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DEVELOPMENT OF SCIENTIFICALLY BASE MANAGEMENT TOWARDS A SUSTAINABLE FISHERY OF THE COMMON SEA URCHIN, *PARACENTROTUS LIVIDUS*, IN SARDINIA

Bio/07

Presentata da: Marco Secci

Coordinatore Dottorato: Emanuele Sanna

Tutor: Piero Addis

Tutor estero: Stefano Carboni

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FOREWARD

The body of this thesis is divided into three sections according to the scientific protocol identified at the beginning of my work. The main body of each section comprises of published papers, excluding the third section, which describes *P. lividus* rearing protocol. A general introduction highlights the sea urchin fisheries in the world and in particular in Sardinia. A general conclusion describes how my findings could improve the sustainability of the fishery in Sardinia.

I have also actively participated in other scientific works that are shortly presented (abstract of publications) as annexes.

GENERAL INTRODUCTION

There are long traditions of consuming sea urchin in many cultures, particularly in Asia, Polynesia, the Mediterranean and Chile. Archaeological evidences proved their consumption in prehistory amongst Mediterranean communities (Gutiérrez-Zugasti, 2011). In recent years sea urchins have become a premium commodity in Japan where the 63.9% of the world production is consumed under different forms: live, fresh or chilled (65%), frozen (10%) and fermented or in brine (25 %) (FAO Fisheries commodities production and trade 1976-2009, 2012).

Sea urchin fishery is a roe fishery where both males and females of the species are harvested for their gonads, generally referred to as “roe” (called *uni* in Japan) in the fishery and catering market (Lawrence, 2001). The average price of roes, on the Japanese market, ranges from 18.6 €/kg for the local production (fresh animals considered as top quality), to 7.9 €/kg of fresh imported echinoids (Hagen, 1996). In Sardinia the average price of fresh roe is about 80 €/kg, more than four times the Japanese price (Cau *et al.,* 2007), demonstrating that this small-scale fisheries is characterized by high profitability. Indeed, one of the main driver of sea urchin exploitation is the high return on investment achievable by this specialized and relatively small-scale fishery industry. This is mainly due to the low capital and running costs of the venture and to the relatively high market price of the product (Carboni, 2013c). Water content, texture, firmness, colour, and taste are important quality factors in sea urchin roe marketing and they strongly influence the price (Spirlet *et al.,* 2000; Unuma, 2002; McBride *et al.,* 2004).

One of the main concerns on the sea urchin fishery is related to poor sustainability. Fishing for sea urchins can be a very profitable business during the first 5 to 10 years operations on a new fishing ground. However, after that short period of time, wild populations decline due to the high efficiency and selectivity of the employed fishing techniques: animals are easily picked by hand or using simple equipment at shallow depths (Andrew *et al.,* 2002; Addis *et al.*, 2009).

In several countries such as USA, Ireland, France and Spain, sea urchin fishery declined considerably since their peaks and stocks are now likely to be over-exploited (Andrew *et al.,* 2002; Barnes *et al.,* 2002).

Paracentrotus lividus is the main echinoid consumed in Mediterranean and Atlantic Europe (FAO, 2013; Fig. 1). France is the world's second largest consumer of sea urchin, consuming around 100 t per year (Hagen, 1996; FAO, 2013). Italy does not appear on FAO statistics although sea urchin fisheries occur in the South and the Islands (Guidetti *et al.,* 2004; Addis *et al.,* 2009; Pais *et al.*, 2011).

Sea urchin fishery started in Sardinia at the end of the 1980s. Due to increasing economic importance of the fishery, in 1994 the local Department for Environmental Protection of the Autonomous Region of Sardinia introduced several limitations on fishery operations: regulated of fishing seasons (November-March), limited number of licensed fishermen (161), fixed daily individual quota (2000 sea urchin d^{-1}) and a minimum size limit of 50 mm of test diameter (Table 1).

Despite the fact that a knowledge-gap on the production as well as on the conservation status of the populations over time in Sardinia exists, limits for fishery changed in 2001 resulting in increased daily quota (1500 sea urchin d^{-1} per fisherman; 3000 sea urchin d^{-1} fisherman with a boatman) and in 2009 in extended fishing season length (November-May) and number of licensees (180) (RAS, 2001; 2009) (Table 1).

Table 1 - Key management strategy of sea urchin fishery in Sardinia according to RAS (1994; 2001; 2006; 2008; 2009; 2010; 2011; 2014).

Year	Key management strategy
1994	Fishing season (Nov-Mar), size limit (50 mm), individual daily quota (2000)
2001	Individual daily quota (1500/3000 d^{-1}), number of licenses (115)
2006	Fishing season (Nov-Apr)
2008	Number of licenses (161)
2009	Number of licenses (180)
2010	Number of licenses (189), Fishing season (Nov-May)
2011	Log-book
2014	Daily fishing time (Sunrise-2:00 p.m.)

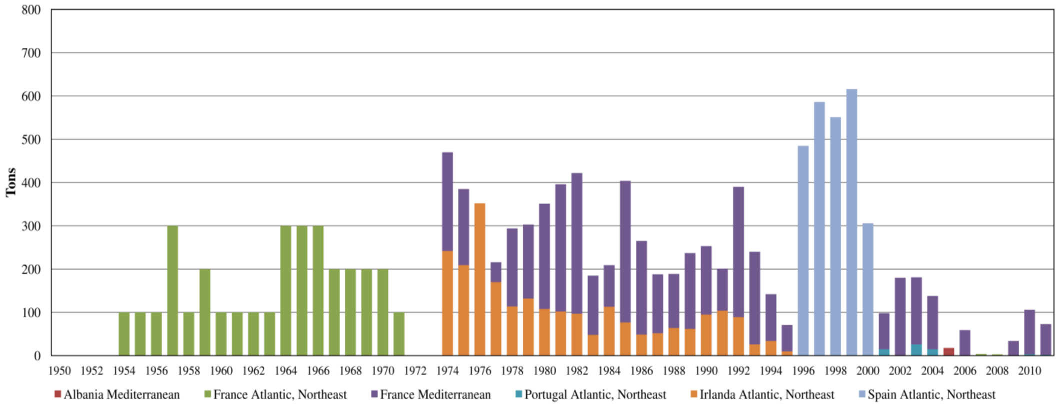

Figure 1 - Capture of *Paracentrotus lividus* during the period 1950-2011 according to FAO Capture Production 1950-2011 (release date 2013).

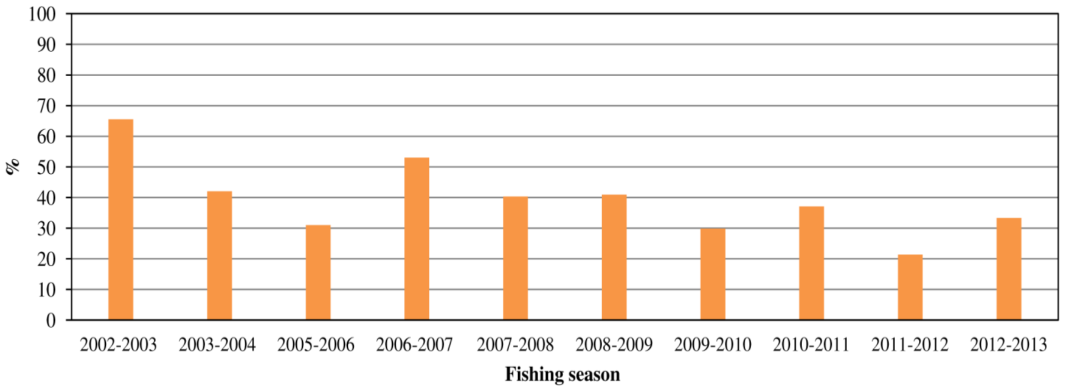

Figure 2 - Percentage of sea urchin larger than 50 mm in test diameter landed in Sardinia according to the voluntarily monitoring of Department of Life and Environmental Sciences during the period 2002-2012.

Nonetheless, studies conducted in north Sardinia confirmed that the harvesting is causing a negative effect on sea urchin populations (Pais *et al.*, 2007; 2011). Additional information come from two investigations conducted in 2002 and in 2006 by the Department of Life and Environmental Sciences of the University of Cagliari within an agreement with the Regione Autonoma della Sardegna - Fishery Service (Cau *et al.*, 2003, 2007). Such investigations were aimed to identify some reference points of the sea urchin fishery. Afterwards, the absence of a regional monitoring plan has led voluntarily the Department of Life and Environmental Sciences to conduct monthly samplings in the sea urchin market in Cagliari. On this issue, time series analysis on test diameter data, have shown a negative trend of the percentage of legal size during the period 2002-2013 (Fig. 2). It must be pointed out that the reduction in the marketable sizes has been recognized as an indicator of over-fishing of sea urchin wild stocks (Carter and VanBlaricom, 2002). These findings suggest that the risk of collapse of the sea urchins stocks in Sardinia could be imminent, unless the fishery agencies take on immediate strategies for the species conservation.

Considering such preconditions, it is imperative to guarantee a sustainable fishing, via adaptation of the sea urchin exploitation levels to the carrying capacity of the resource, and combine this with the economic profitability, and social benefits.

In my thesis, I conducted the work towards the development of possible alternative strategies to improve the sustainability of current management practices for sea urchin fishery in Sardinia. In detail, I have considered two main approaches:

1) To develop a practical tool for the stock assessment of the resource useful to determine the carrying capacity of the stocks;

2) To implement juvenile production protocols within a suitable land-based holding system designed for sea urchins breeding and on-growing. Juveniles produced in such system will be used for reseeding purposes and culture to market size with the final aim of reducing harvesting of wild populations.

Stock assessment options for invertebrates or finfish, includes the so called Biomass Dynamic Models, including the Schnute model, the Time series fitting, and the Bayesian model. All of these require systematic scientific or/and fishery-dependent data to obtain long term CPUE (Catch per Unit of Effort) data series (Perry *et al.*, 2002; Chen and Hunter, 2003).

Failing a systematic scientific or/and fishery-dependent data monitoring, a pragmatic (practical and fast) tool of stock assessment is a key requisite to preserve sea urchin stocks. In this sense, fisheries managers need to develop efficient tools in response to the sharp changes in abundance caused by fishing (see: Perry *et al.*, 1999). A useful method comes from geostatistic, a branch of applied statistic that focuses on detecting, modelling and estimating spatial patterns of geo-referenced environmental data (Rossi *et al.*, 1992). This technique was initially developed to be used in the mining industry during the 1950s. Over the past 20 years, however, there has been an increase in the use of geostatical analysis to study marine systems (Petitgas, 2001) and to solve fishery related problems, ranging from estimating abundances from survey data (Simard *et al.*, 1992; Barange and Hampton, 1997), to planning resource management (Castilla and Defeo, 2001), reducing the risks of overfishing (Orensanz *et al.*, 1998) and quantifying relationships between abiotic variables and species distributions (Maravelias *et al.*, 1996). In 2009 the method has been tested for the first time for *P. lividus* (Addis *et al.*, 2009) in order to predict harvestable stocks on a fine resolution scale in Sardinia. The technique requires a relatively limited number of samples; no complex statistical decisions and the final output (kriging maps) are easier to read, making it an accessible tool for fishery managers (Petitgas, 1993; Defeo and Rueda, 2002; Addis *et al.*, 2009).

The habitat plays a key role in the ecological behaviours of *P. lividus* providing sea urchin with shelter and influencing the size, movement, and spawning (Bonaviri *et al.*, 2005, Hereu *et al.*, 2005; Boudouresque and Verlaque, 2001; Ceccherelli *et al.*, 2009; Ouréns *et al.*, 2011). Colour and gonad index are important quality factors in sea urchin roe marketing (Shpigel *et al.*,, 2006). In the wild, they strictly depend on the quality and quantity of food available in the habitat where they live (Ouréns *et al.*, 2011, Shpigel *et al.*, 2006). Therefore, fishery managers could use information on how different habitats affect the main quality factors of sea urchin gonad to improve sustainability and efficiency of the industry.

Due to the serial depletion of sea urchin stocks and the increasing demand, mariculture could have an important role to play to fulfil the growing gap between market demand and supply (Andrew *et al.,* 2002; Carboni *et al.,* 2013a). Sea urchin aquaculture represents the 0.9 % of the world aquaculture production. China is the world's largest producer (99.5 %), followed by Russia (0.4 %) and Ireland (0.1 %). The price depends largely on the cultured species and the quality of gonads (FAO Aquaculture production, 2013).

Due to its high roe content and the high prolificacy, *P. lividus* has been identified by aquaculturists as an ideal candidate to satisfy increasing demand in Europe (Watson and Stokes, 2000). Recently many studies have focused on culture methods for both larvae (Leighton, 1995; Kelly *et al.,* 2000; Kirchoff *et al.,* 2008; Carboni *et al.,* 2012a, 2012b), adults (Grosjean *et al.,* 1998) and nutritional requirement/roe enhancement (Shpigel *et al.,* 2006; Carboni *et al.,* 2013a). In general, sea urchin hatchery techniques are well established, but cost effective grow-out strategies are still under development (Kirchoff *et al.,* 2008). Culture methods from aquaculture and research of sea urchin could be used as operational tool to stock enhancement adding cultured juveniles into the wild. Few examples where aquaculture has been employed for restocking sea urchin population come from Japan and Philippines. The effectiveness of these stock enhancement programmes in Japan has not yet been evaluated comprehensively (Saito, 1992; Kitada, 1999; Agatsuma *et al.,* 2003; Bell *et al.,* 2005). In Philippines the sea urchins *Tripneustes gratilla* has been successfully cultured and then released in the wild. However very high mortality (91-99%) was observed (Juinio-Menez *et al.,* 2008).

The aim of this thesis is to encourage a science-based management of the sea urchin fishery in Sardinia in order to avoid the collapse of this resource. This overarching objective has been achieved through: a) investigation the pre- and post-effects caused by fishing harvesting on spatial patterns, abundance fluctuations and total mortality rate; b) improvement of the knowledge of the effect of habitat on the ecology behaviour of the species; c) introduction of aquaculture as operational tool to improve wild stocks numbers via restocking and to provide viable alternatives to commercial fishery.

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BIOLOGY OF *PARACENTROTUS LIVIDUS*

Paracentrotus lividus (Lamark, 1816) (Echinodermata: Echinoidea: Echinidae) is a regular echinoid distributed throughout Mediterranean Sea and in the North-Eastern Atlantic, from Scotland and Ireland to southern Marocco and the Canary Islands, including the Azores Islands. It is particularly common in the Mediterranean Sea where inhabits rocky sea-bed areas and meadows of *Posidonia oceanica* up to a depth of 40 m. It is one of the most important grazers in shallow rocky reefs, and may play a part in the transition from macroalgae beds to coralline barrens (Sala *et al.*, 1998; Boudouresque and Verlaque, 2001; Guidetti *et al.*, 2003).

Sexes are separate and fertilization is external. The reproductive cycle is highly dependent on the location. Generally, it has one or two seasonal peaks in early spring and in autumn (Boudouresque and Verlaque, 2001).

In Sardinia, an investigation conducted on seven month samplings on gonads (Cau *et al.*, 2003), revealed two peaks of the relative gonad index, GI (i.e., gonad weight/body weight * 100), which occurred at the end of January and June. Although such information was not complete, demonstrated that the species is locally affected by environmental conditions in the process of gametogenesis, as also resulted from the literature (see Ouréns *et al.,* 2011).

Eggs are fertilized in the water column and develop into free-swimming planktotrophic larva characteristic of echinoids: the echinopluteus (Fig. 3).

This larva develops 4, 6 and 8 arms supported by calcareous skeletal rods. After a few weeks (2-4), the echinopluteus will develop a rudiment inside the wall of an epidermic invagination (the vestibule) located on the right-hand side of the body.

When the larva becomes competent, it seeks a solid substrate to settle and metamorphose (Fig. 3).

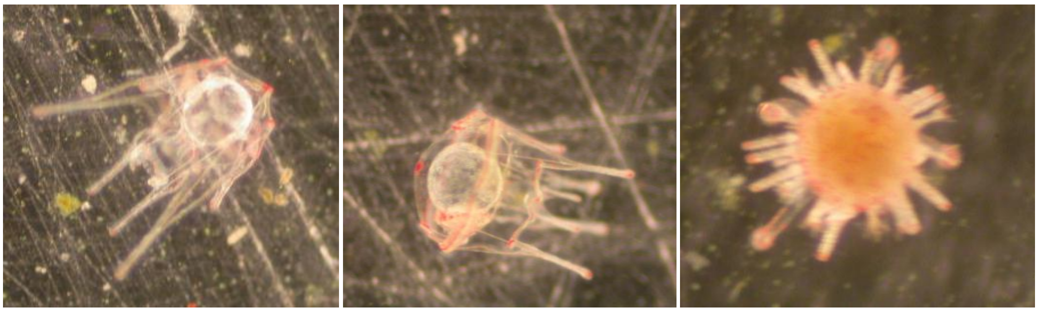

Figure 3 - From left to right: echinopluteus with 8 arms; echinopluteus with rudiment; postlarva one hour post metamorphosis.

Metamorphosis lasts less than one hour: the echinoid rudiment is evaginated and most larval tissues are reabsorbed. The postlarva resembles a miniaturized adult (Fig. 3) but has no mouth and no anus, and is thus endotrophic. After a week the postlarva has undergone some major changes and becomes an exotrophic juvenile with a fully developed and functional digestive tract (Grosjean, 2001).

The body of *P. lividus* has pentaradial symmetry (Fig. 4). The test is composed by an endoskeleton, formed of calcareous ossicles, and the epidermis. Movable spines cover the body of the animal. *P. lividus* can reach a maximal test diameter of 70 mm (Boudouresque and Verlaque, 2001). The mouth is a complex system called Aristotle's lantern, composed by 5 pyramids each ending with a tooth (Fig. 5). From the mouth starts the pharynx followed by digestive tract composed by the esophagus, the oral and the aboral intestine. The feaces are expelled by anus in the aboral pole.

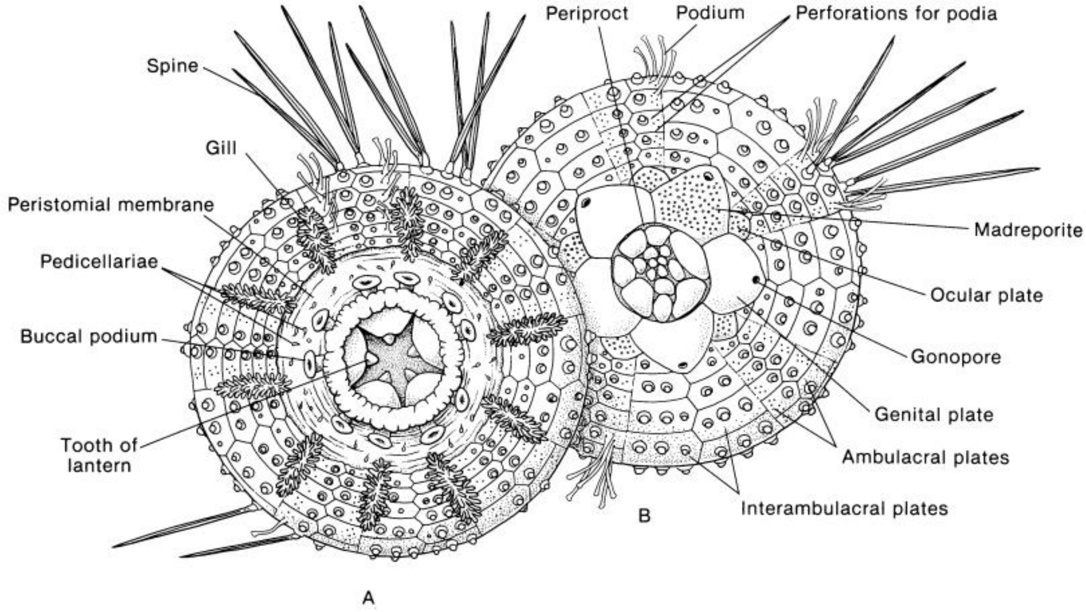

Figure 4 - External anatomy of a regular sea urchin. A. Oral view. B. Aboral view. (after Reid, W.M,. In: Ruppert and Barnes, 1994).

