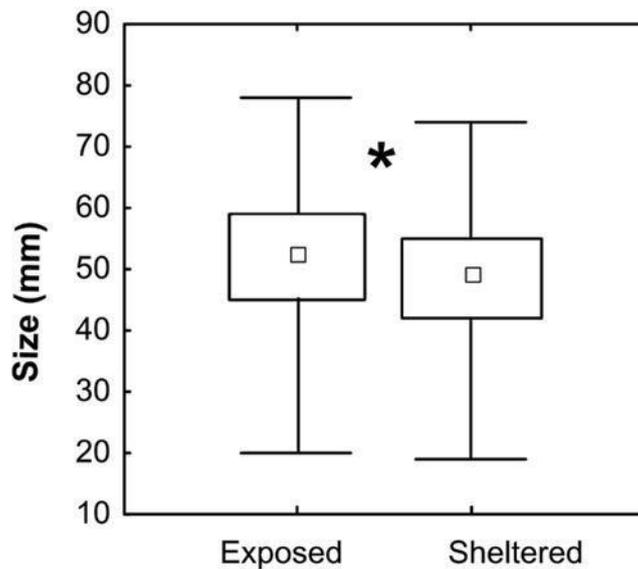


Fig. 6. Box-and-whisker plots of *Patella ferruginea* shell length in exposed (225° – 44°) and sheltered (45° – 224°) sites. Boxes are inter-quartile ranges; open squares within boxes are medians; whisker endpoints are high/low extremes. *, $p < 0.05$.

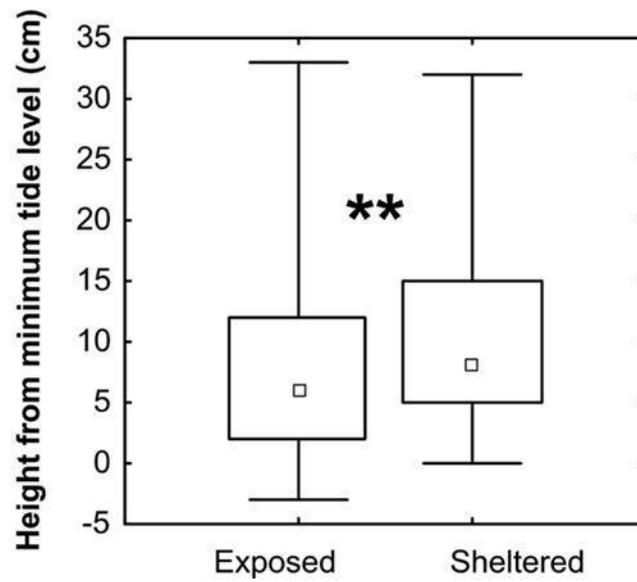


Fig. 7. Box-and-whisker plots of *Patella ferruginea* height from the minimum tidal level in exposed (225°–44°) and sheltered (45°–224°) sites. Boxes are inter-quartile ranges; open squares within boxes are medians; whisker endpoints are high/low extremes. **, $p < 0.01$.

GENERAL DISCUSSION

This study advances the knowledge on the distribution, abundance and ecological traits of *Pinna nobilis* and *Patella ferruginea* in the Sinis MPA (see Annex I). It clarifies the main constraints for conservation effectiveness of these species and proposes specific protection measures. Here are summarized the main findings that have led to answer to the three main questions underlying this thesis.

WHICH IS THE CURRENT HEALTH OF THE TWO SPECIES WITHIN THE SINIS MPA?

Pinna nobilis

Although fairly widespread in the Sinis MPA, this species shows a significant density only within the Gulf of Oristano with mean value of 1 ind.100 m⁻² in line with the majority of the Mediterranean populations (Šiletić & Peharda, 2003 and literature therein). This value did not show significant differences between years (2007 and 2009) however, the observed variability in recruitment success may be considered a potential factor of vulnerability. The low recruitment, which could be an indication of scarce health at population level, is not recorded at individual level. On the contrary, a normal growth over the years, more rapidly in juveniles than in adults, is demonstrated (Coppa *et al.*, 2011). The lack of recruitment must be searched in other causes than those strictly related to the habitat and could be linked to specific conditions of hydrodynamic, or other disturbing factors (biotic or abiotic), which have unpredictably occurred in some years during larval development, settlement and/or subsequent recruitment. Thus, the Sinis MPA and particularly the Gulf of Oristano appear to be a suitable area for the growth of *P. nobilis*.

Patella ferruginea

Patella ferruginea in the Sinis MPA was only found in a restricted area of the western coastline of Mal di Ventre Island. The studied population showed the lowest average density

(0.02 ind/m) ever reported for this species confirming the highly endangered status of *P. ferruginea* on Sardinia rocky shores where it is reported at serious risk of extinction. Moreover the size distribution of *P. ferruginea*, with fewer individuals >70 mm (mainly females), further indicate a sex ratio disequilibrium and a low reproductive potential supported by the limited presence of small specimens (<30 mm). Our results constitute the first evidence of the extinction threat of *P. ferruginea* in the Sinis MPA and probably along the whole western Sardinian coast where no other large breeding population has been described.

