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Larval fish assemblages in the central Mediterranean Sea:  
spatio-temporal dynamics and biological effects on early life stage of  
*Sardinella aurita* (Pisces, Clupeidae)

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## ABSTRACT (Italiano)

Le risorse ittiche marine rappresentano da secoli un importante settore sul quale molti Paesi industrializzati e Paesi in via di sviluppo basano una buona parte della loro economia. Pesca ed acquacultura sono alcuni importanti esempi di un'ampia gamma di business che coinvolgono lo sfruttamento delle specie ittiche. L'incremento della popolazione umana, il miglioramento di tecniche e tecnologie relative alle attività di prelievo ed un sempre crescente aumento delle attività commerciali a livello globale hanno dato slancio ad un settore che tuttavia paga uno sfruttamento rivolto al profitto in modo poco lungimirante delle risorse su cui si basa.

A lungo si è discusso sul ruolo svolto dalla pesca e dai fattori ambientali naturali nell'influenzare le fluttuazioni inter-annuali di biomassa che notoriamente contraddistinguono la maggior parte delle risorse ittiche. Sebbene la maggioranza degli stock ittici sottoposti a sfruttamento siano in stato di “*overfishing*”, l'influenza della variabilità naturale sulle fluttuazioni registrate attraverso stime di biomassa sembra giochi un ruolo fondamentale, tanto certo quanto difficile da valutare. Numerosi sono gli studi che hanno evidenziato come le variabili ambientali incidano su tali popolamenti agendo soprattutto sulla sopravvivenza dei primi stadi del ciclo vitale, uova e larve, notoriamente critici e contraddistinti dai tassi di mortalità più alti dell'intero il ciclo vitale (circa il 99%). L'ambiente agisce da regolatore di questi tassi attraverso numerose forme, le quali, in maniera più o meno diretta, possono essere ricondotte alle mortalità dovute soprattutto alla predazione e alla starvazione. Questi processi si innescano coinvolgendo una serie di fattori che ne regolano l'impatto sul popolamento adulto attraverso una modifica dei tassi di reclutamento dei giovanili. Fluttuazioni del tasso di reclutamento dunque possono incidere in maniera diretta sulla biomassa dello stock adulto, soprattutto in quelle specie semelpare caratterizzate da ciclo vitale breve, grande produzione di uova e larve e la cui popolazione è composta maggiormente dagli individui di un anno di età, come ad esempio i piccoli pelagici.

*Performance* di crescita allo stadio larvale, capacità di alimentarsi e di sfuggire ai predatori sono solo alcuni esempi di meccanismi che possono agevolare o ostacolare lo sviluppo ontogenetico degli individui. Buona parte della variabilità dei fattori che contribuiscono ad influenzare il reclutamento è dato dalla componente stocastica legata all'idrodinamismo che coinvolge lo strato superiore della colonna d'acqua, in cui la maggior parte di uova e larve si concentra in quanto legata al fito e allo

zooplankton durante le prime fasi di alimentazione esogena. Correnti, fronti termoclinici, *gyres* e *upwelling* possono trasportare questi stadi planctonici lontani dalle aree di deposizione e concentrarle altrove, dove le condizioni ambientali possono essere più o meno favorevoli allo sviluppo larvale.

In questo contesto, il presente studio si è proposto come primo obiettivo di esaminare l'effetto che le strutture oceanografiche a mesoscala possono esercitare sulla distribuzione spaziale dei primi stadi di sviluppo dei pesci e sulle modificazioni ambientali indotte, ponendo particolare attenzione sui meccanismi che intercorrono tra questi fattori e le capacità delle larve di fronteggiare questo periodo critico. Tale studio è stato promosso da una serie di campagne oceanografiche portate avanti annualmente dall'Istituto per l'Ambiente Marino Costiero del Consiglio Nazionale delle Ricerche (IAMC-CNR) finalizzate alla raccolta di dati ittioplanctonici e ambientali nel Canale di Sicilia e in diverse zone del Mar Tirreno.