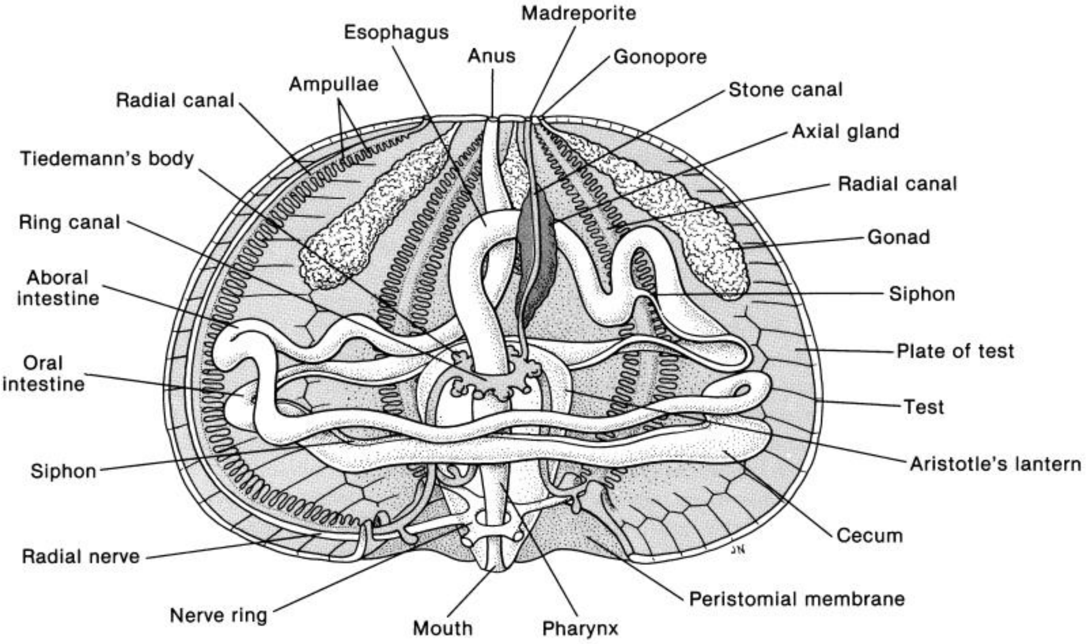

Figure 5 - Internal anatomy of a regular sea urchin, side view. (modified after Reid, W.M., In: Ruppert and Barnes, 1994)

Figure 6 - Mature adult *P. lividus* showing the five gonads.

Five gonads and relative genital pores form the reproductive organs. Gonads are disposed radially in the coelomic cavity. Gametes are emitted by the genital pores disposed around the anus (Fig. 5). *P. lividus* reaches the size at first maturity at 20-25 mm showing a 1 cm variation between geographic areas (Ouréns *et al.*, 2011). Sea urchin gonads consist of two major cell types: 1) germinal cells, which produce gametes, and 2) nutritive phagocytes, which provide the nutrients and energy required by the developing gametes (Walker *et al.*, 2001). Maternal provisioning of nutrients is an important factor also in the development of the embryo (Carboni *et al.*, 2013b).

The factors involved in the reproductive biology of sea urchins are still poorly understood (Wasson *et al.,* 2000). Reproductive cycles have been correlated with changes in a variety of envinromental cues including temperature, salinity, photoperiod and food availability (Pearse and Cameron, 1991). Wasson *et al.* (2000) suggested that the environmental cues are transduced presumably to endogenous chemical signals, which regulate gamete differentiation and growth of gonad but the action mechanism of steroids in echinoderms is still to be explained. Barbaglio *et al.* (2007) found remarkable sex steroid levels of Testosterone (T) and Estradiol (E2) on gonads of *P. lividus*. Sugni *et al.* (2012) found a slight decrease in the gonad index and an increase in lipid content due to E2 treatment. Their results suggested that E-2 could have a function different from that reported for vertebrates and suggested for other echinoderms such as asteroids.

Sea urchins have a water-vascular system, which is used for locomotion. The system is composed of canals connecting numerous tube feet (podia) with sucker-shaped tip. Contracting or relaxing muscles they force the water into the tube feet, causing the extension or the retraction of podia. Spines can be used to aid the locomotion and to improve the grip on the substratum (Grosjean, 2001).

In general sea urchin populations show patchy spatial distribution rather than uniform in their density distribution (Addis *at al.*, 2009; Guidetti *et al.*, 2003; Barnes and Crook, 2001). Average density vary between geographic areas, in general it ranges from a few to a dozen individuals per m^2 (Boudouresque and Verlaque, 2001). Sometimes small individuals tend to stay under larger ones, including inside holes, as a protective mechanism against predators (Tegner and Dayton, 1977; Grosjean, 2001).

Sea urchins show the behaviour called “spine canopy” where specimens cover their exposed aboral surfaces with shells, stones, and algae for camouflage by predators (Boudouresque and Verlaque, 2001; Grosjean, 2001) (Fig. 7).

For the most part, food preferences have been determined by means of aquarium experiments in adults (Boudouresque and Verlaque, 2001). *P. lividus* is basically herbivourous. Macroalgae (i.e. *Cystoseira amentacea*, *Padina pavonica*, *Rissoella verrucolosa* etc.) and the seagrasses (i.e. *Cymodocea nodosa* and *Posidonia oceanica*) are clearly “preferred” as feed (Boudouresque and Verlaque, 2001). Sea urchins are the main herbivores and often influence the abundance and composition of algal assemblages. The effects of sea urchins on seaweeds are well- documented phenomena in many types of habitats (see Hereu, 2006). At high densities, they are capable of depleting all erect algae, which leads to the appearance of barren areas dominated by calcareous encrusting algae (see Hereu, 2006). As regards larvae nutrition, they are planktotrophic. Several different microalgae species such as *Cricosphaera elongata*, *Pleurochrisys carterae* and *Dunaliella tertiolecta*, insure normal growth, survival rate and development (Gago *et al.,* 2009; Carboni *et al.,* 2013b). The presence of live macroalgae (i.e. *Ulva lactuca*, *Corallina elongata*) is a recognized metamorphosis inducing factor (Grosjean, 2001; Vaïtilingon *et al.,* 2001). Diatoms seem to play an important role as feed in the early stage of post- larvae nutrition (Pers. comm. J. Chamberlain, Dunmanus Sea-Foods, Ireland). The potential value of benthic diatoms as food for metamorphosed sea urchin has been well studied on the genus *Strongylocentrotus* (Xing *et al.*, 2007; Onitsuka *et al.*, 2013). Adults are macroalgivores in the field, only switching to becoming a detritivores and browser when macroalgae becomes limiting (Boudouresque and Verlaque, 2001).

Natural mortality is mainly driven by predation. Efficient sea urchin predators has been recognized in the sparids *Diplodus sargus*, *Diplodus vulgaris*, *Sparus aurata* able to kill adult and juveniles and the labrids *Coris julis* and *Thalassoma pavo*, which feed on juveniles (Sala, 1997; Guidetti *et al.*, 2005; Guidetti, 2006). Other causes of mortality are related to diseases, dynoflagellate's bloom and storms (Boudouresque and Verlaque, 2001). *P. lividus* is a stenohaline species, sensitive to a drop in salinity which can cause mass mortality of populations in coastal lagoon (Fernandez *et al.*, 2006).

Figure 7 - Spine canopy in *Paracentrotus lividus*.

CHAPTER 1:

SPATIAL DISTRIBUTION PATTERNS AND POPULATION STRUCTURE OF THE SEA URCHIN, *PARACENTROTUS LIVIDUS* (ECHINODERMATA: ECHINOIDEA), IN THE COASTAL FISHERY OF WESTERN SARDINIA: A GEOSTATISTICAL ANALYSIS

Addis P., **Secci M.**, Angioni A., Cau A. (2012). *Scientia Marina* **76**: 733-740.

ABSTRACT

The identification of appropriate spatial distribution patterns for the observation, analysis and management of stocks with a persistent spatial structure, such as sea urchins, is a key issue in fish ecology and fisheries research. This paper describes the development and application of a geostatistical approach for determining the spatial distribution and resilience of the sea urchin *Paracentrotus lividus* population in a fishing ground of western Sardinia (Western Mediterranean). A framework, combining field data collection, experimental modelling and mapping, was used to identify the best-fit semivariogram, taking pre-fishing and the post-fishing times into consideration. Variographic analyses indicate autocorrelation of density at small distances, while the isotropic Gaussian and spherical models are suitable for describing the spatial structure of sea urchin populations. The point kriging technique highlights a generally patchy population distribution that tends to disappear during the fishing season. Kriging maps are also useful for calculating predictable stock abundances, and thus mortality rates, by class diameters within six months of fishing. We conclude that the framework proposed is adequate for biomass estimation and assessment of sea urchin resource. This can therefore be regarded as a useful tool to encourage a science-based management of this fishery.

Keywords: Paracentrotus lividus, spatial distribution, geostatistics, biomass estimation, management, Western Mediterranean.

INTRODUCTION

Over the past decade there has been an increasing interest in modelling and measuring spatial patterns (e.g., gradients and patches) in biotic variables as a means of understanding the mechanisms that control critical aspects of the ecology of species, such as spatial distribution (Legendre and Legendre, 1998).

With the advancement of computer science, geostatistics has become a powerful tool for estimating the spatial distribution of marine populations (Conan, 1985; Maynou, 1998), for predicting stock abundances (Petitgas, 1993, 2001; Megrey and Moksness, 2009) and to assess marine reserves benefits (Stelzenmüller *et al.*, 2007). Indeed, the identification of appropriate spatial scales for the observation, analysis and management of stocks with a persistent spatial structure, is a key issue in fish ecology and fisheries research (Orensanz *et al.*, 2006; Ciannelli *et al.*, 2008).

Due to their low mobility, numerous benthic commercial species can be considered suitable for geostatistical applications (Jensen and Miller, 2005; Adams *et al*., 2010), and the purple sea urchin, *Paracentrotus lividus*, represents an ideal species for a case-study. *P. lividus* is common throughout rocky intertidal and shallow subtidal zones of the Mediterranean Sea and north-eastern Atlantic Ocean where is generally associated with erect macro-algae. Its spatial distribution can vary on both small and large scales in relation to the interaction of abiotic and biotic factors (Boudouresque and Verlaque, 2001). For example, the variability of water temperature and solar radiation can partially explain intraspecific variation in terms of covering behaviour type in *P. lividus* (Crook, 2003). The heterogeneity of the substratum plays a key role in providing *P. lividus* with shelter, thus influencing the structuring of populations, where predation pressure (which includes human harvesting) is particularly high (Bonaviri *et al.*, 2005; Hereu *et al.*, 2005). Among human-related impacts, site accessibility during harvesting by diver fishermen, significantly affects the structuring of sea urchin populations in a fishing ground in northern of Sardinia (Ceccherelli *et al.*, 2011). Recent studies on sea urchin predation (Sala and Zabala, 1996; Guidetti *et al.*, 2004), recruitment (Tomas *et al.*, 2004), migration (Palacín *et al.*, 1997; Crook *et al.*, 2000), competition (Guidetti, 2004) and harvesting (Pais *et al.*, 2011), employ conventional approaches that assume spatial independence of a measured variable (specifically abundance indices), i.e. values at one location are independent of values at neighbouring locations. Although conventional approaches are equally valid, they raise some constraints about the usefulness of the ecological data gathered for biomass estimates, in terms of spatial scales and the setting of their confidence limits (Addis *et al.*, 2009). In this respect, geostatistical techniques constitute powerful tools for estimating the spatial distribution of marine benthic communities than conventional statistical methods because they explicitly consider spatial correlation between observations (Warren, 1998; Rueda, 2001).

According to Boudouresque and Verlaque (2001), the spatial domain of *P. lividus* populations range from fishing areas where the stock is sufficiently abundant to support a commercial fishery (an isolated bay of a few km^2) to small scale aggregations within a bed or "patches", measuring tens to hundreds of m^2, where ecological experiments are usually developed. However, there have been no attempts to describe spatial structure of *P. lividus* populations by geostatistics, for instance the estimation of semivariograms and its descriptors (nugget, sill and range) which are useful to evaluate the extent of spatial correlation in the data. Such applications are the basis for spatial perception of sea urchin stocks and thus for the successful of fisheries management (Chen and Hunter, 2003; Grabowski *et al*., 2005).

P. lividus is the main echinoid exploited in Europe (FAO, 2013), but the information we have on the current status of populations along the coastal areas of the Mediterranean are scant (Andrew *et al.*, 2002). Trends in relative abundance or stock assessments never have been estimated, nevertheless the decline of landings in few fisheries indicate that populations have been severely depleted (Andrew *et al.*, 2002). Major concerns regards the lack of time series data, in terms of both commercial landings and fishery-independent surveys, which is indispensable for the assessment of sea urchin stocks by means of catch-per-unit-of-effort (CPUE) (Perry *et al.*, 2002; Chen and Hunter, 2003).

We underline that sea urchin fisheries management for *P. lividus* are in need of a precautionary approach 'sensu FAO' (1996) in order to avoid the risk of stock collapse, as has occurred in some fisheries of northern Europe where studies on the impacts of sea urchin harvesting were neglected (Sloan, 1985; Byrne, 1990). Our case-study refers to the sea urchin dive fishery of Sardinia (southern Italy) where fishing for *P. lividus* has a significant social-economic impact, but the management has been largely unsuccessful at conserving the stock and ensuring a sustainable fishery (Cau *et al*., 2007; Pais *et al*., 2011).

Since the elucidation of spatial distribution patterns is essential for abundance estimates of sea urchins stocks, the objectives of this study were to a) determine, model and map the spatial structure of *P. lividus* population in a fishing ground in western Sardinia, b) assess the spatial patterns by size considering the pre- and post-effects caused by fishing harvesting and c) evaluate the predictable number of specimens to detect abundance fluctuations due to harvesting and assess the total mortality rate.

MATERIALS AND METHODS

Study area and field samplings

The study area is located in central-western Sardinia (Fig. 8). Capo Pecora is a shallow open bay where the geomorphology of the sea bottom is characterized by pebbly, metric and decimetric blocks that are highly eroded and rich in caves and rock shelters, providing suitable habitat for certain benthic organisms, particularly sea urchins**.** The biocenosis includes upper subtidal algae exposed to high wave action and *Posidonia oceanica* meadows (Pérès and Picard, 1964). The most representative algae are in the genus *Cystoseira,* with a prevalence of *Cystoseira stricta* var. *amantacea.* Other important algal genera are *Laurencia*, *Dictyota*, *Dictyopteris*, *Codium*, *Stypocaulon*, *Padina*, *Acetabularia*, *Halimeda* and *Amphiroa*.

Surveys were conducted in an area encompassed by a 1.5 km stretch of shoreline to a depth of 10 m (with total surface area of 0.2 km^2). We superimposed a regular grid, subdivided into 30 m×30 m cells, and selected 90 of the grid's 270 cells, representing one-third of the whole area. Stations were randomly selected as starting points from where underwater counts of sea urchins were carried out within three random replicate quadrates of 1 m^2. Each station was geo-referenced (latitude–

longitude) by GPS using Universal Transverse Mercator projection (UTM). Size data were obtained for each station by measuring all individuals >1 cm in diameter, using a Vernier calliper (mm). Data on diameter size were grouped into three size classes: 10-29.9 mm (Juvenile), 30-49.9 mm (Medium) and ≥50 mm (Adult), which corresponds to the minimum size for commercial fishing.

Experimental surveys were conducted in October 2010, prior to the beginning of the fishing season (pre-fishing) and in May 2011 at the end of the fishing season (post-fishing) for all of the 180 stations investigated.

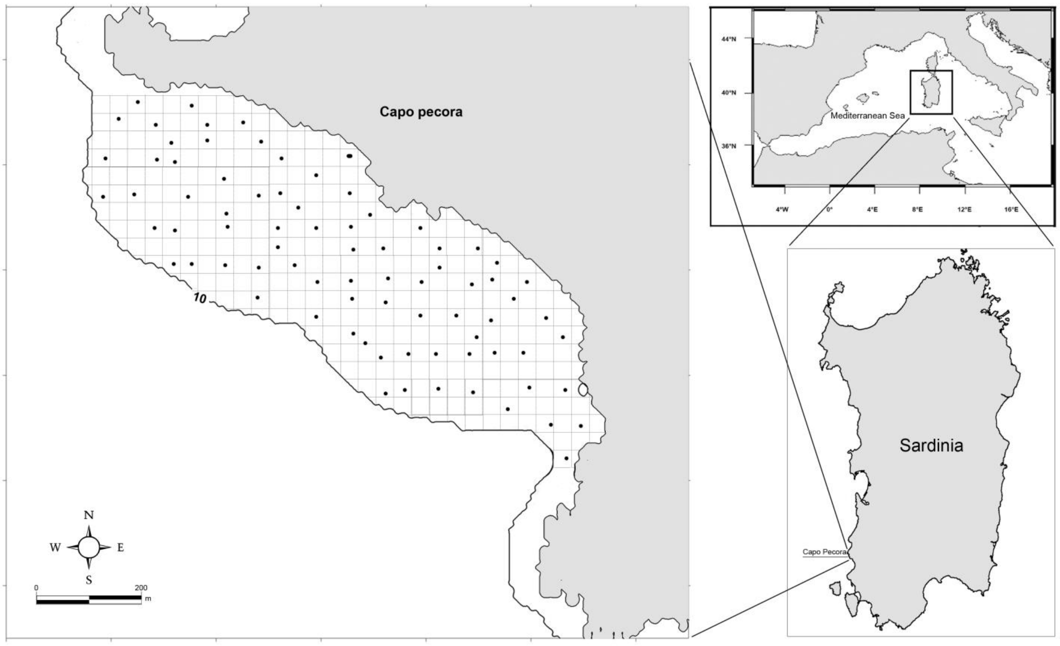

Figure 8 - Study area at Capo Pecora bay (W Sardinia, Mediterranean Sea) showing the fixed grid of 270 cells and the 90 sampling stations (•) randomly selected during the pre-fishing survey.

Statistical analysis

Mean density (±se) for three size classes was calculated; results were plotted on histograms and classed post–maps, to check for errors in the raw data and to verify whether geo-statistical analysis could be applied. A preliminary Z-test was performed to detect differences between mean pre-fishing and post-fishing density.

Density was successively defined as degrees of autocorrelation among measured data points for each size class diameter. This was obtained by a non-directional experimental semivariogram $\gamma(h)$ computing the variance of a population, while taking the spatial position of the sampled stations into account, making use of the following equation (Matheron, 1965):

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} \left[Z(x_i + h) - Z(x_i) \right]^2$$

where $Z(x_i)$ represents the density of sea urchins at sampled station x_i, $Z(x_i+h)$ is a variable value separated from x_i by a distance h (measured in meters), and N(h) is the number of pairs of observations separated by h. To avoid decomposing semivariograms at large lag intervals, the default active lag distance was set close to 70% of the maximum lag distance. We undertook the following estimation for each experimental semivariogram: the nugget effect (C_0) is attributable to measurement error, micro-scale variability or small-scale spatial structure; the sill ($C+C_0$) can be defined as the maximum variability point beyond which the semivariance values become asymptotic; the range (A_0) represents the distance within which the data remain autocorrelated (Maynou 1998). The model that best explained the spatial structure of each case was selected on the basis of values for the reduced sum of squares (RSS) and the coefficient of determination (r^2)

(Cressie, 1991).