WHICH ARE THE MAIN BIOTIC OR ABIOTIC FACTORS THAT CAN AFFECT THE POPULATIONS SUCCESS?

Pinna nobilis

The effects of substrate type, meadows characteristics and hydrodynamics on *Pinna nobilis* distribution were investigated. The substrate type resulted a considerable factor in defining *P. nobilis* distribution especially where the meadow is quite fragmented. Indeed in those sites the specimens colonize preferentially the meadow edge. Otherwise density was higher within dead *matte* than inside the meadow, whereas bare sand showed the lowest values. The substrate type and particularly its compactness influences also the shell stability; in fact, burial level of individuals decreases with increasing fine sediment content. A specimen not sufficiently deeply buried can be more susceptible to predation and hydrodynamics (Richardson *et al.*, 1999). This thesis demonstrated that wave actions can be neglected as a potential factor affecting *P. nobilis* orientation and density distribution due to their low energetic features in the northern part of the Gulf of Oristano. Conversely bottom current direction and speed resulted the main factors influencing shell directions. Where water speed exceeded 0.07 m/s specimens resulted disposed in line with the prevalent current direction with the ventral side exposed to incoming flow. Moreover a key role in determining

high density values is ascribable to the input of the lagoon waters (Cabras and Mistras lagoon) and to sedimentation areas due to gyre formation (i.e. eddy located in the site of Tharros) since they could constitute the major food sources. Thus, the feeding availability seem to be a driving force in determining the patchiness distribution of *P. nobilis* population with higher values in sites close to the mouth of the lagoons, in sedimentation area or in the meadow edge (i.e. *intermatte* channel) where the water flow is conveyed.

Patella ferruginea

The main constraint affecting the population success in the Mal di Ventre Island is human exploitation in relation to the accessibility of the coast. Illegal catches cause the low population density and the absence of largest individuals (>80 mm) which constitute the common fishing target, even though the study area is a no take-no entry zone. Also the exposure of the coast resulted a noteworthy factor in influencing density, mean size and morphotypes distributions of *P. ferruginea*. Higher density values and mean size were recorded on northwestern exposed coast where Lamarki form is significantly confined. These results could suggest that a strong wave motion can support a higher population fraction and the occurrence of the less adaptable morphotype (Lamarki), however this pattern could be largely influenced by the human exploitation which is lower in exposed site.

CURRENTLY, CAN THE SINIS MPA BE CONSIDERED AN EFFECTIVE TOOL FOR THEIR CONSERVATION?

Pinna nobilis

In order to preserve *P. nobilis* population and its habitat, the municipality of Cabras had supplied of free buoy fields the main sheltered bays of the MPA since 2005. Therefore, for many years, these mooring structures have largely contributed to minimize the anchorage impact on the seabeds.

Moreover the actual surface of the MPA, effective from 15 November 2011 (M.D. 20/07/2011; M.D. 20/07/2011 n. 188), further contributes to the protection of *P. nobilis*. The enlargement need of the MPA was justified to the Ministry of environment also by means of the results of this thesis which explain the spatial distribution of the population in the Gulf of Oristano. Thus the protection of the Mar Morto and Mistras sites allows to preserve also *P. oceanica* and *Cymodocea nodosa* meadows which constitute a refuge area not only for *P. nobilis* but also for a large amount of commercial species as the sea urchin *Paracentrotus lividus*.

Using the results and recommendations of this thesis a large amount of informative materials (i.e. roll up, posters, brochures) were produced by the MPA staff in collaboration with the Oristano section of the IAMC-CNR (see Annex II). In the last years the MPA management body has given great importance to scientific dissemination on conservation topics organizing theme days where I had explained to citizen, vigilance bodies and politicians the main results of the present study. The main aim of this collaboration between Sinis MPA and IAMC-CNR was the increase in the awareness on the protection needs of vulnerable species, such as *P. nobilis*.

Despite the large effort of the Sinis MPA in activating awareness campaigns and in implementing protection measures to preserve *P. nobilis* and the seagrass meadows habitat, the local surveillance carried out by the national enforcement bodies to avoid poaching is inadequate. This constitutes the real constraint to the conservation effectiveness in the Sinis MPA. Finally, it remains still unknown the impact caused by the fishing gears (gillnets and pots) on the *P. oceanica* meadow and particularly on *P. nobilis* population. Due to the widespread use of gillnets and pots in the Gulf of Oristano (see Annex III) we infer that a study aimed to the damage evaluation on *P. nobilis* population could be fundamental for the conservation of this species in the Sinis MPA.

Patella ferruginea

While the importance of no take areas for the conservation of marine ecosystem is increasingly worldwide (e.g. Manríquez & Castillia, 2001; Claudet *et al.*, 2008; Chittarro *et al.*, 2009), we consider the local protection measures of the no take-no entry zone not effective to protect an overexploited species. Indeed, we demonstrated that illegal catches constitute the main risk for the *P. ferruginea* population extinction in Mal di Ventre Island (see Annex III). This is due to the low frequency and the high predictability of local surveillance, as well as the modest fines provided by the Italian legislation to poachers. Our results constitute a clear evidence of how an inadequate surveillance, combined with a lack of environmental awareness, can lead to limit the effectiveness of a MPA as a flagship tool of marine conservation.

With the exception of the surveillance, where no power is given to the Italian MPAs, the management body of the Sinis Marine Protected Area is commissioning new monitoring and research studies on this species and organizing awareness campaign and activities of environmental education aimed to the protection of the *P. ferruginea* population (see Annex II). Moreover, in agreement with the other Sardinian Marine Protected Areas, local research institutions (IAMC-CNR and University of Sassari) and regional government, Sinis MPA is working on a network project aimed to define a common conservation strategy for *P. ferruginea* in Sardinia. Indeed, due to the general regression of the geographic distribution of this species, the protection of the hot spots of *P. ferruginea* have to be considered a priority. In this sense MPAs have to play a fundamental role as a refuge area for the remaining populations. Only the understanding of the degradation causes and the formulation of shared conservation strategies, as suggested in this thesis, may prevent the extinction of this species in Sardinia.

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ANNEX I

List of publications (2009-2012)

ISI ARTICLES

Coppa S., de Lucia G.A., Magni P., Domenici P., Antognarelli F., Satta A. and Cucco A. The effect of hydrodynamics on shell orientation and population density of *Pinna nobilis* in the Gulf of Oristano (Sardinia, Italy). *Journal of Sea Research*, submitted.

Coppa S., de Lucia G.A., Massaro G. and Magni P. (2012) Density and distribution of *Patella ferruginea* Gmelin, 1791 in a Marine Protected Area (western Sardinia, Italy): constraint analysis for population conservation. *Mediterranean Marine Science*, in press.

Coppa S., Guala I., de Lucia G.A., Massaro G. and Bressan M. (2010) Density and distribution patterns of the endangered species *Pinna nobilis* within a *Posidonia oceanica* meadow in the Gulf of Oristano (Italy). *Journal of the Marine Biological Association of the United Kingdom* 90, 885 – 894. doi: 10.1017/S002531540999141X.

ISBN ARTICLES (PEER REVIEWED)

Coppa S., Massaro G., Bressan M., Mascia L. and de Lucia G.A. (2011) Studio della popolazione di *Pinna nobilis* (L., 1758) (Mollusca, Bivalvia) nel Golfo di Oristano: analisi dei *pattern* di distribuzione spaziale in relazione all'habitat. *Studi Trentini di Scienze Naturali* 89, 123 – 130.

Camedda A., **Coppa S.**, De Lucia G.A., Brundu R., Mascia L. and Massaro G. (2011) *Paracentrotus lividus* nell'Area Marina Protetta "Penisola del Sinis - Isola di Mal di Ventre" (Sardegna occidentale): prelievo e permessi. *Biologia Marina Mediterranea* 18, 296 – 297.

Coppa S., de Lucia G.A., Camedda A., Massaro G., Petrocelli A., Cecere E. and Magni P. (2011) *Patella ferruginea* nell'AMP del Sinis: caratterizzazione

dell'habitat e cause della sua rarefazione. *Biologia Marina Mediterranea* 18, 244 – 245.

ATTENDANCE AT CONFERENCES

Coppa S., De Lucia G.A., Camedda A., Massaro G., Petrocelli A., Cecere E. and Magni P. (2011) *Patella ferruginea* nell'AMP del Sinis: caratterizzazione dell'habitat e cause della sua rarefazione. Poster presented at the 42° Congresso della Società Italiana di Biologia Marina. Olbia, 23-28/05/2011.

Camedda A., **Coppa S.**, De Lucia G.A., Brundu R., Mascia L. and Massaro G. (2011) *Paracentrotus lividus* nell'Area Marina Protetta Penisola del Sinis – Isola di Mal di Ventre (Sardegna occidentale): prelievo e permessi". Poster presented at the 42° Congresso della Società Italiana di Biologia Marina. Olbia, 23-28/05/2011.

Bussotti s., Baroli M., **Coppa S.**, Di Franco A., Guala I., Panzalis P., Sahyoun R. and Guidetti P. (2011) Relationships between sea urchins and their predatory fishes in the Marine Protected Area Tavolara – Punta Coda Cavallo. Poster presented at the 42° Congresso della Società Italiana di Biologia Marina. Olbia, 23-28/05/2011. Extended abstract, 2 pp.

Boscolo S., **Coppa S.**, Falconi A., Motta E., Ferro A. and Baroni A. (2010) Safeguarding of the Venice coastal area through Habitat® blocks. Talk presented at the 3rd International Conference on the Management of Coastal Recreational Resources. Grosseto 27-30/10/2010. Book of abstract.