Semivariogram parameters of the selected model for each size class at each time were employed using the spatial estimation technique known as 'point-kriging'. This enabled us to create bidimensional density maps. The kriging estimate of Z(x) at each node was obtained by a linear combination of the samples, each weighted by a factor (λ_i), which depends on the combination of the relative position of the sampling points, the theoretical semivariogram, and the $Z(x_i)$ values at the sampling points (Matheron, 1965). The estimated density values Z(x) were given by:

$$Z(x)=\sum_{i}^{n}\lambda_i Z(x_i)$$

The validity of the models in the variographic analysis and kriging interpolations was evaluated using jack-knife cross-validation, performed by sequentially deleting one datum and using the remaining data to predict the deleted density value; the selected semivariogram model and kriging parameters were applied to this end (Maravelias *et al.*, 1996). The observed (O) and estimated (E) densities were plotted and fitted to a linear regression $O=\alpha+\beta E$; the significance of α and β was tested (t-test) under the null hypotheses $\alpha=0$ and $\beta=1$ ($P=0.05$) (Power, 1993).

The predictable number of specimens was calculated by scaling the surface of kriging maps with mean densities for each countering layer, including the confidence limits (mean±se). Predictable number of specimens of pre-fishing (October) and post-fishing (May) by size has been used to estimate the total mortality rate ($Z=M+F$) (Ricker, 1975) by:

$$Z = -\ln N_t/N_0$$

Where N_t is the estimated number of sea urchin in the post-fishing period and N_0 is the number in the pre-fishing period. Since Juveniles and Medium individuals should be only affected to removal not associated with fishing, the total mortality rate (Z) for these classes corresponds to the natural mortality (M); F is the fishing mortality.

Calculations of semivariograms and kriging maps were carried out using geostatistics software Gs+ ver. 7 (Gamma Design Software, LLC) and Surfer8 (Golden Software, Inc.).

RESULTS

The proportion of sea urchins by size classed assessed in the pre-fishing and post-fishing phase, are illustrated in Fig 9. The most representative class in both surveys was that of Medium (~57%) followed by Juvenile (~29%) and Adults (~14%) (Fig. 9). Mean densities (mean+se ind./m^2) of Juvenile in the pre- and post-fishing periods were 1.16±1.24 ind./m^2 and 0.70±0.11 ind./m^2, respectively; the mean densities of the Medium class in pre- and post-fishing periods were 2.03±1.73 ind./m^2 and 1.51±0.16 ind./m^2, respectively; mean density range of Adults in pre- and post-fishing periods was 0.50±0.53 ind/m^2 and 0.35±0.04 ind./m^2ind, respectively. Mean density of pre-fishing and post-fishing populations indicated significant differences for all classes ($P<0.05$).

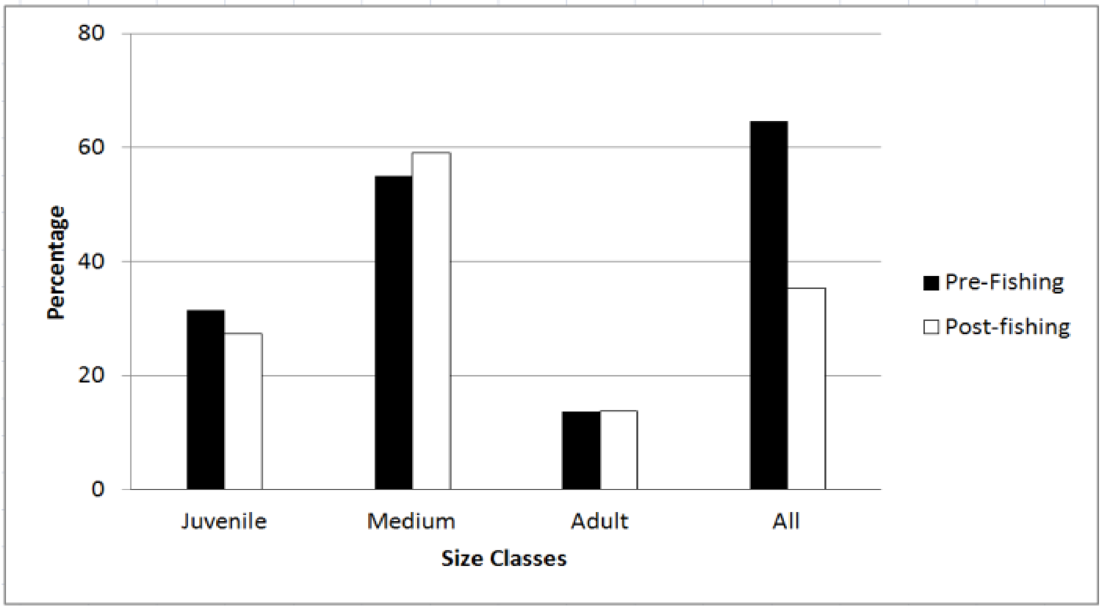

Figure 9 - Percentage size distribution of sea urchins in pre-fishing and post-fishing.

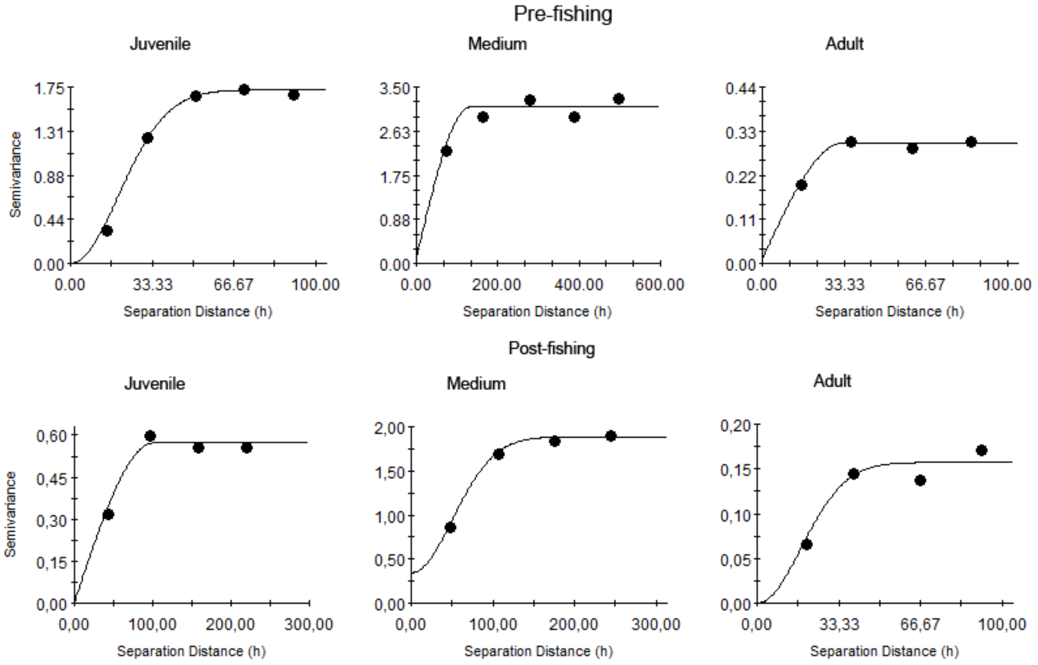

Figure 10 - Experimental semivariograms estimated with spherical and Gaussian models for each size class during pre-fishing and post-fishing. Model parameters are listed in Table 1.

The calculation of experimental semivariograms revealed that densities of the three size classes of *P. lividus* were spatially structured (Fig. 10). Isotropic Gaussian and spherical models provided the lowest RSS and the highest r^2 of all analyzed models, successfully explaining the spatial population structure of the three size classes analyzed from the two periods (Table 2).

Table 2 - Summary of the semivariogram models, Gaussian (Gaus) and spherical (Sph); relative descriptors (lag, nugget, sill and range) and goodness of fit criteria based on sea urchin density by size. Lag (in meters); C_0: nugget effect; C_0+C: sill; A_0 (in meters): range; C/(C_0+C) (in percentage): spatially structured component; r^2: coefficient of determination; RSS: reduced sum of squares.

	Pre-fishing			**Post-fishing**		
Size class	Juvenile	Medium	Adult	Juvenile	Medium	Adult
Model	Gaus	Sph	Sph	Sph	Gaus	Gaus
Lag	20	110	24	63	70	26
(C_0)	0	0.11	0.01	0	0.34	0
(C0+C)	1.72	3.1	0.3	0.57	1.88	0.15
A_0	48.32	132	33.2	100.8	124.53	44.16
C/(C_0+C)	99.9	96.3	96.3	99.8	81.7	99.9
r^2	0.99	0.81	0.97	0.97	0.99	0.91
RSS	6.13E-03	0.12	2.16E-04	1.42E-03	1.69E-03	5.78E-04

In all cases, there was no significant discontinuity at origin ($C_0 \leq 19\%$) thus indicating that the sampling spatial resolution used was appropriate (Fig. 3). In the pre-fishing phase the semivariogram of Medium provided the highest range value (A_0=132.00 m) while that associated with Adult was the lowest (A_0=33.20 m); a similar result occurred in the post-fishing period but in this case the size class of Juvenile showed an increase, with the distance of spatial influence varying from 48.32 m to 100.8 m (Table 1). All the size classes showed the lower values of sill in the post-fishing period (Table 2). The spatially structured density component [C/(C_0+C)] varied between 81.70% and 99.90%, indicating that the sea urchin population that we studied had a well-defined spatial structure (Table 2).

Cross-validation analysis supported the appropriateness of the Gaussian and spherical models and kriging predictions; in all cases the values tested by the t-test revealed no-significant differences ($P>0.05$).

The estimated densities by kriging allow for visualisation of spatial distribution by size over time (Fig. 11). During pre-fishing, the Juvenile density remained within the range of 0-2 ind./m^2 throughout the study area. A few sporadic 'density hot-spots' (with densities up to 5 ind/m^2) are localized along the coastline. Individuals belonging to the Medium class showed a patchy distribution, with densities generally ranging between 1 and 3 ind/m^2, but with some hot-spots where densities higher than 4 ind./m^2 were recorded. The density of Adults was consistently low (<2 ind./m^2), with only hot-spot density reaching levels as high as 3 ind./m^2 (Fig 11).

Densities in the post-fishing phase were lower than those of the pre-fishing phase. In details, Juveniles were missing from the northern area and occurred in low numbers, never exceeding 4 ind/m^2, in the southern areas, with only two isolated higher-density hot-spots appearing in the post-fishing map. Densities in the Medium class showed a similar pattern to that of the Juvenile class, but in the latter case the hot-spots had almost disappeared, with only one case (of 5 ind/m^2) occurring in a large area. The density in the Adult class ranged from 0 to 3 ind/m^2, with a patchy distribution during the periods studied. The number of patches was also noted to be lower than that measured during the pre-fishing period (Fig 11).

The predicted numbers (mean±se) of Juveniles in the pre- and post-fishing periods were 535,274±37,016 and 408,331±31,879, respectively, indicating a total mortality rate Z=0.27. The estimated numbers for the Medium class in the pre- and post-fishing periods, indicated values of 1 119,652±68,854 and 907,796±59,005, respectively, with Z=0.21. The ranges of estimated number of harvestable sea urchins (Adult) in the pre- and post-fishing periods were 19,068±1 580 and 11,701±951, respectively, indicating a total mortality rate Z=0.49.

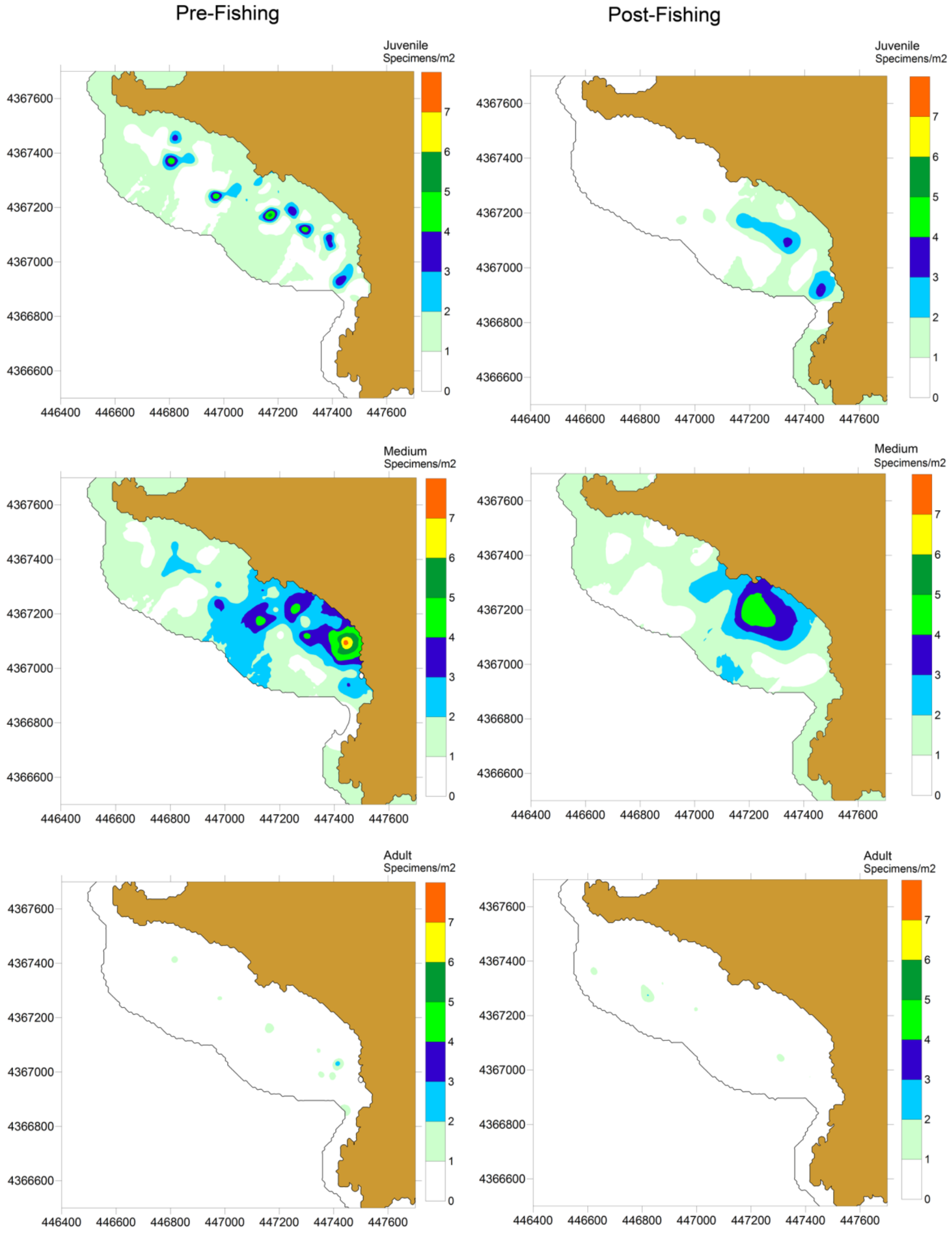

Figure 11 - Kriging maps for *P. lividus* population at Capo Pecora bay based on geostatistical interpolation at the pre-fishing (October 2010) and post-fishing (May 2011). Mean densities of Juvenile (10-29.9 mm), Medium (30-49.9 mm) and Adult (≥50) mm are shown.

DISCUSSION

Through the use of geostatistics we have described the patterns of spatial distribution of the purple sea urchin in an area of the western coast of Sardinia. In this region the commercial harvesting of sea urchin is commonly practiced by the dive fishery. The case-study thus reflects a true condition of an exploited stock. The geostatistics application and the kriging maps explain the spatial characteristics of the resource, on both spatial and time scales. Density maps were useful for predicting the likely stock biomass by surface, which is important key information for the development of spatial management-based quotas.

Results of variographic analyses show autocorrelation of density at small distances. The isotropic Gaussian and spherical models describe the spatial structure of sea urchins in the area, confirming a previous study conducted in Sardinia (Addis *et al.*, 2009). We observed a general reduction of semivariance and changes in the spatial structure of sea urchins from the pre-fishing to the post-fishing, which is a consequence of the decrease of sea urchin densities due to fishing over a 6 month period.

The most important changes relate to spatial patterns for the Juvenile class. Indeed, the kriging map in the pre-fishing phase shows a clear hot-spot pattern with a regular spread of contour levels (Fig. 11). This result is particularly interesting because it highlights the existence of nursery sites, as a result of the good fit of settlers for the seabed. In the study area the geomorphology of the bottom is characterized by caves and rocky shelters, which provide protection for small sea urchins ‘post-settlers’ from predators and destructive wave action, the main perturbation affecting populations (Sala *et al.*, 1998; Hereu *et al.*, 2004). In the scientific literature, attempting to explain the settlement process of in sea urchins has raised conflicting opinions. Some authors have pointed out that there is an absence of significant autocorrelations between sampling sites located even tens of meters apart, demonstrating a high level of settlement heterogeneity over very small patch sizes (tens of meters) (Hereu *et al.*, 2004). On the other hand, other authors claim that settlement of echinoids only shows spatial variability on a scale of thousands of meters, rather than on smaller scales (Keesing *et al.*, 1993). Considering that the home-range of sea urchins is generally low, varying from 50 and 300 cm (Hereu, 2005), we can assume that the Juveniles were ‘new settlers’. Our results concurred with those of Hereu *et al.* (2004), who found high spatial variability over small distances and very small patch sizes.

Analysis of the post-fishing phase for Juveniles suggested a change in the spatial pattern. Considering that this class would not be affected by fishing harvesting, but only by natural mortality, specimens from this class would be expected to be distributed over the whole area. Nevertheless, our results indicated that these specimens disappeared from many areas and were confined to the southern area. We propose two assumptions to explain this observation. Firstly, that these specimens had shifted into the Medium class as a consequence of growth during the 6 month experimental period, in which case we would expect an increase of diameter lengths of 4 mm based on the growth parameters proposed by Allain (1978). Our second statement explains the pattern identified as a consequence of mass-mortality caused by strong events of Mistral wind (NW) during the studied period. This process, which is common in shallow exposed habitats (Turon *et al.*, 1995), might affect the Juvenile class and thus the population size structure and density over time.

Sea urchins belonging to the Medium class indicated differences in spatial distribution between pre-fishing and post-fishing. As noted for the previous class, the semivariogram described the autocorrelation of data changes from the spherical to the Gaussian model. The main changes encompass a decrease in the estimated number of sea urchins (from 1 119,652±68,854 to 907,796±59,005) and the occurrence of only one density hot-spot. Besides natural mortality and the shift into the next size class, this group could be also subjected to fishing mortality since specimens are illegally harvested by commercial fishing operators (Cau *et al.,* 2007).

The spatial structure for large specimens (Adult) did not show significant changes over time. A decline in semivariance of this group was due to a decrease in the population densities, based on densities measured during the pre-fishing and post-fishing periods. The patchy distribution identified was relatively stable over time but there was a substantial decrease in the estimated number of sea urchins (from 19,068±1,580 to 11,701±951). In such case the total mortality for the Adult group is quite high (0.49): double that of the Medium class. At this size sea urchins are affected by natural and fishing mortality (Z=M+F). Considering a steady condition, we expected to identify a total mortality similar to the previous classes (0.27 and 0.21 respectively). Since we observed a total mortality rate of 0.49, it is very likely that fishing mortality is approximately 0.2.