Coppa S., Massaro G., Bressan M., Mascia L. and de Lucia G.A. (2010) Studio della popolazione di *Pinna nobilis* nel Golfo di Oristano: analisi dei pattern di distribuzione spaziale in relazione alle caratteristiche dell'habitat. Poster presented at the XIX meeting of GADIO "il ruolo delle Aree Marine Protette per

la tutela della biodiversità". Olbia, 21-23/05/2010. Book of abstract. Poster awarded as one of the best works presented by young researchers.

Perilli A., Antognarelli F., de Lucia G.A., Satta A., **Coppa S.**, Sinerchia M., Murgia R. and Mossone P. (2009) Sea & Science in Sardinia: Experience on the dissemination of scientific culture to students. Poster presented at the meeting of Geoitalia. Rimini 9-11/9/2009. Book of abstract.

TECHNICAL-SCIENTIFIC REPORTS

Coppa S., Wrachien F., Camedda A., Massaro G. and de Lucia G.A. (2010) Indagine sullo stato della popolazione del riccio di mare *Paracentrotus lividus* nell'AMP Penisola del Sinis-Isola di Mal di Ventre. Technical-scientific report, pp 61.

de Lucia G.A., Camedda A., **Coppa S.** and Massaro G. (2010) Monitoraggio della risorsa ittica e dell'effetto riserva nell'AMP "Penisola del Sinis – Isola di Mal di Ventre". Technical-scientific report, pp 70.

de Lucia G.A., **Coppa S.**, Camedda A. and Massaro G. (2010) Monitoraggio dei siti diving nell'Area Marina Protetta Penisola del Sinis-Isola di Mal di Ventre. Technical-scientific report, pp 68.

Coppa S., Massaro G. and de Lucia G.A. (2009) Indagine preliminare sullo stato della popolazione di *Patella ferruginea* nella zona integrale dell'Isola di Mal di Ventre. Technical-scientific report, pp 42.

Coppa S., Antognarelli F., Satta A., Camedda A. and de Lucia G.A. (2009) Monitoraggio dell'Indice Gonado – Somatico del riccio di mare edule *Paracentrotus lividus* nell'Area Marina Protetta "Penisola del Sinis – Isola di Mal di Ventre". Technical-scientific report, pp 32.

ANNEX II

Informative material produced using the results of the present thesis



LA PATELLA
aiutaci a proteggerla






La *Patella ferruginea* è un mollusco gasteropode endemico del Mediterraneo. È una specie erbivora che popola le coste rocciose e si trova, generalmente, nella fascia compresa tra il livello della bassa e dell'alta marea. La *Patella ferruginea* era una specie originariamente diffusa in tutto il Mediterraneo ed ora presente in poche e ristrette zone costiere del bacino occidentale. Attualmente risulta l'invertebrato marino più a rischio di estinzione di tutto il Mediterraneo occidentale. La drastica diminuzione di questa specie è causata:

- dal prelievo illegale da parte dell'uomo;
- dal degrado dell'habitat;
- dalla bassa fecondità e dalla scarsa capacità dispersiva.

Ormai la popolazione dell'isola di Mal di Ventre è costituita da pochi esemplari e le possibilità che nuove larve possano arrivare da luoghi vicini sono scarsissime. È quindi necessario non prelevare alcun esemplare: ogni singolo individuo è importante per consentire la sopravvivenza di questa specie nell'isola di Mal di Ventre. Molte volte la specie *Patella ferruginea* non viene distinta da altre specie simili, che pur se non in pericolo di estinzione non possono essere prelevate, per nessuna ragione, all'interno dell'Area Marina Protetta.

NON RACCOGLIERE NESSUN ESEMPLARE DI PATELLA FERRUGINEA

"Aiutaci anche tu a conservare la popolazione di *Patella ferruginea* dell'Isola di Mal di Ventre sensibilizzando tutti e segnalando i comportamenti scorretti".
Il prelievo di *Patella ferruginea* è un reato punito ai sensi dell'art.30 della Legge 394/91.
Per qualsiasi segnalazione:

VIGILI URBANI 800 125 018	CORPO FORESTALE 15 15	CAPTANERIA DI PORTO 15 30
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AREA MARINA PROTETTA
PENISOLA DEL SINIS
ISOLA DI MAL DI VENTRE

Comune di Cabras
Ministero dell'ambiente

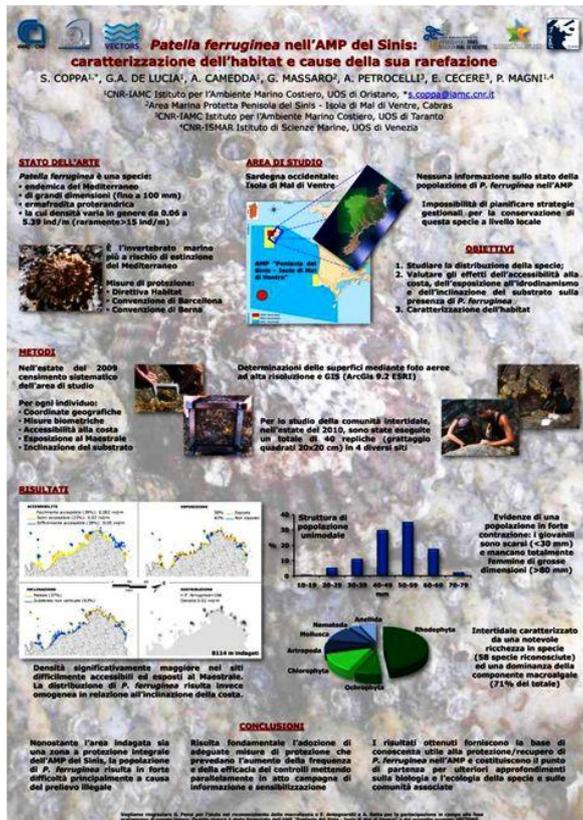
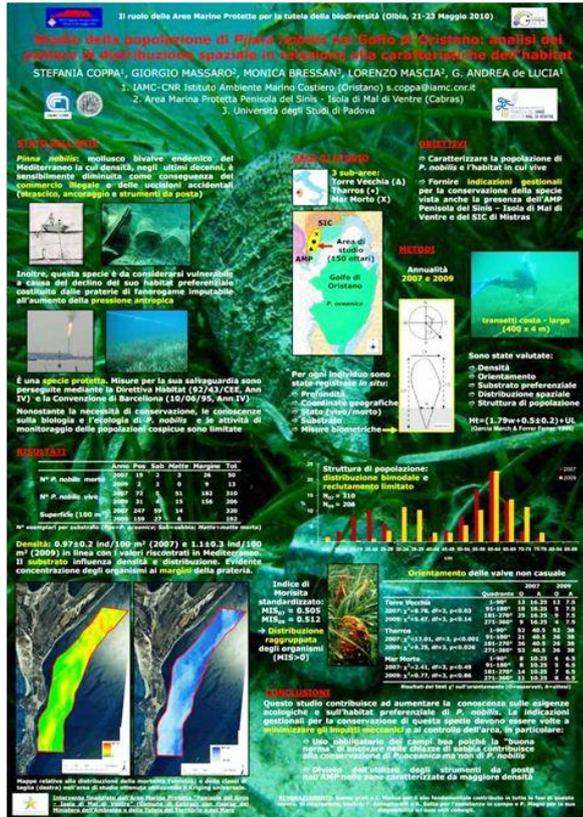
www.areamarinasinis.it - info@areamarinasinis.it

La tutela e la valorizzazione delle caratteristiche biologiche e genetiche sono tra gli scopi istituzionali dell'Area Marina Protetta "Penisola del Sinis - Isola di Mal di Ventre"

Postcard for the preservation of *Patella ferruginea* in the Sinis MPA.



Titles of the talks useful to scientific dissemination and environmental education on the problematic of endangered species conservation.



Posters on the population health of *Pinna nobilis* and *Patella ferruginea* in the Sinis Marine Protected Area.

LA PATELLA

aiutaci a proteggerla



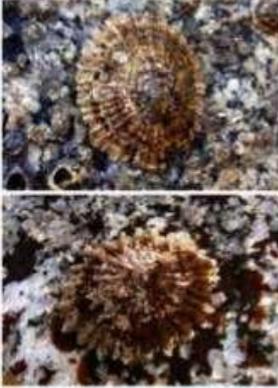


Importante è conoscere la Patella ferruginea: la biologia e l'ecologia.

Patella ferruginea è un mollusco patellomorfo endemico del Mediterraneo. È una specie estrema che popola le coste rocciose, si trova spesso nella fascia compresa tra il livello della bassa e dell'alta marea. Raggiunge dimensioni più grandi rispetto alle altre patelle che vivono nelle nostre coste (oltre la più estrema, infatti, Patella ferruginea può superare i 10 cm di lunghezza anche se, di norma, le sue dimensioni non superano i 7-8 cm).

Di Patella ferruginea sono stati osservati due varianti: l'arctica e rosei già descritti da Poyardou nel 1826. La prima, caratterizzata da conchiglie molto marcate e dal riccio depressa, è presente in microambienti ripari di zone battute da azzurra, più alta e con un'azione meno forte, e la forma più frequente.

Patella ferruginea cresce molto lentamente e la Patella femmine viene raggiunta tra il secondo e il terzo anno di vita quando la conchiglia misura 2,5 - 3 cm. È una specie che cambia sesso con l'età, assumendo prima il sesso maschile e poi quello femminile. In natura si osservano anche il dimorfismo sessuale e il dimorfismo successivamente femminile di raggiungimento di una dimensione taglia (da 3 - 5 cm). Nel periodo della loro primaverili è possibile osservare piccoli esemplari di Patella ferruginea (3 anni) sul riccio di forte stallo, infatti, questa specie è molto trasparente sulla superficie esterna del guscio in forme giovani. I principali predatori sono granchi, gastropodi, galathea ed epatolite (uomini).



La popolazione di Patella ferruginea dell'Isola di Mal di Ventre: una popolazione a rischio di estinzione.