Reduction in density, size structure and biomass of *P. lividus,* as a consequence of fishing, has been recorded in the literature (Guidetti *et al.,* 2004; Pais *et al.,* 2011) but the method proposed in the present paper provides a useful technique that permits the quantification of these reductions, highlighting where and how they occurred. On the other hand, kriging maps are easier to read than other statistic outputs, and can therefore become a useful tool for the main stakeholders tasked with the management of the sea urchin dive fishery.

Spatially explicit management strategies are a key requisite for maintaining persistent stocks, such as sea urchins, that are dominated by a low mobility, and fragmentary and restricted fishing grounds. According to scientific surveys, conducted on the whole of the Sardinia cost in 2006-2007 (Cau *et al.,* 2007), the domain of sea urchin population would be on a scale of a few kilometres (mesoscale). This spatial scale corresponds to the domain of the sea urchin dive fisheries that harvest the resource in fixed fishing grounds. The assessment of sea urchin stocks around Sardinia, using the model we tested, thus represents a pragmatic tool for predicting abundances per fishing ground. In terms of developing a scientific-based management of the sea urchin fishery (Perry *et al*., 2002; Chen and Hunter, 2003), we stress the development of a systematic scientific program with the purpose of acquiring substantial data that should be interpreted together with fishery-dependent statistics.

CHAPTER 2:

THE EFFECT OF TWO DIFFERENT HABITATS ON SPINE AND GONAD COLOUR IN THE PURPLE SEA URCHIN *PARACENTROTUS LIVIDUS*

Addis P., Moccia D., **Secci M.** (2014). *Marine Ecology* 1-7.

ABSTRACT

This study investigates the effect of different Mediterranean habitats on spine and gonad colouring and the gonad index in the purple sea urchin, *Paracentrotus lividus* (Echinodermata: Echinoidae). Specimens were collected in winter 2012 in *Posidonia oceanica* meadows and rocky bottom areas in two sites in Southern Sardinia (Italy, Tyrrhenian Sea). Colorimetric analyses of spines and gonads were conducted by a digital colorimeter using CIELAB colour spaces, which defines the colour in terms of 3 coordinate values: lightness (L^*), redness (a^*) and yellowness (b^*). Lightness and redness of the spines significantly differed between habitats, whereas colour spaces of the gonads did not. A careful analysis of the gonad index showed that it is significantly affected by habitat, registering higher values in rocky bottom locations. In conclusion, it seems that habitat does affect spine colour but not gonad colour. Spine colour seems to be site dependent while the gonad index depends solely on the habitat.

Keywords: Paracentrotus lividus, habitat, spine colour, gonad colour, gonad index.

INTRODUCTION

Paracentrotus lividus (Lamark, 1816) is a common Mediterranean sea urchin that inhabits rocky sea-bed areas and meadows of *Posidonia oceanica* up to a depth of 40m. It is one of the most important grazers in shallow rocky reefs, and may play a part in the transition from macroalgae beds to coralline barrens (Sala *et al.*, 1998; Boudouresque and Verlaque, 2001; Guidetti *et al.*, 2003).

P. lividus is the most commercially exploited echinoid in Europe (FAO, 2013), where both the male and female of the species are harvested for their gonads, generally referred to as “roe” in fishery and catering markets (Lawrence, 2001).

In spite of its popular name - the purple sea urchin - the colour of spines and testes is in fact highly variable: black-purple, purple, red-brown, dark brown, light brown or olive green (Boudouresque and Verlaque, 2001). The variability of external colour in sea urchins may be the result of phenotypic plasticity or it may be associated with genetic divergence between morphs (Calderon *et al.*, 2010). In some species, sea urchins display a colour variation related to differences in habitat or distribution behaviour (Lindahl and Runnström, 1929; Tsuchiya and Nishihara, 1984; 1985). The pigments responsible for colouration in *P. lividus* have already been identified (Goodwin and Srisukh, 1950; Lederer, 1952), and a genetic basis of their variation has been found (Louise and Benard, 1993). However, the possible relationship between coloration and habitat has not yet been investigated. In fact, it is well known that the habitat or substrate affect some of the ecological behaviours of *P. lividus* such as size, movement and spawning (Boudouresque and Verlaque, 2001; Ceccherelli *et al.*, 2009; Ouréns *et al.*, 2011).

The study of the colour of gonads has been the focus of most of the studies on sea urchin nutrition because it is one of the most important quality factors of its marketability (McBride *et al.,* 2004; Shpigel *et al.,* 2006, Carboni *et al.,* 2013a). In the last few years, there has been an increase in the use of colorimetric methods to assess the colour of sea urchin gonads, in relation to artificial or natural diets (McBride *et al.,* 2004; James, 2006; Shpigel *et al.,* 2006; Woods et *al.,* 2007; Carboni *et al.,* 2013b).

The scientific literature has not focused on the effect that different habitats have on the colour of both spines and gonads in wild *P. lividus* and it is a novelty in the study of its ecological behaviour.

The aim of this study was to evaluate the colour of the spines and the gonads of *P. lividus* using colorimetric methods and our case study refers specifically to the sea urchin population of Sardinia (southern Italy), where fishing for *P. lividus* has a significant socio-economic role (Addis *et al*., 2009; 2012).

It was hypothesized that specimens collected in different habitats, *P. oceanica* meadows and rocky bottom areas may be differentiated by the colour of spines and the roe. The effect of habitat on the gonad index has also been investigated and possible explanations are discussed.

MATERIALS AND METHODS

Sample collection

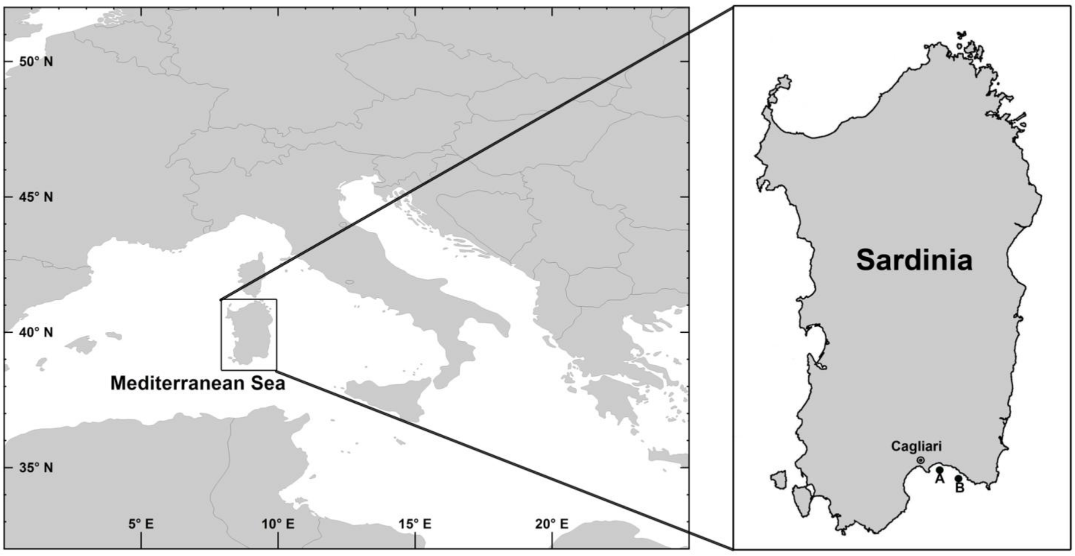

Figure 12 - Location of sites where the effect of habitat on sea urchin colour has been studied.

The study was conducted in the Gulf of Cagliari (Southern Sardinia) in January 2012. Sea urchins (>35 mm test diameter) were collected in two sites (A, B) spaced about 20 km apart (Fig. 12) at 3-4 m depth. A total of 40 specimens were collected in equal numbers between the two sites and two habitats, i.e. *P. oceanica* meadows and rocky bottom areas, which were characterized by a prevalence of *Cystoseira spp*. Sea urchins were collected inside a quadrate of 1 m^2 cast by scuba divers; the quadrate was launched until a total of 10 specimens had been reached from each habitat and specimens were brought back to the laboratory alive.

Spine colour

All individuals were measured to test diameter using a Vernier caliper (±0.01 mm), wet weight (±0.001 g) and were then classified into four colour categories (brown, green, purple, red). Spines were removed and left to dry before taking the colorimeter measurements.

Gonad colour and gonad index

The sea urchins were dissected and the five gonads were weighed. The gonad index (GI) was calculated as: GI=gonads (g wet)/whole urchin (g wet)*100.

Colorimetric analysis

Colorimetric analyses were conducted for both spines and gonads by a digital colorimeter (Chroma meter CR-400, Konica Minolta, Tokyo, Japan), which specifies the colour according to the *Commission Internationale de l'Eclairage* (CIE, Vienna, Austria) L*a*b* colour space (CIELAB). The Chroma meter CR-400 measures colour with six silicon photocells and a standard luminant D65. The light source is a pulsed xenon lamp, used to take readings calibrated against a white plate. The colour formula defines colour in terms of 3 coordinate values (L*, a*, b*), which locate the colour of an object within a 3-dimensional colour space. The *L** component represents the lightness; *a** is the component from green (negative values) to red/magenta (positive values); *b** is the component between blue (negative values) and yellow (positive values) (CIE, 1978).

Statistical analysis

The distribution of four external colour categories of sea urchin collected in *P. oceanica* meadows and rocky bottom areas was compared by means of the Kolmogorov-Smirnov test. One-way ANOVA (α=0.05) was performed to test the differences in sea urchin spine and gonad colour spaces L*, a* and b* between habitats (Pos and Rock) and sites (A and B) (factors=Pos-A, Pos-B, Rock-A, Rock-

B). Two-way analysis of variance (ANOVA, α=0.05) was performed to test the differences in the sea urchin GI between habitats (Posidonia vs Rock) and sites (A vs B). Prior to the analysis, Cochran's C test (α=0.05) was used to check the assumption of the homogeneity of variances and, whenever necessary, data were appropriately transformed (i.e. L* of gonads). Where data transformation did not correct violations in the assumption of homogeneous variances (i.e. GI), an alpha-level adjustment to 0.01 was used to compensate for increased type I errors (Underwood 1997). Post-hoc multiple comparisons were performed using Tukey's test; STATGRAPHICS PLUS 5.1 professional edition (Statistical Graphics Corp., Rockville, MD, USA) was used for statistical analysis.

RESULTS

The mean size and wet weight (mean±SD) of sea urchins in *P. oceanica* meadows was 42.58±3.61 mm and 35.336±9.064 g, respectively. Mean size and wet weight in rocky bottom areas was 44.29±3.70 mm and 39.712±11.161 g, respectively.

Spine Colour

Specimens collected from the rocky sea-bed area were mainly brown (55%), followed by red (30%), green (10%) and purple (5%) (Fig. 13). In the *P. oceanica* meadows, the distribution of colour categories (Fig. 13) was the same in both habitats: 35% purple, 25% brown, 20% green, 20% red (Kolmogorov-Smirnov test, *P*>0.05).

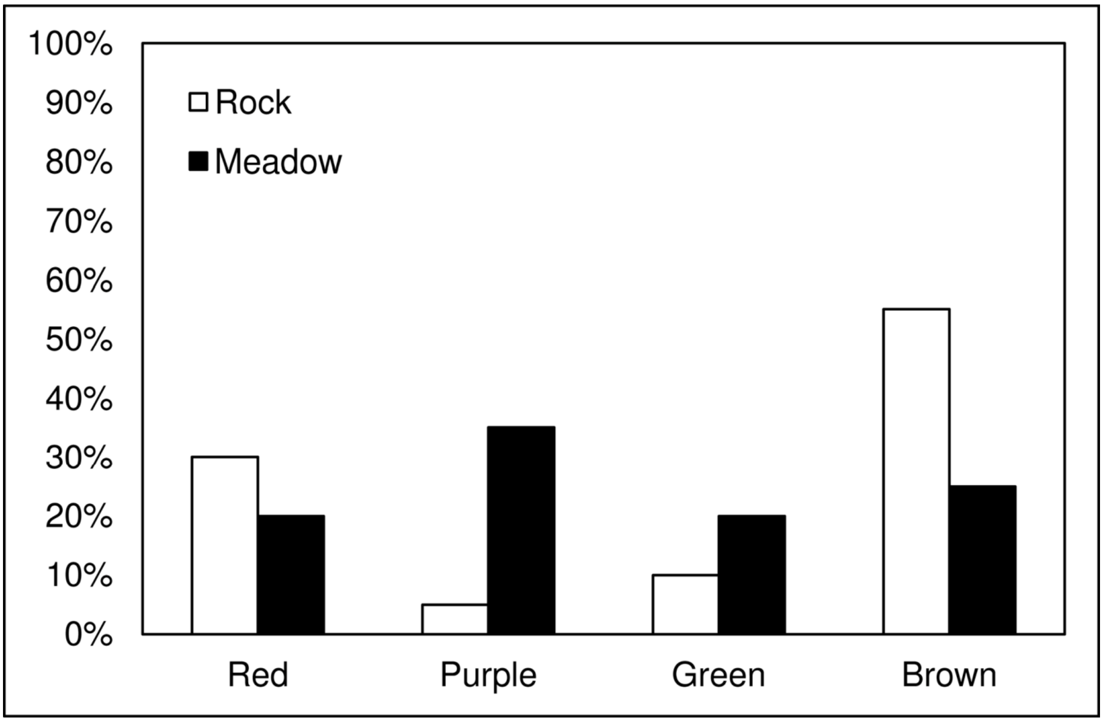

Figure 13 - Distribution of external colour of sea urchin collected in *Posidonia oceanica* meadows and rocky bottom areas.

The mean (±SD) spine colour spaces of specimens collected in *P. oceanica* meadows was as follows: L*=25.80±10.39; a*=3.97±1.71 and b*=3.32±1.85 (Fig. 14), while for spines of sea urchin collected in the rocky bottom areas, the measurements obtained were L*=35.59±3.73; a*=2.53±1.09 and b*=3.75±1.92 (Fig. 14). Colour-space parameters L* and a* significantly differed between factors (Table 3). Lightness (L*) showed significant differences between Pos-B and the other factors and also differed between the two rocky bottom sites (Table 3). As regards component a*, there were differences in the pair-wise comparisons: Pos-A vs Pos-B, Pos-B vs Rock-A and Pos-B vs Rock-B (Table 4).

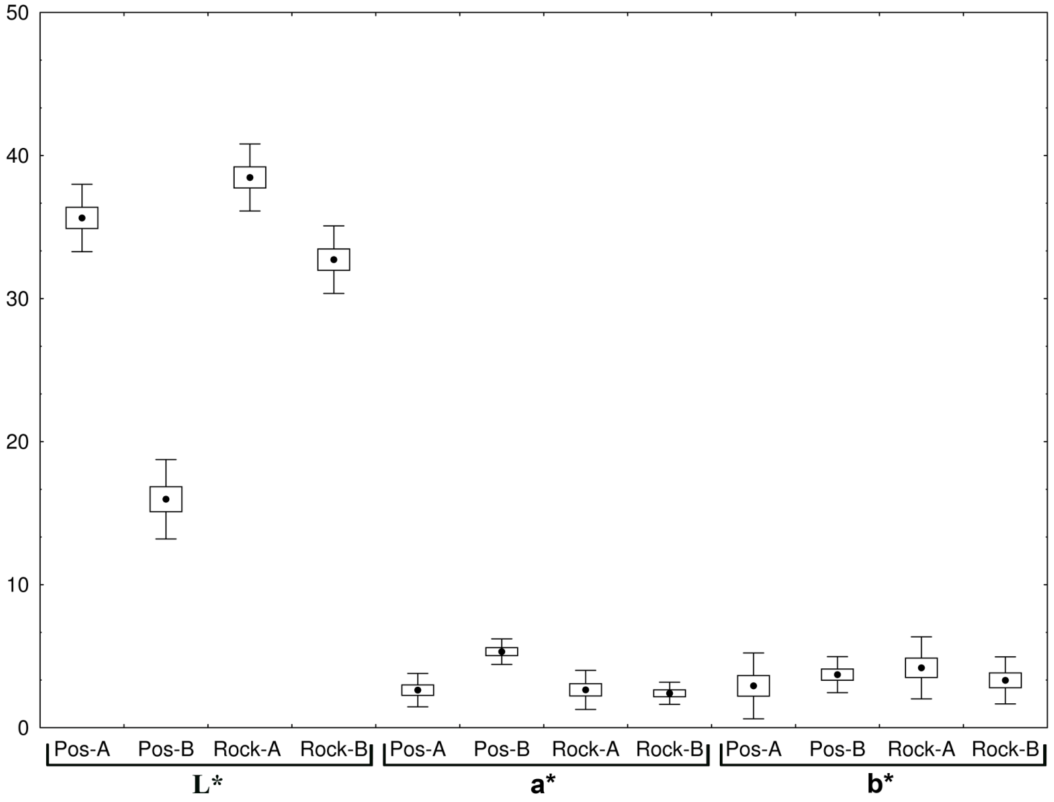

Figure 14 - Box-plot of colour spaces of spines.

Table 3 - Results of one-way ANOVA for the effect of habitat in the colour spaces of sea urchin spines L*, a* and b*.

		L*			a*			b*		
Source	*df*	MS	F	*p*	MS	F	*p*	MS	F	*p*
Between groups	3	1018.83	167.53	<0.05	19.11	16.54	<0.05	2.92	0.82	0.49
Within groups	36	6.08			1.15			3.56		
total	39									
Transformation			none			none			none	
Cochran test				>0.05			>0.05			>0.05

Table 4 - Results of Tukey's test of L* and a* of sea urchin spines collected in *P. oceanica* meadows and rocky bottom sites.

	L*		a*	
Contrast	Difference	*P*	Differences	*p*
Pos-A vs Pos-B	19.66	<0.05	-2.69	<0.05
Pos-A vs Rock-A	-2.83	ns	-0.02	ns
Pos-A vs Rock-B	2.91	ns	0.22	ns
Pos-B vs Rock-A	-22.49	<0.05	2.67	<0.05
Pos-B vs Rock-B	-16.75	<0.05	2.91	<0.05
Rock-A vs Rock-B	5.75	<0.05	0.24	ns

n.s. - nonsignificant

Gonad colour

10 sea urchins collected in *P. oceanica* meadows and 5 from rocky bottom areas did not have a sufficient quantity of gonads for colorimeter measurement and were excluded from further analysis. The colour of gonads from specimens collected in *P. oceanica* meadows revealed a mean (±SD) of colour spaces as follows: L*=37.52±6.06; a*=12.56±6.45 and b*=17.77±7.06, while for sea urchin collected in the rocky bottom areas, the following values were obtained: L*=42.77±8.71; a*=12.20±5.06 and b*=18.04±6.72.

No significant differences resulted in gonad colour after one-way ANOVA between factors (Table 5).

Table 5 - Results of one-way ANOVA for the effect of habitat on sea urchin gonads L*, a* and b*.

		L*			a*			b*		
Source	*df*	MS	F	*p*	MS	F	*p*	MS	F	*P*
Between groups	3	0.04	0.98	0.42	29.68	0.97	0.43	24.39	0.51	0.68
Within groups	21	0.04			30.69			47.99		
Total	24									
Transformation			ln(x)			none			none	
Cochran test				>0.05			>0.05			>0.05

Table 6 - Results of two-way ANOVA for the effect of habitat and site in gonad index (GI).