Patella ferruginea era una specie originariamente diffusa in tutto il Mediterraneo ed era presente in pacche e ristrette zone costiere del bacino occidentale. È ancora possibile osservare popolazioni lungo la costa che va dal Marocco alla Tunisia, in Spagna (regioni di Murcia e dell'Andalusia), nei Isole Baleari, Corsica e nelle Sardegna, nell'isola di Portofino, nel Canale di Sicilia e nell'arcipelago toscano anche se le popolazioni della Sardegna e della Corsica sono in chiara regressione. Al momento, Patella ferruginea risulta l'endemismo marino più a rischio di estinzione di tutto il Mediterraneo occidentale.

La drastica diminuzione di questa specie è causata:

- dal prelievo illegale di questo mollusco;
- dal degrado del habitat;
- dalla bassa fertilità e dalla scarsa capacità di dispersione.

Il prelievo di questa specie è legato ai suoi utilizzi come uscio, a scopo alimentare e per fini ornamentali. Essendo la specie che raggiunge le dimensioni più grandi risulta quella maggiormente interessata dal prelievo. Inoltre, considerato che in questa specie gli individui più grandi sono femmine, le culture da parte dell'azione influenzano direttamente sul rapporto tra maschi e di conseguenza, sul successo riproduttivo delle popolazioni nelle aree maggiormente sfruttate. La distruzione dell'habitat ed il prelievo da parte dell'uomo sono stati infatti come le principali cause del degrado delle popolazioni di Patella ferruginea. Infatti, risulta una specie molto sensibile all'inquinamento: la presenza di Patella ferruginea diminuisce drasticamente in acque turbide, poco ossigenate e caratterizzate da livelli concentrati di ammoniaca e nitrato organica.

Patella ferruginea sembra caratterizzata da bassa fertilità e da scarsa capacità di dispersione. Tali caratteristiche, in caso di prelievo un effettivo controllo della popolazione, dell'eliminazione un riparo e scure recupero e sono quindi causa notevole della vulnerabilità della specie.

Cosa fare per non perderla per sempre: l'azione di sensibilizzazione dell'Area Marina Protetta.

Nell'Isola di Mal di Ventre è presente una ridotta popolazione di Patella ferruginea, monitorata in questi anni dall'Istituto per l'Area Marina Protetta - Consiglio Nazionale delle Ricerche di Genova, per conto dell'Area Marina Protetta, e l'agente della attività di ricerca scientifica del Dipartimento di Zoologia e Genetica Evolutiva dell'Università di Sassari. Questa specie sembra essere fortemente minacciata dal continuo prelievo, fatto da qualche pescatore e anche da altri. Ormai la popolazione di Mal di Ventre è costituita da pochi esemplari e la possibilità che nuove forme possano arrivare da luoghi vicini non è certa.

Molto utile in questa Patella ferruginea non viene dipinto da altre specie simili, oltre per la non il periodo di estinzione non possono essere prelevate, per essere regolate, all'interno dell'Area Marina Protetta.

È quindi necessario educare e sensibilizzare la Patella ferruginea, riportare le disposizioni del decreto ministeriale italiano dell'Area Marina Protetta di Mal di Ventre (2003), che tutela la cultura, la ricerca e il miglioramento delle specie animali, più in generale, monitorare le attività commerciali e ricreative e prendere serie considerazione di questo importante specie. Salvo ogni singolo esemplare di Patella ferruginea è assolutamente importante per consentirne la sopravvivenza di questa specie nell'Isola di Mal di Ventre.



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La tabella e la tabella di distribuzione delle caratteristiche biologiche e genetiche sono tra gli scopi del progetto dell'Area Marina Protetta "Penisola del Sinis - Isola di Mal di Ventre"

NON RACCOLGERE NESSUN ESEMPLARE DI PATELLA FERRUGINEA

"Aiutaci anche tu a conservare la popolazione di *Patella ferruginea* dell'Isola di Mal di Ventre sensibilizzando tutti e segnalando i comportamenti scorretti".
Il prelievo di *Patella ferruginea* è un reato punito ai sensi dell'art.30 della Legge 394/91.

Per qualsiasi segnalazione:

VIGILI URBANI Numero Verde 800 125 018	CORPO FORESTALE 15 15	CAPITANERIA DI PORTO 15 30
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Roll up on the protection need of *Patella ferruginea*.



LA PINNA NOBILIS
aiutaci a proteggerla

Per la salvaguardia di questa preziosa specie della Riserva (Pinna Nobilis)
La Pinna Nobilis è il mollusco bivalve più grande del Mediterraneo, tutti pensano che può superare i metri d'altezza! In sua famiglia ha una forma irregolare che ne permette di agganciarlo saldamente sul substrato favorendo il colonizzare anfratti, rovine e fessure del suolo. Questa specie, endemica per il Mare Mediterraneo, vive in una collina fino a 80 m di profondità. Si trova soprattutto su praterie di fanerogame marine come la Posidonia oceanica, ma anche su sabbia, fango e ghiaia, o nelle grotte tra le rovine. Può raggiungere 30 anni di età, la maturità sessuale avviene tra l'anno e l'anno e mezzo, quindi, in termini di taglia, vengono riferiti a 20 per cento i vari esemplari giovani. Si tratta di un mollusco "beno effluente", perché aderente che vive parzialmente "dentro nel foro". La stessa famiglia è diffusa in tutto il mondo, nei 4 generi Pinna, Arca e Spondylus. Nel Mediterraneo si trovano solo tre specie di questo famiglia: Pinna nobilis (Linnaeus, 1758), Pinna sarda (Fabrizio, 1788) e Arca inflata (Ponslet, 1777). La specie che produce il nobile vino è il padre (Oliviero) originario, l'Arca (Arca arca), il nobile (Pinna nobilis) e l'Arca, in particolare l'Arca è la base della costituzione di Pinna sarda per il periodo degli usi alimentari ed ornamentali, sia per le usanze occidentali dovute a pratiche medievali (ric. modificazioni dei liquori, impieghi, usanze delle stoffe...). In tutto il Mediterraneo, negli ultimi 30 anni, l'abbondanza di Pinna nobilis ha subito una drastica riduzione. Inoltre, il declino del suo habitat preferenziale (le praterie di fanerogame), impedito all'aumento della pressione antropica, ne ha compromesso la sopravvivenza e la sostenibilità.

Beneficiaria per la tutela di Pinna nobilis
È stata riconosciuta come specie protetta e ritenuta per la sua salvaguardia viene paragonata mediante la Direttiva Habitat (2003/43/CE, Direttiva) e la Convenzione di Barcellona (1995/96, Art. 10).

Caratteristiche del Gabbio di Cristallo
• Densità media: 1 individuo ogni 100 mq.
• Le pinne sono concentrate ai bordi delle macchie di sabbia nella prateria.
• Le pinne giovani sono quasi esclusivamente a forma "tecnica" e "fianco".
• A Mal di Ventre prevalgono gli individui grandi dimensioni.
• La mortalità è aumentata nel 2007 e del 10%.
• La percentuale di pinne morte aumenta procedendo da sud verso nord e raggiunge i valori massimi a Mal di Ventre (30%).

Cosa fare per contribuire alla conservazione di questa specie
• Non toccare e non prelevare dal fondo alcun individuo.
• Non ancorare sulla Posidonia o comunque nemmeno nelle macchie di sabbia all'interno della prateria.
• Accettare sempre di buon umore il rifiuto di cariche, se presenti.
• Evitare l'utilizzo di qualsiasi attrezzo che possa danneggiare le rovine (se di carta, metallo, plastica, ecc.).
• Segnalare alle autorità competenti comportamenti inopportuni, riferiti ai numeri gratuiti 150 della Capitaneria di Porto e al 115 del Corpo Forestale e di Vigilia Ambientale della Sardegna.

Progetto ANTIPIG e finanziato nel quadro del programma



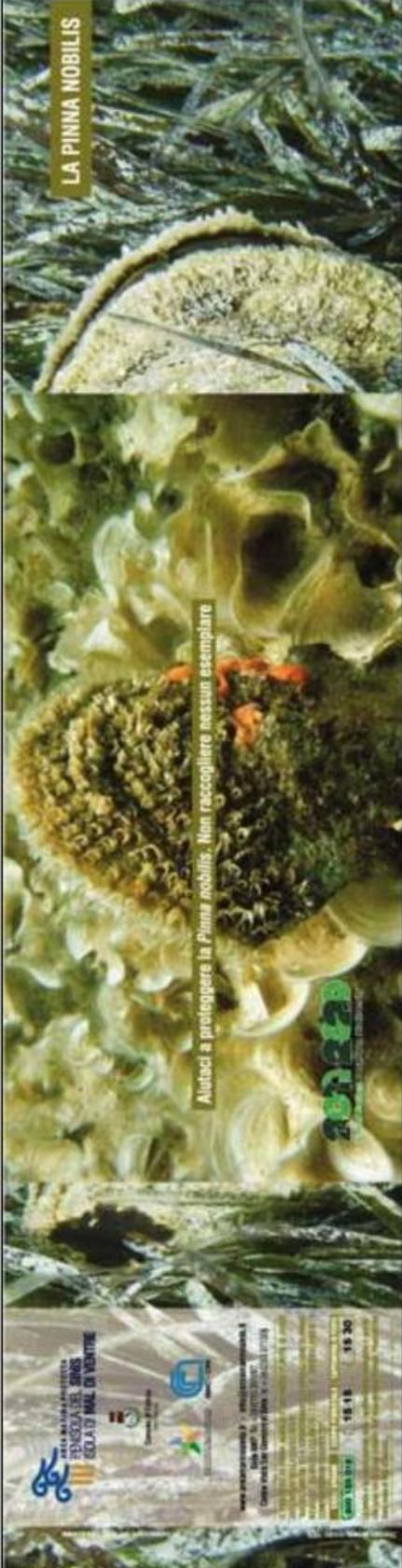
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La tutela e la valorizzazione delle caratteristiche biologiche e genetiche sono tra gli scopi primari dell'Area Marina Protetta "Parco del Sinis - Isola di Mal di Ventre".