		GI		
Source	*df*	MS	F	*p*
Habitat	1	59.93	10.51	0.002
Site	1	3.11	0.61	0.441
Habitat x Site	1	13.11	2.55	0.118
Residual	36	5,13		
Transformation			none	
Cochran test				<0.05

Gonad index

Specimens collected in the rocky bottom areas were all mature, though one specimen in *P. oceanica* meadows had not gonads. GI (mean ± SD) was 1.18±0.94 and 3.48±3.14 in *P. oceanica* meadows and rocky bottom sites, respectively. GI was greater in rocky bottom areas than in *P. oceanica* meadows ($P<0.01$) and did not differ by site ($P>0.05$) (Table 6).

DISCUSSION

The colour of spines and gonads of the sea urchin *P. lividus* were measured by CIELAB colour space colorimetric methods, which have the advantage of eliminating the subjective aspects of colour measurement (Douglas *et al.,* 2007).

For both habitats studied, four subjective colour categories of spines have been identified. The body colour of sea urchin specimens collected in rocky bottom sites was mainly brown, while in the *P. oceanica* meadows the distribution of colours seems to be more homogeneous. Statistical analysis did not reveal any significant differences between habitats in the distribution of colour categories.

The high variability of tone within a single colour rank displayed by sea urchins depends on individual subjectivity, i.e. relying on the human eye can be a source of misinterpretation.

Colour measurements using digital instrumentation enabled us to overcome this disadvantage, revealing significant differences in the L* and a* colour spaces of the spines. Although L* differed significantly between habitats and sites, indicating a high variability in the parameter, the value of redness (a*) showed statistical difference between habitats in one site only. It may be the case that these differences are related to genetic or ecological factors, though information on possible genetic divergence between colour morph of *P. lividus* is not yet available. However, as reported by Lopes and Ventura (2012) there is a small but measurable degree of genetic divergence between colour morphs of the allopatric species *P. gaimardi* (Calderon *et al.,* 2010). They believe that this divergence is due to the presence of imperfect gene flow barriers such as reproductive timing and habitat segregation (see

Palumbi,1994; Lessios, 2007). With regard to the differences in GI between habitats, our results seem to support the hypothesis that different reproductive timing affects colour morphing and its relationship with the habitat for *P. lividus*. Although the variations in the GI are not strictly related to reproduction, the gonad index is an indicator that can be easily estimated, and thus allows us to define the reproductive cycle of sea urchin with some measure of accuracy (Ouréns *et al.*, 2011). As regards possible habitat segregations, there is no evidence that *P. lividus* inhabiting one given habitat cannot migrate to another one.

A further ecological factor affecting colour variation in sea urchins could be the need for camouflage against predators. Recent studies have provided evidence that only a few fish species are efficient sea urchin predators and have the potential to directly control sea urchin populations in the Mediterranean (Sala, 1997; Guidetti *et al.,* 2005; Guidetti, 2006). They include the sparids *Diplodus sargus*, *Diplodus vulgaris*, *Sparus aurata* and the labrids *Coris julis* and *Thalassoma pavo* (Guidetti *et al.,* 2005). In rocky sea-bed areas, the presence of brown specimens, analogous to the colour of brown algae such as *Cystoseira spp*, means that sea urchin are not easily seen by predators. In meadows where specimens are protected by *P. oceanica* leaves, the body colour has no bearing on their ability to avoid predation.

The Lightness (L*) and Red (a*) and yellow (b*) colouring in gonads were the same in *P. oceanica* meadows as in rocky sea-bed sites, as well as in sites A and B. Since the carotenoid content of sea urchin gonads has been recognized as a major factor influencing roe colouration (Shpigel *et al.,* 2006; Symonds *et al.,* 2007), it may be tempting to assume that the content of carotenoids in the food available in *P. oceanica* meadows and rocky sea-bed areas should be the same; unfortunately, this is not easy to verify. Although the carotenoid content of *P. oceanica* has been well researched (Dalla Via *et al.,* 1998; Marín-Guirao *et al.,* 2011; Li *et al.,* 2012), information on carotenoid content of brown algae in rocky bottom sites is scant and difficult to assess. The only such data we have was reported by Carboni *et al.* (2013b) on the pigment profile of North Atlantic brown algae *Laminaria digitata*, resulting in a pigment profile of urchin gonads. Rocky bottom habitats are generally characterized by a large variety of algal species that vary during the year, making it fairly difficult to estimate the possible contribution and the rate of carotenoids for each algal species in the field.

As regards the gonad index, results showed that it is affected by habitat and not by site, since habitat has indeed been recognized as an important factor in the gonad growth of Mediterranean populations (Ouréns *et al.*, 2011). Although in vitro experiments have shown that an increase in the gonad index occurs when food availability is high (Lawrence *et al.,* 1992), contradictory results have been obtained from field data. Several authors (San Martín, 1995; Fernandez and Boudouresque, 1997; Boudouresque and Verlaque, 2001; Sellem and Guillou, 2007) found that the gonad indices in sea-grass beds are larger than those reached by their counterparts living on subtidal rocks. Guettaff (1997) found that the gonad index in populations from *P. oceanica* meadows, dense algal stands or barren-grounds did not differ. He surmised that populations in barren-grounds integrate their diet with drift algae and drift leaves of *P. oceanica*. In contrast to these findings, our results indicate that sea urchin in southern Sardinia that live in rocky bottom sites had a higher gonad index than those that lived in *P. oceanica* meadows. These contrasting results may be put down to differences in food availability between location or depend on the different reproductive timing between habitats, as described above.

In conclusion, it seems to be the case that habitats affect the colour of spines and the gonad index but does not affect the colour of the gonads. In sum, spine colour seems to be site dependent while the gonad index depends only on habitat. There is a lot of literature on how habitats affect phenotypic plasticity in sea urchin ecology and our findings suggest that this also occurs for spine colour and the gonad index. The corroboration of histological data, carotenoid profile on gonads and a larger spatial-temporal scale in the study, would improve knowledge on how habitat affects these ecological features.

P. lividus is an incredibly opportunistic generalist, with a wide range of adaptive responses to environmental conditions (Boudouresque and Verlaque, 2001). These responses are in general variable even between highly close location and habitats, a fact that means the processes studied are not always applicable to all populations of this species. Further information about possible genetic divergences, reproductive timing, predatory selectivity of the colour morphs would improve the understanding of the behavioural ecology of this species.

CHAPTER 3:

A REARING PROTOCOL FOR SEA URCHIN AQUACULTURE

INTRODUCTION

Sea urchin have been employed by generations of molecular, cellular, and developmental biologists as models in studies of gametogenesis, egg and sperm interaction and activation, fertilization and early development (Bottger *et al.,* 2004). Due to the increasing interest of aquaculture of sea urchins, such information can be used to support a successful intensive commercial production. Although the biology of sea urchins is well known, many aspects still require investigation. The high mortalities during larviculture remain the major bottleneck in aquaculture that limits production (Dhert *et al.*, 2001; Carboni *et al.*, 2013b). In fact, the production of high quantities of larvae and juveniles represents the first step in any culture activity. Recently, most studies have focused on investigation of feeding (Pedreotti and Fenaux, 1993; George *et al.*, 2004; Liu *et al.,* 2007; Carboni *et al.,* 2012a, 2012b) and culture methods for larvae of sea urchins (Kelly *et al.,* 2000; Carboni *et al.*, 2012a; 2013b). Most of these studies focused on the purple sea urchin, *P. lividus*, an ideal candidate for aquaculture due to its high roe content and prolificacy. Its culture credentials have been investigated and are now well established in Scotland and Ireland (Cook and Kelly 2007; Liu *et al.,* 2007).

In Sardinia the consumption of *P. lividus* has a significant social-economic impact. Annual per-capita consumption is about 1.1 kg, about four times the Japanese consumption. Resource management attempts have been largely unsuccessful at conserving the stock and ensuring a sustainable fishery (Pais *et al.*, 2011; Cau *et al.*, 2007). Aquaculture can be useful to reduce harvesting pressure on wild populations and meeting the increasing demand for sea urchin roe. Set up a pilot hatchery to produce large quantities of juveniles represents the first challenge to introduce sea urchin as candidate species for aquaculture in Sardinia.

Hatcheries and rearing procedure can vary greatly in their design, configuration and methods from site to site. It depends on species cultured, target production levels and, most of all, the local conditions and personal preferences of owner/operators. This chapter is a summary of my experience gained over the last three years in sea urchin culturing. The general aim was to set up a land-based rearing system in Sardinia to produce juveniles of *P. lividus* for restocking purposes and develop and test protocols on echiniculture. The first challenge has been the recognition of a suitable site for the experimental hatchery; second, the setting up of the hatchery; third, the acquisition and implementation of the echiniculture protocol which included my training period at the Ardtoe Marine Laboratory, Argyll, Scotland, UK.; finally, the application of echiniculture protocol in the land-based rearing system in Sardinia.

MATERIALS AND METHODS

The Facilities

The facilities are located in the establishments belonging to the Consorzio Ittico Santa Gilla close to the Santa Gilla lagoon, Sardinia, Italy (Lat: 39°13'48.15"N; Long: 9° 4'41.56"E; Fig. 15). The establishment is mainly used as dispatch and purification centre for bivalve molluscs *Mytilus galloprovincialis* and *Ruditapes decussatus*. The lagoon of Santa Gilla occupies an area of 1300 ha. The mean depth is 1.17 m characterized by muddy/sandy bottom.

Figure 15 - Buildings of the Consorzio Ittico Santa Gilla where the hatchery is located.

The site has been selected because ensure some advantages: it is located close to the sea (the distance required to pump water is kept to minimum) ensuring good sea water quality over the year; high availability of wild macroalgae i.e. *Ulva lactuca* and the proximity to relevant University department. Periods of heavy rainfall may cause low salinity, associated with an increase of quantities of silt and other materials which may create problems in the hatchery. For these reasons the hatchery has been also provide by a closed recirculation system.

Seawater is pumped directly from the sea and it is passed through sand filters (20-40 µm) before being micro filtrated by cartridges filters (1 µm) and UV sterilised (30mJ/cm^2; Delta Ultraviolet Corporation, Grenada, CA, USA) (Fig. 16). Pipes and seawater were been disinfected to avoid disease problems prior to the commencement of the operation.

The facilities are equipped with two 10.000 l concrete ponds and a hatchery of 17 m^2 (Fig. 17-18-19). The hatchery design includes the systems to spawn the adults, rear the larvae and produce large quantities of microalgae. The hatchery has been designed to be worker friendly and efficient to allow for convenient and efficient operations (Fig.18-19). It is insulated, thermoregulated at 17.8±1.5 °C, correctly aerated, and exposed to a 24h photoperiod. The ponds include the systems for broodstock, settlement and growth of juveniles. Larval rearing methods were conducted according to Carboni *et al.* (2012b).

Figure 16 - Sea water treatment composed by filters (60 µm, 10 µm, 1 µm filters), activated carbon; UV light sterilization.

Figure 17 - The ponds.

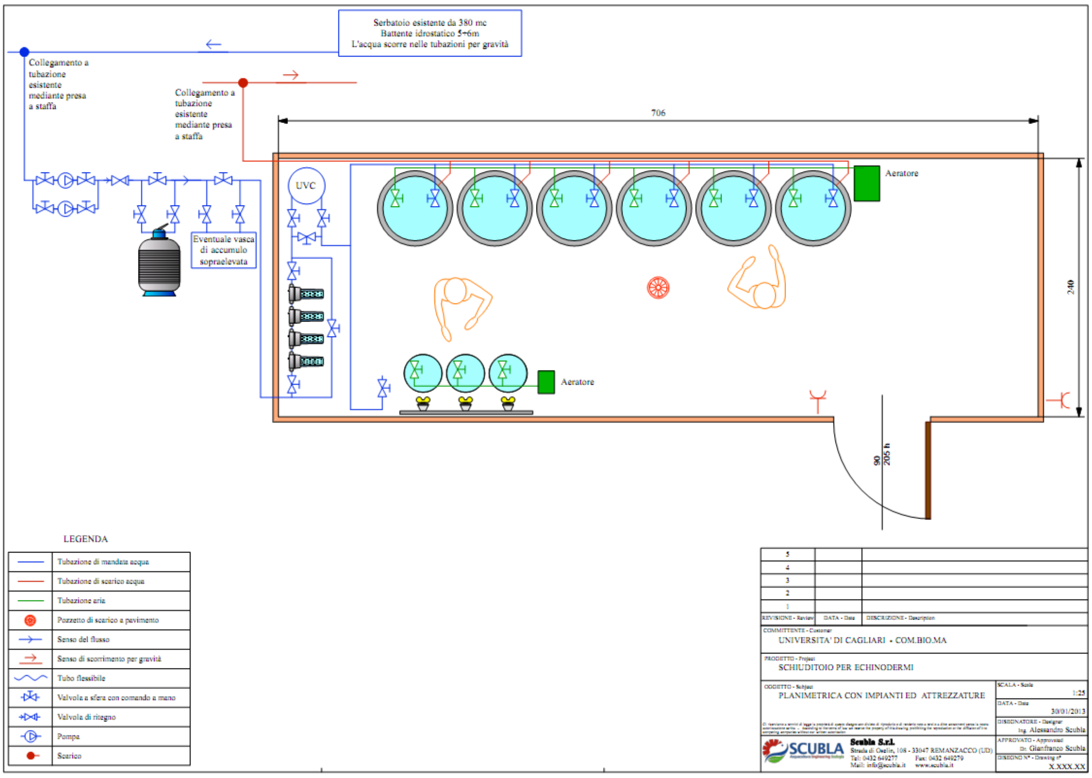

Figure 18 - Graphic design of the experimental hatchery adopted in Sardinia (original project by SCUBLA S.r.l.).

Figure 19 - View of the hatchery. Right: microalgae facilities. Left: conical paxton.

Fertilization

Broodstock were held in flow-through seawater tanks and were fed with sea lettuce, *Ulva lactuca* Linnaeus 1753. The broodstock came from a wild single population located in the Gulf of Cagliari (Sardinia, Italy). *P. lividus* were induced to spawn in November 2013 by injection of 1M KCl (40 µl per g of body weight) into the coelom via the peristomial membrane. The eggs were fertilized by adding few drops of diluted sperm. Fertilization rate was assessed 2 h post fertilization. The fertilized eggs were left to hatch in static seawater without aeration for 24 h in the dark.

Figure 20 - Mesh basket used for tank cleaning.

Larval rearing systems, physical requirements, and feeds

Larvae were stocked at a density of 4 per ml in 150 L in conical fibreglass tank (Fig. 21) and maintained in aerated ambient filtered seawater in continuous light. The cleaning of the tanks and survival counting were carried every three days.

Figure 21 - Conical fibreglass tanks of 150 l volume.

Figure 22 - Transferring of larvae.

The larvae were fed by micoralgae *Dunaliella tertiolecta* (7 μm Equivalent Spherical Diameter, 180 μm3 Volume) and *Pleurochrysis carterae* (8.9 μm ESD; 380 μm3 Volume) prepared as mix diet (50/50). The species, sourced from the Culture Collection for Algae and Protozoa (CCAP: Oban, Scotland), were grown in 150 L polyethylene bags in sterilized seawater enriched with the f/2 medium. Larvae were fed every day. For larvae with two, three and four pairs of arms, the daily feeding rate of *D. tertiolecta* and *P. carterae* was 750, 2250, and 3500 cells/ml and 325, 1125, 1750 cells/ml, respectively. Microalgae culture densities were made using light microscopy and cell counting via hemocytometer. The equation used to calculate feed ration was: Volume of algae given=(number of algae cells x rearing volume) / Algae culture concentration.

Early juvenile rearing systems, physical requirements, and feeds

When the larvae reached competence for settlement, i.e. when the rudiment was equal in size or larger than the stomach, were located in a tank of 150 l equipped with 2 plate holders designed to hold 6 plates of 35x50 cm "corrugated" sheet of fibreglass (Fig. 23). Plates were kept in the seawater thus representing a basibiont for epi-biosis colonization. Plates provided the substrata for metamorphosis and also supplied post-met morphic larvae with a varied natural diet (Fig. 24-25).

Figure 23 - Plate holder with plates encrusted by algae for settlement.

Figure 24 - Post-larve 24 h post metamorphosis.

Figure 25 - Post larva of 0.8 mm of test diameter.

Growth of juveniles

Once post-larvae became exotrophic juveniles they were fed with *U. lactuca*, collected in the wild. Juveniles were left in the settlement tank until the mean individual size reaches the 15 mm.

RESULTS

2 females (47.0±1.7 g) and 2 males (45.0±1.5 were induced to spawn. Each female spawned approximately 500.000-2.000.000 eggs. Fertilization rate was 97.5±1.0%. Hatching rate was 76.8±1.8%. Survival rate was 24% when larvae were competent, 31 days post fertilization (Fig. 26). The 6% of which became a post-larvae (Fig. 26). Over 8000 post-larvae have been successfully cultured.

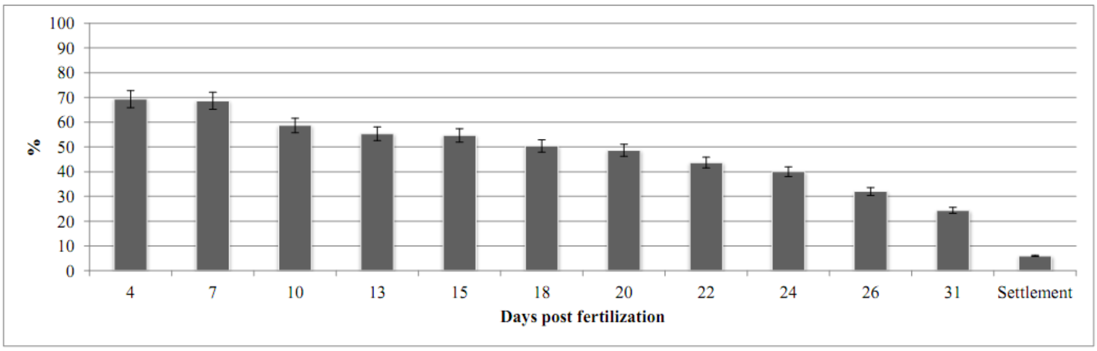

Figure 26 - Survival rate of *P. lividus* larvae and post-larvae. Data are expressed as Mean±SD (n=2).

DISCUSSION

Obtaining gametes of *P. lividus* in large amounts and rearing larvae is an easy task. The success of rearing larvae depends largely on the production of large quantities of high quality algae. Several different species of microalgae can be used as feed. Some species have been reported to produce better results, improving the survival rate and development of larvae i.e. *P. carterae*, *C. elongata* (Carboni *et al.*, 2012b). Mixes of different microalgae have never been investigated, our results compared with the findings of Carboni *et al.* (2012b) seems to suggest that mix diet could improve the survival rate.

Settlement represents a critical phase but can be achieved with the presence of macroalgae such as *U. lactuca* or *Corallina elongata*.

We obtained over 8,000 post-larvae for each of the 150 l tanks. This can probably be improved using a flow trough system (Carboni *et al.*, 2012b). The long larval rearing period (31 days) is probably due to a drop in water temperature (15° C) caused by a wrong setup of the thermoregulation system at the second week of rearing. The results of the first trial are promising; once fully operational, the hatchery is able to produce 48,000 post-larvae per larval trial and 4-5 trials can be carried out per year; that means at least 250,000 postlarvae will be cultured per year. However some problems remain to be solved. One of the major constraints regards the growth of juveniles at an acceptable size both for restocking and for commercial purposes. The high survival rate and the speed of growth on juveniles are important parameter for the profitability of sea urchin aquaculture (Grosjean *et al.*, 1998; Vaïtilingon *et al.*, 2001). Experienced sea urchin aquaculturists feed post-larvae with diatoms until they reach 15 mm of test diameter improving the survival rate (Pers. comm. J. Chamberlain, Dunmanus Sea-Foods, Ireland). Natural diet ensures an healthy growth of juveniles but the growth rate is too low.

At the moment specific diet for sea urchin has not been developed. Artificial diets must be developed to substitute wild collected macroalgae for *P. lividus* aquaculture to become commercially and environmentally sustainable (Carboni *et al.*, 2013a). These diets should be able to promote good somatic and gonadal growth. Actually, prepared diets resulted in large but pale, unmarketable gonads (Shpigel *et al.*, 2006). Recent studies revealed that the biochemistry of the gonads can be changed in 4-5 weeks with the diet (Shpigel *et al.*, 2005; Hughes *et al.*, 2006; Carboni *et al.*, 2013b). Prepared diet could be used until the sea urchins reach the commercial size while natural can be used to promote good gonad quality before the commercialization.

Another major aspect hindering sea urchin commercialization is the lack of diets with long shelf-life (Pearce *et al.*, 2002). In the literature has been recognized that fatty acid profiles of sea urchin eggs and embryos can be controlled through broodstock nutrition (Gago *et al.*, 2009: Carboni *et al.*, 2013b). This could be considered in the development of new feeds and protocols for the first feeding of sea urchin larvae (Carboni *et al.*, 2013b).

We started in January 2014 an international EU-funded project (within the FP7-SME-2013 call) called RESURCH, Research and Technological Development to Improve Economic Profitability and Environmental Sustainability of Sea Urchin Farming. Our hatchery facilities and Department laboratories are involved in the test of new diets and grow out techniques from juvenile to market size.

Finally, I should mention that cleaning tanks, feeding larvae and juveniles, growing and counting microalgae and all the routine activities in a hatchery are very labor-intensive. That was possible thanks to the voluntary help of our students.

GENERAL CONCLUSION

In general the sea urchin fishery is part of the so called small-scale and artisanal fisheries. It is a fishery which develops faster than the ecology is assessed, raising uncertainty and delay over proper management actions. For this reason it is recommended a precautionary approach and management framework which take into account some focal points: (1) identification of appropriate spatial scales for the species distribution and for the fishery; (2) development of regulatory strategies, which require some form of stock assessment or analysis of biological knowledge (3) acknowledgement and consideration of uncertainty in biological and fishery information, and uncertainty in the effectiveness of management actions; and (4) constant collaborative interactions between scientists, fishery managers, producers and stakeholders useful to identify alternative strategies for the resource conservation (Perry *et al.*, 1999; 2002).

Today, most worldwide sea urchins fisheries must deal with overexploitation and there is a knowledge-gap on the conservation status of the populations. Exceptions include a few jurisdictions that have conducted scientifically base stock assessments and have developed a precautionary approach to management. These include the green sea urchin fisheries in British Columbia and New Brunswick, where estimates of the current biomass are made, and the fisheries are allowed exploitation rates by year (DFO, 2008; Miller and Nolan, 2008).

In Sardinia the management of sea urchin fishery occurs by passive and approximate measures, i.e. minimum size limit, seasonal closures and inconsistent individual quotas. These measures are insufficient to prevent the imminent crisis of the fishery. Development of scientifically base management towards a sustainable sea urchin fishery is not an easy task. Fishermen, stakeholder, researcher and policymakers must be involved in a consultative process to review and provide advice regarding management issues and to propose a new management plan for the commercial fishery.

The aims of my PhD project have been to identify alternative tools for the species conservation. My research activities were carried out within the project C.A.M.P., Coastal Area Management Programme financed by the Regional Agency for Coastal Conservation (within the Mediterranean Action Plan UNEP, United Nations Environment Programme) and the project "Integrated approach to the protection, management and enhancement of the sea urchin resource in Sardinia" financed by the Autonomous Region of Sardinia. I had the opportunity to face some of the issues previously described also involving fishermen, which have been sensitize toward a sustainable exploitation of sea urchin stocks. The managers of the fishery have been made aware of the results which provide scientifically information and tools to improve the management of the resource. Scientific research and the joint stock assessment activities are of vital importance to this fishery as it moves towards a biologically based fishery.

Geostatistics has been confirmed a good estimator of fishable biomass and population dynamics in response to the resource exploitation. The small autocorrelation founded and the high variability in the ecology and the biology, such as the external colour and gonad index, suggest that the small scale management is the best prospect for long-term sustainability of sea urchin fishery in Sardinia. The studied area (Capo Pecora Bay) is one of the typically sea urchin fishing zone of

Sardinia. The time required carrying out the sampling, the geostatical analyses and the stock assessment by contour maps was relatively smaller, about 7 maximum 10 days.

Due to the relatively limited number of fishing zones in Sardinia, it is desirable to plan an annual or biannual stock assessment program before the fishing season in order to evaluate the exploitable biomass. Such information, together with fishery-dependent statistics by log-book will provide vital information about the carrying capacity of the resource and in the planning the exploitation rate by year.

Regarding the potential of aquaculture for reseeding, the results coming from the hatchery are promising. It permits to culture abundant juveniles both for reseeding and research. Regarding the release of reared juveniles in the wild, the methods to test the effectiveness of stock enhancement are relatively scant and the results are contrasting (Saito, 1992; Kitada, 1999; Agatsuma *et al.,* 2003; Bell *et al.,* 2005; Juinio-Menez *et al.,* 2008). One of the major concerns is to recognize reared specimens to wild ones on the field. Different tagging methods have been tested for sea urchins such as PIT tag, tetracycline injection and anchor tag (Hagen, 1996; Kalvass *et al.,* 1998; Duggan and Miller, 2001; Woods and James 2005; Lauzon-Guay and Scheibling 2008;). Each method has its advantages and disadvantages, in particular in relation to the test diameter. For example, PIT tags need test diameter of 20-25 mm for effectiveness tag retention (Sonnenholzner *et al.,* 2010). When cultured specimens are used for restocking some concerns need to be considered: 1) increasing abundances of target species may have negative impacts on other species through interactions such as competition for food and space or predation; 2) the risk of spreading disease; 3) large scale releases of hatchery-reared juveniles can affect genetic diversity of wild population (Bell *et al.*, 2005). All these concerns must be considered when juveniles will release in the wild.

Recently, sea urchins have been selected as new candidate species for aquaculture in European countries. Due to the increasing demand for sea urchin roe in European and international markets, there is a general perception within the sector for a need to innovate and to increase profitability and enhancing the competitiveness of involved companies. Sea urchin producers have identified the costs of the grow out period as a major expense and a constraint on the development of the industry. They also have identified significant cost savings when sea urchin production can be moved from land to sea. However, such transfer requires significant development of cage and feed technologies. The development of sea urchin diet technology aims also to produce good gonad growth to create gonads with colour, taste and texture that is acceptable to the market. At this time, all these technologies are not fully available. The economic feasibility of these innovations will be tested, developed and validated by the ongoing project RESURCH "Research & technological development to improve economic profitability and environmental sustainability of sea urchin farming”, within the FP7 EU framework. The aim of the project will be the development for net-working in the aquaculture industry for the rapid expansion and dissemination of the technology of the European sea urchin cultivation industry.

In conclusion, my suggestion to guaranty the sustainability of the fishery in the future should be:

1. Start a collaboratively consultative process between fishermen, researchers and policymakers to review and provide advice regarding the current

management issues in order to propose a new management plan for the fishery;

2. Develop a fishery-independent stock assessment in Sardinian seas;
3. Develop a plan for the fishery-dependent monitoring in order to reduce/avoid the poaching and to provide appropriate data for assessment strategy;
4. Testing the effectiveness of restocking by cultured juveniles in overfished areas;
5. Increasing the profitability of sea urchin roe production by echiniculture, aimed to reducing harvesting pressure on wild stocks

Moreover, a precautionary approach is highly recommended, i.e. reducing the fishing season, number of licenses and the individual quota of exploitable sea urchins.

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REFERENCES

Adams C. F., Harris B. P., Marino M. C., Stokesbury K. D. E. (2010). Quantifying sea scallop bed diameter on Georges Bank with geostatistics. *Fishery Research* **106**: 460-467.

Addis P., Secci M., Manunza A., Corrias S., Niffoi A., Cau A. (2009). A geostatistical approach for the stock assessment of the edible sea urchin, *Paracentrotus lividus*, in four coastal zones of Southern and West Sardinia (SW Italy, Mediterranean Sea). *Fishery Research* **100**: 215-221.

Addis P., Secci M., Angioni A., Cau A. (2012). Spatial distribution patterns and population structure of the sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea), in the coastal fishery of western Sardinia: a geostatistical analysis. *Scentia Marina* **76**: 733-740.

Agatsuma Y., Sakai Y., Andrew N., Saiko Y. (2003). Enhancement of Japan's sea urchin fishery. In J. M. Lawrence (Ed). *Sea urchins: Fisheries and Ecology. Procceding of the international Conference on Sea Urchin Fisheries and Aquaculture*. CRC Press, Boca Raton, Florida: 18-36.

Allain J. Y. (1978). Age et croissance de *Paracentrotus lividus* (Lamarck) et de *Psammechinus milaris* (Gmelin) des côtes nord de Bretagne (Echinoidea). *Cahiers de Biologie Marine* **19**: 11-21.

Andrew N. L., Agatsuma Y., Ballesteros E., Bazhin A. G., Creaser E. P., Barnes D. K. A., Botsford L. W., Bradbury A., Campbell A., Dixon J. D., Einarsson S., Gerring P. K., Hebert K., Hunter M., Hubert S. B., Johnson C. R., Juinio-Menez M. A., Kalvass P., Miller R. J., Moreno C.A., Palleiro J. S., Rivas D., Robinson S. M. L., Schroeter S.

C., Steneck R. S., Vadas R. L., Woodby D. A., Xiaoqi Z. (2002). Status and management of world sea urchin fisheries. *Oceanography and Marine Biology - An Annual Review* **40**: 343-425.

Barange M., Hampton I. (1997). Spatial structure of co-occurring anchovy and sardine populations from acoustic data: implications for survey design. *Fisheries Oceanography* **6**: 94-108.

Barbaglio A., Sugni M., Di Benedetto C., Bonasoro F., Schnell S., Lavado R., Porte C., Candia Carnevali D. M. (2007). Gametogenesis correlated with steroid levels during the gonadal cycle of the sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea). *Comparative biochemistry and physiology Part A*: 466-474.

Barnes D., Crook A. (2001). Quantifying behavioural determinants of the coastal European sea urchin *Paracentrotus lividus*.” *Marine Biology* **138** (6): 1205-1212.

Barnes D. K. A., Verling E., Crook A., Davidson I., O’Mahoney M. (2002). Local population disappearance follows (20 yr after) cycle collapse in a pivotal ecological species. *Marine Ecology Progress Series* **226**: 311-313.

Bell J., Rothlisberg P., Munro J., Loneragan N., Nash W., Ward R., Munro N. A. (2005). *Restocking and Stock Enhancement of Marine Invertebrate Fisheries*. Elsevier, San Diego: 392 pp.

Bonaviri C., Agnetta D., Gianguzza P., Vega Fernàndez T., Raggio S. (2005). Effetto della complessità del substrato sulla distribuzione di *Paracentrotus lividus* e *Arbacia lixula* nel barren dell’AMP ‘‘Isola di Ustica’’. XV Congresso della Società Italiana di Ecologia, Torino 2005.

Bottger A., Walker C. W., Unuma T. (2004). Care and maintenance of adult echinoderm. In:

Ettensohn C. A., Wray G. A., Wessel G: M. (Eds). *Development of sea urchins, ascidians, and other invertebrate deuterostomes: Experimental Approaches*. Elsevier, San Diego: 18-33.

Boudouresque C. F., Verlaque M. (2001). Ecology of *Paracentrotus lividus*. In: J.M. Lawrence (Ed). *Edible Sea Urchins: Biology and Ecology vol. 32*. Elsevier, Amsterdam: 177-216.

Byrne M. (1990). Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. *Marine Biology* **104**: 275-289.

Calderón I., Ventura C. R. R., Turon X., Lessios H. A. (2010). Genetic divergence and assortative mating between colour morphs of the sea urchin *Paracentrotus gaimardi*. *Molecular Ecology* **19**: 484-93.

Carboni S., Vignier J, Chiantore M., Tocher D. R., Migaud H. (2012a). Effects of dietary microalgae on growth, survival and fatty acid composition of sea urchin *Paracentrotus lividus* throughout larval development. *Aquaculture* **324-325**: 250-258.

Carboni S., Kelly M. S., Hughes A. D., Vignier J., Atack T., Migaud. H. (2012b). Evaluation of flow through culture technique for commercial production of sea urchin (*Paracentrotus lividus*) larvae. *Aquaculture Research*: 1-5.

Carboni S., Hughes A., Atack T., Tocher D., Migaud H. (2013a). Influence of broodstock diet on somatic growth, fecundity, gonads carotenoids and larval of sea urchin. *Aquaculture Research*: 1-8.

Carboni S., Hughes A., Atack T., Tocher D., Migaud H. (2013b). Fatty acid profiles during gametogenesis in sea urchin (*Paracentrotus lividus*): effects of dietary inputs on gonad, egg and embryo profiles. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology* **164**: 76-82.

Carboni S. (2013c). *Research and development of hatchery techniques to optimise juvenile production of the edible sea urchin, Paracentrotus lividus*. Ph.D thesis, University of Stirling.

Carter, S. K., VanBlaricom G. R. (2002). Effects of experimental harvest on red sea urchins (*Strongylocentrotus franciscanus*) in northern Washington. *Fishery Bulletin* **100**(4): 662-673.

Castilla J. C., Defeo O. (2001). Latin-American benthic shellfisheries: emphasis on co-management and experimental practices. *Reviews in Fish Biology and Fisheries* **11**: 1-30.

Cau A., De Miranda M. A., Masala M. L., Serra E. (2003). *Valutazione degli stocks ed allevamento estensivo del riccio di mare Paracentrotus lividus (Lamarck) per fini gestionali e produttivi nei mari circostanti la Sardegna*. Rapporto Finale per la Regione Autonoma della Sardegna, Assessorato della Difesa dell’Ambiente, Servizio Pesca Acquacoltura e Stagni.

Cau A., Addis P., Corrias S., Corpino G., Manunza A., Secci M., Niffoi A., Atzori F., Corrias S., Lai L., Sechi N., Ceccherelli G., Pinna S., Mura F., Pala D. (2007). *Valutazione degli stocks del riccio di mare (Paracentrotus lividus): dinamica di popolazione, reclutamento, valutazioni economiche, gestione sostenibile della* *risorsa*. Rapporto Finale per la Regione Autonoma della Sardegna, Assessorato della Difesa dell'Ambiente, Servizio Pesca Acquacoltura e Stagni.

Ceccherelli G., Pinna S., Sechi N. (2009). Evaluating the effects of protection on *Paracentrotus lividus* distribution in two contrasting habitats. *Estuarine, Coastal and Shelf Science* **81**: 59-64.

Ceccherelli G., Pais A., Pinna S., Sechi N., Chessa L. A. (2011). Human impact on *Paracentrotus lividus*: the result of harvest restrictions and accessibility of locations. *Marine Biology* **158**: 845-852.

Chen Y., Hunter M. (2003). Assessing the green sea urchin (*Strongylocentrotus droebachiensis*) stock in Maine, USA. *Fishery Research* **60**: 527-537.

Ciannelli L., Fauchald P., Chan K. S., Agostini V. N., Dingsor G. E. (2008). Spatial fisheries ecology: recent progress and future prospects. *Journal of Marine Systems* **71**: 223-236.

CIE, Commission Internationale de l'Eclairage (1978). Recommendations on uniform color spaces, color-difference equations, psychometric color terms. In: Paris (Ed). *Supplement No. 2 of publication CIE: No. 15 E-1.3.1*.

Conan G. Y. (1985). Assessment of shell fish stocks by geostatistical techniques. *ICESCM 1985/K:30*.

Cook E. J., Kelly M. S. (2007) Effect of variation in the protein value of the red macroalga *Palmaria palmata* on the feeding, growth and gonad composition of the sea urchins *Psammechinus miliaris* and *Paracentrotus lividus* (Echinodermata). *Aquaculture* **270**: 207-217.

Cressie N. (1991). *Statistics for Spatial Data*. Wiley-Intercience, New York.

Crook A. C. (2003). Individual variation in the covering behaviour of the shallow water sea urchin *Paracentrotus lividus*. *Marine Ecology* **24**(4): 275-287.

Crook A. C., Long M., Barnes D. K. A. (2000). Quantifying daily migration in the sea urchin *Paracentrotus lividus*. *Journal of the Marine Biological Association of the United Kingdom* **80**: 177-178.

Dalla Via J., Sturmbauer C., Schonweger G., Sotz E., Mathekowitsch S., Stifter M., Rieger R. (1998). Light gradients and meadow structure in *Posidonia oceanica*: ecomorphological and functional correlates. *Marine Ecology Progress Series* **163**: 267-278.

Defeo O., Rueda M. (2002). Spatial structure, sampling design and abundance estimates in sandy beach macroinfauna: some warnings and new perspectives. *Marine Biology* **140**: 1215-1225.

DFO, 2008. *Green Sea Urchin Integrated Fisheries Management Plan*. Fisheries and Oceans, Canada: 79 p.

Dhert P., Rombaut G., Suantika G., Sorgeloos P. (2001). Advancement of rotifer culture and manipulation techniques in Europe. *Aquaculture* **200**: 129-146.

Douglas R., Duane T., Steinhauer J., Wee A. G. (2007). Intraoral determination of the tolerance of dentists for perceptibility and acceptability of shade mismatch. *The Journal of prosthetic dentistry* **97**: 200-208.

Duggan R. E., Miller R. J. (2001). External and internal tags for the green sea urchin. *Journal of Experimental Marine Biology and Ecology* **258**: 115-122.

FAO (1996). *FAO Technical Guidelines for Responsible Fisheries No. 2*. FAO, Rome: 54pp.

FAO (2011). www.fao.org/fishery/statistics/software/fishstat/en. Capture Production 1950-2009.

FAO (2012). http://www.fao.org/fishery/statistics/software/fishstatj/en#6. *Fisheries Commodities Production and Trade 1976-2009*.

FAO (2013). www.fao.org/fishery/statistics/software/fishstat/en. *Capture Production 1950-2011*.

FAO (2013). www.fao.org/fishery/statistics/software/fishstat/en. *Aquaculture Production (Quantities and values) 1950-2011*.

Fernandez C., Boudouresque C. F. (1997). Phenotypic plasticity of *Paracentrotus lividus* (Echinodermata: Echinoidea) in a lagoonal environment. *Marine Ecology Progress Series* **152**: 145-154.

Fernandez C., Pasqualini V., Boudouresque C. F., Johnson M., Ferrat L., Caltagirone A., Mouillot D. (2006). Effect of an exceptional rainfall event on the sea urchin (*Paracentrotus lividus*) stock and seagrass distribution in a Mediterranean coastal lagoon. *Estuarine Coastal and Shelf Science* **68**(1-2): 259-270.

Gago J. M., Luis O. J., Repolho T. R. (2009). Fatty acid nutritional quality of sea urchin *Paracentrotus lividus* (Lamarck, 1816) eggs and endotrophic larvae: relevance for feeding of marine larval fish. *Aquaculture Nutrition* **15**: 379-389.

George S. B., Lawrence J. M., Lawrence A. L. (2004). Complete larval development of the sea urchin *Lytechinus variegatus* fed an artificial feed. *Aquaculture* **242:** 217-228.