NON RACCOLGERE NESSUN ESEMPLARE DI PINNA NOBILIS

"Aiutaci anche tu a conservare la popolazione di Pinna Nobilis dell'Isola di Mal di Ventre sensibilizzando tutti e segnalando i comportamenti scorretti".
Il prelievo di Pinna Nobilis è un reato punibile ai sensi dell'art.30 della Legge 394/91.
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Roll up on the protection need of *Pinna nobilis*.



LA PINNA NOBILIS

Aiutaci a proteggere la *Pinna nobilis*. Non raccogliere nessun esemplare

Per la salvaguardia di questa preziosa specie protetta: il caso della Naucoria (*Pinna nobilis*)

La Naucoria (*Pinna nobilis*) è il mollusco bivalve più grande del Mediterraneo, bast pensare che può superare il metro d'altezza! La sua conchiglia ha una forma triangolare che ne permette il parziale inaffondamento nel sedimento: l'accoppiamento avviene mediante i filamenti del bisso. Questa specie, endemica per il Mare Mediterraneo, colonizza aree costiere fino a 60 m di profondità. Si trova soprattutto su praterie di lavenogamie marine come la Posidonia oceanica, ma anche su sabbia, fango e ghiaia, e nella spaccatura tra le rocce. Può raggiungere 30 anni di età, la maturità sessuale avviene tra il primo e il secondo anno, quindi, in termini di taglia, organismi inferiori a 20 cm sono considerati giovani.

Si tratta di un bivalve della "zona-elfana", perché aderisce che vive parzialmente infossato sul fondo". La stessa famiglia è diffusa in tutto il mondo, con i generi *Perna*, *Atrina* e *Stropaterna*. Nel Mediterraneo si trovano solo tre specie di questa famiglia.

Consigli per contribuire alla conservazione di questa specie

- Non staccare o prelevare dal fondo alcun individuo.
- Non ancorare sulla Posidonia oceanica e nemmeno ai bordi delle macchie di sabbia all'interno della prateria.
- Ancorare su ampie distese sabbiose o utilizzare campi boia, se presenti.
- Evitare l'effetto di qualsiasi attracco che possa danneggiare le macchie (feti da posta, nasse, strascico).
- Segnalare alle autorità competenti comportamenti irresponsabili, telefonando ai numeri gratuiti 1530 della Capitanata di Porto o al 1515 del Corpo Forestale e di Vigilanza Ambientale della Sardegna.

Il caso del Goleto di Ortistene

- Densità media: 1 individuo ogni 100 mq.
- La prateria è concentrata ai bordi delle macchie di sabbia nella prateria.
- La prateria giovane sono quasi esclusivamente nelle aree di frontiera "Sani Vecchia e a Tharros".
- A Mar Moro privilegiano gli individui di grandi dimensioni.
- La mortalità riscontrata nel 2007 è del 14%.
- La percentuale di prateria morta procedendo da sud verso nord e raggiungendo i veltri massimi a Mar Moro (33%).

Normative per la tutela di *P. nobilis*

È stata riconosciuta come specie protetta e inclusa per la sua salvaguardia sono previste mediante la Direttiva Habitat (92-43/EEC, Annesso IV) e la Convenzione di Barcellona (10/06/96, Annesso B).

Pinna nobilis (Linnaeus, 1758), *Pinna nodis* (Linnaeus, 1758) e *Atrina hepia* (Ponslet, 1777). Le specie che presentano *P. nobilis* sono il porro (*Cicopus vulgaris*), l'orata (*Spondylus auratus*), il muscio (*Myoplasma musculosus*) e l'uomo. In particolare l'uomo è causa della capillazione di *P. nobilis* sia per il prelievo legato al suo alimentare ed ornamentale sia per le uccisioni accidentali dovute a pratiche invasive (es. modificazioni del fondo, inquinamento, utilizzo dello strascico...).

In tutto il Mediterraneo, negli ultimi 30 anni, l'abbondanza di *P. nobilis* ha subito una drastica riduzione. Inoltre, il destino del suo habitat preferenziale (le praterie di lavenogamie), irrimediabile all'aumento della pressione antropica, ne ha notevolmente compromesso la vulnerabilità.

Brochure on the protection need of *Pinna nobilis*.

ANNEX III

**Exemples of habits limiting the Sinis MPA effectiveness for the
protection of *Pinna nobilis* and *Patella ferruginea***



Images selection as example of how the no take-no entry zones in the Sinis MPA are disregarded.



Images selection on the damage of gillnets and pots on *Pinna nobilis* and its habitat (*Posidonia oceanica* meadow).



Images selection of people collecting invertebrates along the coast of the Sinis MPA, no take-no entry zones included. Some artisanal gears used to collect invertebrates and the effect on the intertidal zone are shown.