Goodwin T. W., Srisukh S. (1950). A study of the pigments of the sea urchins, *Echinus* *esculentus* L. and *Paracentrotus lividus* Lamarck. *Biochemistry Journal* **47**: 69-76.

Grabowski R., Windholz T., Chen. Y. (2005). Estimating exploitable stock biomass for the Maine green sea urchin (*Strongylocentrotus droebachiensis*) fishery using a spatial statistics approach. *Fishery Bulletin* **103**: 320-330.

Grosjean P., Spirlet C, Gosselin P., Vaïtilingon D., Jangoux M. (1998). Land-based closed-cycle echiniculture of *Paracentrotus lividus* (Lamarck) (Echinoidea: Echinodermata): a long-term experiment at a pilot scale 1998. *Journal of Shellfish Research* **17**(5): 1523-1531.

Grosjean P. (2001). Growth model of the reared sea urchin *Paracentrotus lividus* (Lamarck, 1816). PhD in Agronomic Sciences and Biological Engineering, 271pp.

Guettaf M. (1997). Contribution à l'étude de la variabilité du cycle reproductif (índice gonadique et histologie des gonades) chez *Paracentrotus lividus* (Echinodermata: Echinoidea) en Méditerranée sud-occidentale (Algérie). Thèse Doct. Faculté des Sciences, Univ Aix-Marseille 2.

Guidetti P. (2004). Consumers of sea urchins, *Paracentrotus lividus* and *Arbacia lixula*, in shallow Mediterranean rocky reefs. *Helgoland Marine Reserach* **58**: 110-116.

Guidetti P. (2006). Short Communication Estimating body size of sea urchins, *Paracentrotus lividus* and *Arbacia lixula* , from stomach contents of *Diplodus sargus,* a Mediterranean predatory fish. *Terra Nova* **22**: 91-93.

Guidetti P., Fraschetti S., Terlizzi A., Boero F. (2003). Distribution patterns of sea urchins and barrens in shallow Mediterranean rocky reefs impacted by the illegal fishery of the rock-boring mollusc *Lithophaga lithophaga*. *Marine Biology* **143**: 1135-1142.

Guidetti P., Terlizzi A., Boero F. (2004). Effects of the edible sea urchin, *Paracentrotus lividus*, fishery along the Apulian rocky coast (SE Italy, Mediterranean Sea). *Fishery Research* **66**: 287-297.

Guidetti P., Bussotti S., Boero F. (2005). Evaluating the effects of protection on fish predators and sea urchins in shallow artificial rocky habitats: a case study in the northern Adriatic Sea. *Marine Environmental Research* **5**: 333-48.

Gutiérrez-Zugasti F. I. (2011). The Use of Echinoids and Crustaceans as Food During the Pleistocene-Holocene Transition in Northern Spain: Methodological Contribution and Dietary Assessment. *The Journal of Island and Coastal Archaeology* **6**(1): 115-133.

Hagen N. T. (1996). Echinoculture: from fishery enhancement to close-cycle cultivation. *World Aquaculture* **27**: 6-19.

Hereu B. (2005). Movements pattern of the sea urchin *Paracentrotus lividus* in the NW Mediterranean. *Marine Ecology* **26**: 54-62.

Hereu B. (2006). Depletion of palatable algae by sea urchins and fishes in a Mediterranean subtidal community. *Marine Ecology Progress Series* **313**: 95-103.

Hereu B., Zabala M., Linares C., Sala E. (2004). Temporal and spatial variability in settlement of the sea urchin *Paracentrotus lividus* in the NW Mediterranean. *Marine Biology* **144**: 1011-1018.

Hereu B., Linares C., Zabala M., Sala E. (2005). The effects of predator abundance and habitat structural complexity on survival of Juvenile sea urchins. *Marine Biology* **146**: 293-299.

Hughes A. D., Kelly M. S., Barnes D. K. A., Catarino A. I., Black K. D. (2006). The dual functions of sea urchin gonads are reflected in the temporal variations of their biochemistry. *Marine Biology* **148**(4): 789-798.

James P. (2006) The effects of wave and feeding disturbance on roe enhancement of the sea urchin *Evechinus chloroticus* held in sea-cages. *Aquaculture* **252**: 361-371.

Jensen O., Miller T. (2005). Geostatistical analysis of the abundance and winter distribution patterns of the blue crab *Callinectes sapidus* in Chesapeake Bay. *Transactions of the American Fisheries Society* **134**(6): 1582-1598.

Juinio-Menez M. A., Bangi H. G.; Malay M. C.; Pastor D. (2008). Enhancing the recovery of depleted *Tripneustes gratilla* stocks through grow-out culture and restocking. *Reviews in Fisheries Science* **16** (1-3): 35-43.

Kalvass P.E., Hendrix J.M., Law P.M. (1998). Experimental analysis of 3 internal marking methods for red sea urchins. *California Fish and Game* **84**: 88-99.

Keesing J. K., Cartwright C. M., Hall K. C. (1993). Measuring settlement intensity of echinoderms on coral reefs. *Marine Biology* **117**: 399-407.

Kelly M. S., Hunter, A. J., Scholfield C. L., McKenzie J. D. (2000). Morphology and survivorship of larval *Psammechinus miliaris* (Gmelin) (Echinodermata: Echinoidea) in response to varying food quantity and quality. *Aquaculture* **182**: 223-240.

Kirchoff N. T., Eddy S., Harris L.; Brown N. P. (2008). Nursery-phase culture of green sea urchin *Strongylocentrotus droebachiensis* using “On-bottom” cages. *Journal of Shellfish Research* **27**(4): 921-927.

Kitada S. (1999). Effectiveness of Japan’s stock enhancement programmes: Current perspectives. In B. R. Howell, Moksness E., Svåsand T. (Eds). *Stock Enhancement and Sea Ranching*. Blackwell Science, Oxford: 153-167.

Lauzon-Guay J.-S., Scheibling R. (2008). Evaluation of passive integrated transponder (PIT) tags in studies of sea urchins: caution advised. *Aquatic Biology* **2**: 105-112.

Lawrence J. M., Fenaux L., Corre M. C., Larence A. (1992). The effect of quantity and quality of prepared diet on production in *Paracentrotus lividus* (Echinodermata: Echinoidea). In: Scalera L., C. Canicatti (Eds). *Echinoderm Research*. Balkema, Rotterdam: 107-110.

Lawrence J. M. (2001) *Edible Sea Urchins: Biology and Ecology*. Elsevier Science, Amsterdam: 380 pp.

Lederer E. (1952). Sur les pigments naphthoquinoniques des épines et du test des oursins *Paracentrotus lividus* and *Arbacia pustulosa*. *Biochimica et Biophysica Acta* **9**: 92-101.

Legendre P., Legendre L. (1998). *Numerical Ecology*. Elsevier, Amsterdam: 853 pp.

Lessios H. A. (2007). Reproductive isolation between species of sea urchins. *Bulletin of Marine Science* **81**: 191-208.

Li L., Huang X., Borthakur D., Ni H. (2012). Photosynthetic activity and antioxidative response of seagrass *Thalassia hemprichii* to trace metal stress. *Acta Oceanologica Sinica* **31**: 98-100.

Lindahl P. E., Runnström R. (1929). Variation und ókologie von *Psammechinus miliaris* (Gmelin). *Acta Zoologica* **10**: 401-484.

Liu H., Kelly M. S., Cook E. J., Black K., Orr H., Zhu J. X., Dong S. L. (2007). The effect of diet type on growth and fatty-acid composition of sea urchin larvae, I. *Paracentrotus lividus* (Lamark, 1816) (Echinodermata). *Aquaculture* **264**: 247-262.

Lopes E. M., Ventura C. R. R. (2012). Morphology and gametic compatibility of color morphs of *Paracentrotus gaimardi* (Echinodermata: Echinoidea). *Invertebrate Biology* **131**: 224-234.

Louise F., Benard F. (1993). Dététerminisme gétique du polychromatisme de *Paracentrotus lividus* (Lamarck). *Bulletin de la Société Zoologique de France* **118**: 405-408.

Maravelias C. D., Reid D. G., Simmonds E. J., Haralabous J. (1996). Spatial analysis and mapping of acoustic survey data in the presence of high local variability: geostatistical application to North Sea herring (*Clupea harengus*). *Canadian Journal of Fishery Aquatic Sciences* **53**: 1497-1505.

Marín-Guirao L., Sandoval-Gil J. M, Ruíz J. M., Sánchez-Lizaso J. L. (2011). Photosynthesis, growth and survival of the Mediterranean seagrass *Posidonia oceanica* in response to simulated salinity increases in a laboratory mesocosm system. *Estuarine, Coastal and Shelf Science* **92**: 286-296.

Matheron G. (1965). *La théorie des variables régionalisées et ses applications*. Masson et Cie Éditeurs, Paris: 212 pp.

Maynou F. (1998). The application of geostatistics in mapping and assessment of demersal resources. *Nephrops norvegicus* (L.) in the northwestern Mediterranean: a case study. *Scientia Marina* **62**(1): 117-133.

Mcbride S. C., Price R. J., Tom P. D., Lawrence J. M., Lawrence A. L. (2004). Comparison of gonad quality factors: color, hardness and resilience, of *Strongylocentrotus franciscanus* between sea urchins fed prepared feed or algal diets and sea urchins harvested from the Northern California fishery. *Aquaculture* **233**: 405-422.

Megrey B. A., Moksness E. (2009). *Computers in Fisheries Research, 2nd edition*. Springer Science + Business Media B.V, Netherlands: 422 pp.

Miller R. J., Nolan S. C. (2008). Management methods for a sea urchin dive fishery with individual fishing zones. *Journal of Shellfish Research* **27**(4): 929-938.

Onitsuka T., Unuma T., Kawamura T. (2013). Effects of diet and temperature on post-settlement growth and survival of the short-spined sea urchin *Strongylocentrotus intermedius*. *Aquaculture Research* **45**(1): 1-15.

Orensanz J. M., Armstrong J., Armstrong D., Hilborn R. (1998). Crustacean resources are vulnerable to serial depletion - the multifaceted decline of crab and shrimp fisheries in the greater Gulf of Alaska. *Reviews in Fish Biology and Fisheries* **8**: 117-176.

Orensanz J. M., Parma A. M., Turk T., Valero J. (2006). Population, Dynamics and Management of Natural Scallops. In: Shumway S. E., Parsons G. J. (Eds), *Scallops: Biology, Ecology and Aquaculture, Vol 35*. Elsevier, Amsterdam: 765-868.

Ouréns R., Fernández L., Freire J. (2011). Geographic, population, and seasonal patterns in the reproductive parameters of the sea urchin *Paracentrotus lividus*. *Marine Biology* **158**: 793-804.

Pais A., Chessa L. A., Serra S., Ruiu A., Meloni G., Donno Y. (2007). The impact of commercial and recreational harvesting for *Paracentrotus lividus* on shallow rocky reef sea urchin communities in North-western Sardinia, Italy. *Estuarine Coastal and Shelf Science* **73**: 589-597.

Pais A., Serra S., Meloni G., Saba S., Ceccherelli G. (2011). Harvesting effects on

Paracentrotus lividus population structure: a case study from Northwestern Sardinia, Italy, before and after the fishing season. *Journal of Coastal Research* **28**(3): 570-575.

Palacín C., Giribert G., Turon X. (1997). Patch recolonization through migration by the echinoid *Paracentrotus lividus* in communities with high algal cover and low echinoid densities. *Cahiers de Biologie Marine* **38**(4): 267-271.

Palumbi S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology, Evolution, and Systematics* **25**: 547-572.

Pearce C. M., Daggett T. L., Robinson S. M. C. (2002). Optimizing prepared feed ration for gonad production of the green sea urchin *Strongylocentrotus droebachiensis*. *Journal World Aquaculture Society* **33**: 268-277.

Pearse J. S., Cameron R. A. (1991). Echinodermata: Echinoidea. In Giese A. C., Pearse J. S., Pearse V. B. (Eds): *Reproduction of marine invertebrates, Vol. VI. Echinoderms and Lophophorates*. Boxwood press, CA, Pacific Grove: 513-662.

Pedreotti M. L., Fenaux L. (1993). Effects of food on larval survival, development and growth rates of two cultured echinoplutei (*Paracentrotus lividus* and *Arbacia lixula*). *Invertebrate Reproduction & Development* **24**: 59-70.

Pérès J.M., Picard J. (1964). Nouveau manuel de bionomie benthique de la mer Méditerranée. *Recueil des Travaux de la Station Marine d'Endoume* **31**(47): 5-137.

Perry R. I., Walters C. J., Boutillier J. A. (1999). A framework for providing scientific advice for the management of new and developing invertebrate fisheries. *Reviews in Fish Biology and Fisheries* **9**: 125-150.

Perry R. I., Zhang Z., Harbo R. (2002). Development of the green sea urchin (*Strongylocentrotus droebachiensis*) fishery in British Columbia, Canada - back from the brink using a precautionary framework. *Fishery Research* **55**: 253-266.

Petitgas P. (1993). Geostatistics for fish stock assessments: a review and an acoustic application. *ICES Journal of Marine Sciences* **50**: 285-298.

Petitgas P. (2001). Geostatistics in fisheries survey design and stock assessment: models, variances and applications. *Fish and Fisheries* **2**(3): 231-249.

Power M. (1993). The predictive validation of ecological and environmental models. *Ecological Modelling* **68**: 33-50.

RAS (1994). Decreto dell’Assessore della Difesa dell’Ambiente n.276 in data 3 marzo 1994: disciplina della pesca dei ricci di mare. *Bollettino Ufficiale della Regione Autonoma della Sardegna* Parti I e II **8**: 440-441.

RAS (2001). Modifiche ed integrazioni al decreto dell’Assessore della Difesa dell’Ambiente n.277 in data 3 marzo 1994: “Disciplina della pesca professionale subacquea e n.276 in data 3 marzo 1994: disciplina della pesca dei ricci di mare. *Bollettino Ufficiale della Regione Autonoma della Sardegna* Parti I e II **2**: 41-43.

RAS (2008). Ulteriore proroga del calendario della pesca dei ricci di mare per la stagione 2005/2006. *Regione Autonoma della Sardegna - Assessorato della Difesa dell’Ambiente DECRETO N. 13/V DEL 7 aprile 2006*.

RAS (2009). Calendario della pesca del riccio di mare (*Paracentrotus lividus*) per la stagione 2009/2010. *Regione Autonoma della Sardegna - Assessorato Agricoltura e Riforma Agro-Pastorale* Decreto N. 2763/DecA/117 DEL 29.10.2009.

RAS (2010). Calendario della pesca del riccio di mare (*Paracentrotus lividus*) per la stagione 2010/2011. *Regione Autonoma della Sardegna - Assessorato Agricoltura e Riforma Agro-Pastorale* Decreto N. 2599 /DecA/98 DEL 26 ottobre 2010.

RAS (2011). Calendario della pesca del riccio di mare (*Paracentrotus lividus*) per la stagione 2011/2012. *Regione Autonoma della Sardegna - Assessorato Agricoltura e Riforma Agro-Pastorale* Decreto N. 1940/DecA/68 DEL 28 ottobre 2011.

RAS (2014). Modifiche e integrazioni al Decreto n. 1967/DecA/67 del 29 ottobre 2013 “Calendario della pesca del riccio di mare (*Paracentrotus lividus*) per la stagione 2013/2014. *Regione Autonoma della Sardegna - Assessorato Agricoltura e Riforma Agro-Pastorale* Decreto. N. 325/DecA/19 DEL 26 marzo 2014.

Ricker W. E. (1975). Computation and interpretation of biological statistics of fish populations. *Bulletin Fisheries Research Board of Canada* **191**: 1-382.

Robinson S. M. C., Castell J. S., Kennedy E. J. (2002). Developing suitable colour in the gonads of cultured green sea urchins (*Strongylocentrotus droebachiensis*). *Aquaculture* **206**: 289-303.

Rossi R. E., Mulla D. J., Journel A. G., Franz E. H. (1992). Geostatistical tools for modeling and interpreting spatial depen-dence. *Ecological Monographs* **62**: 277-314.

Rueda M. (2001). Spatial distribution of fish species in a tropical estuarine lagoon: a geostatistical appraisal. *Marine Ecology Progress Series* **222**: 217-226.

Ruppert E. E., Barnes R. D. (1994). *Invertebrate zoology*. Saunders College Publishers, Philadelphia.

Saito K. 1992. Japan’s sea urchin enhancement experience. In California Sea Grant College

(Ed). *The management and enhancement of sea urchins and other kelp bed resources: a Pacific rim perspective*. Report number T-CSGCP-028, Scripps Institution of Oceanography, La Jolla: (not paginated).

Sala E. (1997) Fish predators and scavengers of the sea urchin *Paracentrotus lividus* in protected areas of the north-west Mediterranean Sea. *Marine Biology* **129**: 531-539.

Sala E., Ribes M., Hereu B., Zabala M., Alvà V., Coma R., Garrabou J. (1998). Temporal variability in abundance of the sea urchins *Paracentrotus lividus* and *Arbacia lixula* in the northwestern Mediterranean: comparison between a marine reserve and an unprotected area. *Marine Ecology Progress Series* **168**: 135-145.

Sala E., Zabala M. (1996). Fish predation and the structure of the sea urchin *Paracentrotus lividus* populations in the NW Mediterranean. *Marine Ecology Progress Series* **140**: 71-81.

San Martín G.A. (1995). Contribution a la gestion des stocks d’oursins: etude des populations et transplantations de *Paracentrotus lividus* a Marseille (France, Mediterranee) et production de *Loxechinus albus* a Chiloe (Chili, PaciWque). *These de Doctorat.* Faculté des Sciences de Luminy, Université de la Méditerranée Aix, Marseille II: 60 pp.

Sellem F., Guillou M. (2007). Reproductive biology of *Paracentrotus lividus* (Echinodermata: Echinoidea) in two contrasting habitats of northern Tunisia (south-east Mediterranean). *Journal Marine Biology Association of the United Kingdom* **87**: 763-767.

Shpigel M., McBride S. C:, Marciano S., Ron S, Ben-Amotz A. (2005). Improving gonad colour and somatic index in the European sea urchin *Paracentrotus lividus*.

Aquaculture **245**: 101-109.

Shpigel M., Schlosser S.C., Ben-Amotz A., Lawrence A.L., Lawrence J.M. (2006). Effects of dietary carotenoid on the gut and the gonad of the sea urchin *Paracentrotus lividus*. *Aquaculture* **261**: 1269-1280.

Simard Y., Legendre P., Lavoie G., Marcotte D. (1992). Mapping, estimating biomass, and optimizing sampling programs for spatially autocorrelated data: Case study of the northern shrimp (*Pandalus borealis). Canadian Journal of Fisheries and Aquatic Sciences 49*(1):32-45.

Sloan N. A. (1985). Echinoderm fisheries of the world: a review. In: Keegan B.F., O'Connor B.D.S. (Eds). *Proceedings of the Fifth International Echinoderm Conference*. Balkema, Rotterdam: 109-125.

Sonnenholzner J. I., Montaño-Moctezuma G., Searcy-Bernal R. (2010). Effect of three tagging methods on the growth and survival of the purple sea urchin *Strongylocentrotus purpuratus*. *Journal of Aquatic Sciences* **5**: 414-420.

Spirlet C., Grosjean P., Jangoux M. (2000). Optimization of gonad growth by manipulation of temperature and photoperiod in cultivated sea urchins, *Paracentrotus lividus* (Lamarck) (Echinodermata). *Aquaculture* **185**: 85-99.

Stelzenmüller V., Maynou F., Martín P. (2007). Spatial assessment of benefits of a coastal Mediterranean Marine Protected Area. *Biology Conservation* **136**(4): 571-583.

Sugni M., Motta D., Tremolada P., Carnevali M. D. C. (2012) Exploring endocrine regulation of sea urchin reproductive biology: effects of 17 beta-oestradiol. *Journal of The Marine Biological Association of The United Kingdom* **92**(6): 1419-1426.

Symonds R. C., Kelly M. S., Caris-Veyrat C., Young A. J. (2007). Carotenoids in the sea urchin *Paracentrotus lividus*: occurrence of 9'-cis- echinoenone as the dominant carotenoid in gonad colour determination. *Comparative Biochemistry and Physiology-Part B: Biochemistry and Molecular Biology* **148**: 432-444.

Tegner M. J., Dayton P. K. (1977). Sea urchin recruitment patterns and implication of commercial fishing. *Science*, **196**: 324-326.

Tomas F., Romero J., Turon X. (2004). Settlement and recruitment of the sea urchin *Paracentrotus lividus* in two contrasting habitats in the Mediterranean. *Marine Ecology Progress Series* **282**: 173-184.

Tsuchiya M., Nishihara M. (1984) Ecological distribution of two types of sea urchin, *Echinometra mathaei* (Blainville), on Okinawan reef flat. *Galaxea* **3**: 131-143.

Tsuchiya M., Nishihara M. (1985). Agonistic behaviour and its effect on the dispersion pattern of two types of the sea urchin, *Echinometra mathaei* (Blainville). *Galaxea* **4**: 37-48.

Turon X., Giribet G., Lopez S., Palacín C. (1995). Growth and population structure of *Paracentrotus lividus* (Echinodermata: Echinoidea) in two contrasting habitats. *Marine Ecology Progress Series* **122**: 193-204.

Underwood A. J. (1997) *Experiments in Ecology: Their Logic Design and Interpretation using Analysis of Variance*. Cambridge University Press, Cambridge: 504 pp.

Unuma T. (2002). Gonadal growth and its relationship to aquaculture in sea urchins. In: Yukio Y., Matranga V., Smolenicka Z. (Eds). *The Sea Urchin: From Basic Biology to Aquaculture*. Balkema, The Netherlands: 115-128.

Vaïtilingon D., Morgan R., Grosjean P., Gosselin P., Jangoux M. (2001). Effects of delayed metamorphosis and food rations on the perimetamorphic events in the echinoid *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata). *Journal of Experimental Marine Biology and Ecology* **262**: 41-60.

Walker C. W., Unuma T., McGinn N. A., Harrington L. M., Lesser M. P. (2001). Reproduction of sea urchin. In: J.M. Lawrence (Ed). *Edible Sea Urchins: Biology and Ecology vol. 32*. Elsevier, Amsterdam: 5-26.

Warren W. (1998). Spatial analysis for marine populations: factors to be considered. *Canadian Special Publication of Fisheries and Aquatic Sciences* **125**: 21-28.

Wasson K. M., Gower B. A., Hines G.A., Watts S. A. (2000). Levels of progesterone, testosterone and estradiol and androstenedione metabolism in the gonads of Lytechinus variegatus (Echinodermata: Echinoidea). *Comparative Biochemistry and Physiology* **126C**: 153-165.

Woods C. M. C., James P. J. (2005). Evaluation of passive integrated transponder tags for individually identifying the sea urchin *Evechinus chloroticus* (Valenciennes). *Aquaculture Research* **36**:730-732.

Woods C. M. C., James P. J., Moss G. A., Wright J., Siikavuopio S. (2007). A comparison of the effect of urchin size and diet on gonad yield and quality in the sea urchin *Evechinus chloroticus* Valenciennes. *Aquaculture International* **16**: 49-68.

Xing R. L., Wang C. H., Cao X. B., Chang Y. Q. (2007). The potential value of different species of benthic diatoms as food for newly metamorphosed sea urchin *Strongylocentrotus intermedius*. *Aquaculture* **263**(1-4): 142-149.

ANNEXES

Since my scientific activity was much diversified regarding other topics, only a part of my work was included in this thesis. Here are the abstracts of all publications and symposia where I have participated.

International publications:

Addis P., **Secci M.**, Manunza A., Corrias S., Niffoi A., Angelo Cau (2009). A geostatistical approach for the stock assessment of the edible sea urchin, *Paracentrotus lividus*, in four coastal zones of Southern and West Sardinia (SW Italy, Mediterranean Sea). *Fisheries Research* **100**(3): 215-221.

ABSTRACT: Quantitative surveys of the edible sea urchin, *Paracentrotus lividus*, were conducted in four fishing zones of Sardinia (Southern Italy, Mediterranean Sea), in Autumn 2007. Atotal of 120 stations were geo-located along a bathymetric gradient ranging from 0 to 10 m.Ageostatistical method was used to evaluate spatial patterns in density and to estimate harvestable stocks. Variographic analyses showed that the isotropic Gaussian and spherical models successfully explained the spatial structure of sea urchin assemblages in these areas. Density maps obtained by punctual kriging showed that sea urchin populations tend to be patchy rather than uniform in their density distribution. A combination of mapping and size categories was used to generate diverse scenarios of harvestable stocks (specimens ≥50 mm in diameter) before the start of the current fishing season. We conclude that the geostatistical approach, which takes into consideration the spatial autocorrelation structure of the populations in small areas, seems to be a good estimator of *P. lividus*

density and biomass and for the assessment of its harvestable stocks, and thus provides an initial step towards a scientific approach to the management of local sea urchin fisheries.

KEYWORDS: *Paracentrotus lividus*, Geostatistical method, Stock assessment, Sardinia, Mediterranean Sea.

Angioni A., Porcu L., **Secci M.**, Addis P. (2012). QuEChERS Method for the Determination of PAH Compounds in Sardinia Sea Urchin (*Paracentrotus lividus*) Roe, Using Gas Chromatography ITMS-MS Analysis. *Food Analytical Methods* **65**(3): 1131-1136.

ABSTRACT: Sea urchin roe (*Paracentrotus lividus*) is an excellent biomarker, and it has been used as a test for toxicology study of chemical pollution of restricted marine areas; in fact, unlike mussels which filter water, it feeds mainly on micro- and macroalgae. Therefore, it is not influenced by the sea tide and meets better the situation of local pollution. A modified Quick Easy Cheap Effective Rugged Safe extraction method coupled with gas chromatography ion trap mass spectrometry analysis has been used to set up a method for the determina- tion of 17 polycyclic aromatic hydrocarbons (PAHs) in *P. lividus* sea urchin roe. A method validation was performed, and the following parameters have been determined: instrument limit of detection (LOD) and of quantification (LOQ), precision, recovery, and linearity. The method carried out showed good LOD under 1.5 μg/kg and LOQ under 5 μg/kg for all PAHs studied, and these values were in close agreement with quality criteria described in the Commission Regulation (EC) no. 333/2007 concerning the PAH benzo[a] pyrene in foodstuffs. Precision and linearity were in accor- dance with EC SANCO/10684/2009 values. For all PAHs, recoveries ranged from 72%to 119%,with RSDranging from 3% to 17% in the most unfavorable case.

KEYWORDS: QuEChERS, *Paracentrotus lividus*, Sea urchin roe, GC/ITMS/MS, Polycyclic aromatic hydrocarbons, Validation.

Addis P., Corrias S., Garau C., **Secci M.** (2013). Physiologic responses to stress and changes in Atlantic bluefin tuna (*T. Thynnus*) meat color during trap fisheries capture and processing in Sardinia (W. Mediterranean) *Journal of Aquatic Food Product Technology* **22**(3): 298-309.

ABSTRACT: This study investigated plasma cortisol, lactate, and glucose as descriptors of hematological stressors; while the RGB color model using the percentage of monochromatic channels Red (R_p), Green (G_p), and Blue (B_p) was used for the analysis of muscular tissue of the Atlantic bluefin tuna, *Thunnus thynnus*. The experimental design provided a comparison of stressors and color channels *Before* and *After* the stress state and the analysis of variability of monochromatic channels for *Fresh* and *Frozen* specimens collected at three time intervals. Results showed a rapid accumulation of cortisol levels (from 73.3 ± 9.5 to 148.0±21.2 ng/mL), lactate (from 5.7±2.9 to 17.0±2.2 μmol/mL), and glucose (from 83.5±8.0 to 128.6±19.3 mg/dL; $p<0.05$). The colorimetric analysis highlighted that this accumulation did not in fact affect the color variability of muscle. Analysis of variance carried out to test the effects of color variability in *Fresh* samples showed significant differences for R_p, $G_{p,}$ and B_p channels ($p<0.05$), whereas no differences were found in *Frozen* samples. Highly significant differences ($p<0.001$) were found comparing *Fresh* and *Frozen* for R_p and B_p, indicating a drop of these channels under diverse treatments.

KEYWORDS: Atlantic bluefin tuna, stress response, meat color, trap fishery, Mediterranean Sea.

Addis P., **Secci M.**, Locci I., Cau A., Sabatini A. (2012) Analysis of Atlantic Bluefin Tuna Catches From the Last Tonnara in The Mediterranean Sea: 1993-2010. *Fisheries Research* **127-128**: 133-141**.**

ABSTRACT: In the last 20 years, several factors have heavily impacted the Atlantic bluefin tuna fishery. They include management policies, market changes, the ban of the drift-net fishery, expansion of the modern purse seine fleet, and implementation of a stock recovery plan. To enhance current knowledge about the pop- ulation's status, we conducted a long-term analysis (1993–2010) of scientific data and standardized catch-per-unit-of-effort (CPUE) from the traditional trap fishery of Sardinia (Western Mediterranean, Italy), which is the last active bluefin trap fishery in the Mediterranean. We detected a significant increase of the standardized CPUE and a significant decrease in mean weight over time. Cluster analysis con- ducted on 29,000 specimens revealed three different size groups that were distinct by time period: the 1993–1995 period was characterized by a significant presence of large bluefin; a decrease in mean weight occurred in the 1996-2006 period; and 2007-2010 was characterized by the prevalence of young adults in the history in the trap fishery. This trend, which needs to be confirmed over longer time and spatial scales, raises some ecological questions. In particular, is the occurrence of these young adults a conse- quence of changes in the migratory behavior of bluefin tuna in the Mediterranean, or does it reflect the actual demography of this population? The results of this study emphasize that data from traditional traps provide valuable long term scientific information about population parameters through time, and thus the use of traps as monitoring stations should continue in the future.

KEYWORDS: Bluefin tuna Trap fishery Time series Demographic structure

Mediterranean Sea.

Addis P., **Secci M.**, Cau A. (2013) The effect of Mistral (a strong NW wind) episodes on the occurrence and abundance of Atlantic bluefin tuna (*Thunnus thynnus*) in the trap fishery of Sardinia (W Mediterranean). *Scientia Marina* **77**: 419-427.

ABSTRACT: From April to June Atlantic bluefin tuna, *Thunnus thynnus*, migrate along the western Sardinian coastline in a southward direction, where they are intercepted by the trap fishery. Fishermen claim that Mistral episodes facilitate the entry of tuna schools towards the traps, thus increasing capture rates. To test the fishermen's hypothesis we conducted underwater visual counts of tuna in the trap chambers and analysed these data under the effect of wind. The results indicate a "stair-step" pattern in the abundance of tuna, demonstrating that major increases in abundance are associated with the Mistral. The second analytical approach involved a longer time scale to test whether higher Mistral occurrences corresponded to periods when higher captures were recorded. Using a linear regression model we found a significant correlation ($p<0.01$) between catches and the wind speed class ≤15 knots. We hypothesize that the wind-driven current may produce favourable conditions for tuna migration and could play a significant role in enabling fish to save energy. It was also noted that the wind speed class >15 knots seemed to have a negative effect on captures. This pattern may be caused by wind-induced advection of coastal waters generating a physical boundary that may have had a deterrent effect on tuna schools.

KEYWORDS: *Thunnus thynnus*, trap fishery, abundance, visual census, wind effect, Western Mediterranean.

Angioni A., Cau A., **Secci M.**, Addis P. Contamination levels of PAHs in marine environment by sea urchin *Paracentrotus lividus* GCITMS analysis in Sardinia. *Marine Pollution Bulletin* **In press**.

ABSTRACT: This paper describes the results of a two year monitoring study on the pollution of sea urchin *Paracentrotus lividus* by polycyclic aromatic hydrocarbons (PAHs) in Sardinia. GCITMS analysis of sea urchin gonads showed the presence of 11, and 12 PAHs in the samples of Capo Pecora, and Capitana, respectively. Fluorene, naphthalene and its two degradation products 1-methyl-naphthalene, and 2-methyl-naphtalene were detected in all samples analyzed. The PAH residues showed in the two years a similar trend. Furthermore the residues in the first year were slightly higher than in the second year. The information obtained by multivariate statistical analysis PLS-DA allowed to distinguish the samples for the field site, and for the different habitat (rocky reef, and Posidonia seabed). The results of this study showed that Posidonia sea urchins are contaminated by high molecular weight PAHs, and Capitana samples are more contaminated due to the higher level of human activities.

KEYWORDS: *Paracentrotus lividus*, PAH, residues, GC-ITMS, PLS-DA

Reports and other publications

Secci M., Addis P. (2011). Preliminary data on spatial distribution by size of *Paracentrotus lividus* in an area of Sardinia. *Biologia Marina Mediterranea* **18** (1): 288-289.

ABSTRACT: We investigated on spatial distribution of the edible sea urchin *Paracentrotus lividus* (Echinidae) in an area of western Sardinia (Southern Italy, Mediterranean Sea) by a geostatistical approach. The investigation was carried out before the beginning of the fishing season 2010/2011 and considered three size classes of test diameter. This method enables to successfully explain spatial pattern of the species in the given area identifying recruitment areas and adult distribution. We emphasize the use of such a tool to improve management efficiency aimed at the conservation of the resource.

KEYWORDS: *Paracentrotus lividus*, Mediterranean Sea, Geotechnology, spatial distribution.

Secci M., Locci I., Addis P. (2011). Physiological responses in the Atlantic bluefin tuna, *Thunnus. thynnus*, captured in the trap fishery of Sardinia (W. mediterranean). *Biologia Marina Mediterranea* **18**(1): 228-229.

ABSTRACT: The aim of this study has been to improve the knowledge of the physiological responses of the Atlantic bluefin tuna *Thunnus thynnus* (Scombridae), under stress and stress-free state conditions. We investigate plasma lactate, cortisol and glucose as haematological descriptors of stress. The experimental design provides a comparison of indicators Before vs. After the stress state. Finally we argue on the adoption of good fishing practice and handlings of specimens in the pre- and post-fishing phase of specimens, an expedient strategy to obtain the maximum quality of product in this "niche" fishery.

KEYWORDS: Atlantic bluefin tuna, stress response, trap fishery, Mediterranean Sea.

Addis P., **Secci M.**, Locci I., Sabatini A., Dean J. M., Cau A. (2011). Long-term analysis (1993-2010) of the catches of the Atlantic bluefin tuna (*Thunnus thynnus*) from the traditional trap fisheries of sardinia. *Collection Volume of Scientific Papers ICCAT* **67**(1): 295-308.

SUMMARY: In the last decades several changes have heavily impacted the Atlantic bluefin tuna fishery. To contribute to the evaluation of population status and the effectiveness of management policies, a long-term analysis of catch data (1993-2010) and standardized CPUE from the trap fishery of Sardinia (western Mediterranean, Italy) are presented. A significant increase is observed of the standardized CPUE together with a significant decrease of mean weight. The cluster analysis of the mean weight identified three different year groups: 1993-1995, 1996-2006 and 2007-2010. These time periods, which were confirmed by the analysis of the weight frequency distribution, have showed a progressive reduction of larger individuals in the landings. A significant increase of recruits was observed, beginning in 2007, together with a slight increase of mean weight. This trend, which needs to be confirmed over a larger time span of observations, might be the result of new management policies. Results are presented on the length-weight, total weight/gutted weight relationships and the sex-ratio. These results emphasize that traditional traps can provide valuable scientific information on population parameters through time.

KEYWORDS: Bluefin tuna, trap fishery, time series, demographic structure, Mediterranean Sea.

Addis P., **Secci M.**, Locci I., Cannas R., Greco G., Dean J. M., Cau A. (2011). Social, cultural and basic economic analysis of the trap fishery of Sardinia: first step towards parameterization. *Collection Volume of Scientific Papers ICCAT* **67**(1): 380-389.

SUMMARY: In this paper the economic, social and cultural factors engendered by the tuna-trap fishery of Sardinia are studied. To acquire quantitative data, questionnaires were disseminated to stakeholders of the trap fishery and in a sample of 200 inhabitants of the villages of Carloforte and Portoscuso. Results emphasized that some sub-sectors produced direct economic income; others produced an unquantifiable social capital highlighting a strong interdependence of the local community from the “trap network”. We note that the vulnerability of the trap fishery is determined by two intimately related mechanisms: Risks of predictable) and uncertainty (unpredictable). The latter take account the fishing business (likely of the current management policy which generates “the quarrel for quotas”, an unfavourable condition for traps. A review of the fishery policy considering the interconnection between socio-political and economics feedback is essential for the survival of the trap system and to preserve their cultural heritage.

KEYWORDS: Mediterranean Sea, trap fishing, socioeconomic aspects, conservation.

Addis P., **Secci M.**, Locci I., Cau A. (2011). Harvesting, handling practices and processing of bluefin tuna captured in the trap fishery: possible effects on the flesh quality. *Collection Volume of Scientific Papers ICCAT* **67**(1): 390-398

SUMMARY: We investigated the physiological responses of bluefin tuna under stress and stress-free state conditions to assess the possible effect on color variability in muscle tissue. The study was carried out in the trap fishery of Sardinia (western Mediterranean). Here bluefin tuna pass from the wild to conditions of captivity and are then fished out during the “mattanza” and prepared for fresh and cannery markets. We investigated plasma lactate, cortisol and glucose and the color variability of flesh by the RGB color space model. The experiment provides a comparison of stressors and monochromatic channels Before vs. After the stress state. Results showed a significant accumulation of stressors from Before to After. The colorimetric analysis highlighted that this accumulation did not affect the color variability of flesh. Fresh samples showed significant differences for Rp, Gp and Bp over time ($P<0.05$), whereas no differences were found in frozen samples. We suggest that the adoption of good fishing practise and handling of tunas is an expedient strategy to obtain the maximum quality of product and highest market quotation.

KEYWORDS: Bluefin tuna, trap fishing, fish handling, processed fishery products

Addis P., **Secci M.**, Sabatini A., Palmas F., Culurgioni J., Pasquini V., Cau A. (2013). Conventional tagging of bluefin tunas in the trap fishery of Sardinia (W-Mediterranean): a critical review. *SCRS/2013/180*.

ABSTRACT: In May-July 2013 the first conventional tagging of bluefin tuna by divers was conducted in the trap fishery of Sardinia (western Mediterranean). The Isola Piana and the Portoscuso trap, the last active traps in the Mediterranean Sea, were involved in the scientific programme. In order to adapt the fishing gear and the equipment for tagging, a few changes were needed at the earliest tagging design. Major changes regarded the use of modified speraguns, underwater action cameras, and the utilization of underwater laser pointers useful for size estimation of tunas. A comparative analysis was conducted to validate length estimations by direct method (photo referencing techniques) and indirect method (visual estimation of weight). A total of 208 bluefin tunas were tagged and released during four days of tagging. Finally, recommendations and technical advices have been proposed to improve the methodology for future tagging activities by traditional traps.

KEYWORDS: Bluefin tuna, trap fishing, tagging, Sardinia