Nella prima parte della tesi (Capitolo 1), i dati raccolti durante due campagne oceanografiche effettuate nel 2009 hanno permesso di caratterizzare per la prima volta la distribuzione di uova e larve di pesci che depongono durante il periodo estivo nel Canale di Sicilia e nel Tirreno meridionale. I pattern spaziali emergenti sono stati poi discussi alla luce delle forzanti fisiche rilevate nelle due aree di studio. In particolare, il Canale di Sicilia ha mostrato un idrodinamismo superficiale molto complesso, in buona parte dominato dallo scorrimento verso est della corrente superficiale atlantica (AIS) e dalle acque di risalita (*upwelling*) fredde e ricche in nutrienti che influenzano gli strati superficiali della colonna d'acqua nella zona costiera sud-orientale siciliana. A queste strutture sono legate da un lato le correnti dirette verso il largo, le quali trasportano larve deposte da specie demersali e costiere in zone offshore; dall'altro le correnti lungo costa che trasportano larve costiere, ma anche meso e batipelagiche verso est, concentrandole nei pressi di un fronte termoclinico e creando così una zona di ritenzione tra Capo Passero (Sicilia sud-orientale) e Malta. Alcune forzanti fisiche hanno mostrato inoltre la capacità di modificare le condizioni ambientali che caratterizzano i primi stadi della colonna d'acqua, specialmente in termini di temperatura e disponibilità di cibo, che sono parametri fondamentali per lo sviluppo ontogenetico dei primi stadi di vita dei pesci. Situazione differente è stata evidenziata nel Sud-Tirreno. Un anticiclone presente nei pressi della zona centrale della costa settentrionale siciliana è stato identificato come l'unica struttura oceanografica a mesoscala in grado di trasportare, in particolare, larve mesopelagiche nella zona costiera. La distribuzione spaziale delle larve in questa area di studio ha quindi mostrato un legame più

stretto con parametri ambientali locali, risultando poco vincolata all'idrodinamismo superficiale. Accoppiando dati fisici con quelli relativi alla distribuzione dell'ittioplancton, questo studio ha quindi mostrato che le forzanti fisiche che si verificano a livello superficiale possono giocare un ruolo chiave in aree di studio caratterizzata da una circolazione superficiale complessa.

Nell'ottica di esaminare il link esistente tra i meccanismi biologici che caratterizzano le fasi larvali e l'ambiente esterno, il presente studio ha successivamente indirizzato l'indagine verso un Clupeidae in particolare: *Sardinella aurita*. In quanto piccolo pelagico, questa specie presenta caratteristiche del ciclo vitale che la rendono molto sensibile alle fluttuazioni di biomassa del popolamento adulto in relazione ai tassi di reclutamento annuali. Inoltre essa è da sempre una specie *target* per le attività di prelievo da parte della pesca, soprattutto nella zona ionica e levantina del Mediterraneo. Recenti studi hanno evidenziato una sua espansione verso nord legata ai fenomeni di *global warming*. Infine è una specie molto poco studiata se confrontata con altri piccoli pelagici, come la sardina e l'acciuga europea, che sono caratterizzati da un maggiore valore economico e con la quale condivide la zona costiera e, nel caso dell'acciuga, il periodo riproduttivo.

Nel Capitolo 2, per la prima volta, le principali aree di *spawning* di questa specie sono state identificate nel lato italo-costiero del canale di Sicilia. Dai dati raccolti durante due campagne oceanografiche effettuate nel 2010-2011, è emerso che le zone caratterizzate da maggiore concentrazione di uova e larve sono le zone costiere orientali in prossimità di Capo Passero e Malta, contraddistinte da acque superficiali più calde. Inoltre, la distribuzione degli stadi planctonici di questa specie è stata messa in relazione alle forzanti fisiche. In aggiunta a quanto evidenziato nel precedente capitolo, l'analisi del trasporto d'Ekman generato dal vento e le simulazioni Lagrangiane hanno evidenziato l'instaurarsi di filamenti freddi e ricchi di nutrienti nelle principali area di *spawning*, la cui genesi è legata a forti e persistenti venti di Maestrale. È stato evidenziato che tali strutture sono in grado di trasportare uova e larve dalla costa siciliana verso Malta e verso le zone offshore, oltre che abbassare la temperatura ed arricchire le acque superficiali di fito e zooplancton per effetto della risalita di nutrienti.

Lo *step* successivo (Capitolo 3) è stato quindi indirizzato verso la valutazione dell'effetto della temperatura e della disponibilità di cibo sullo sviluppo larvale di questa specie. Al fine di valutare le *performance* di crescita in termini di crescita media giornaliera, tecniche di *otolith microstructure analysis* sono state quindi applicate agli otoliti di larve estratte in due zone del Mediterraneo centrale: lo

Stretto di Sicilia e il Tirreno centrale. I dati relativi alla crescita sono stati successivamente messi in relazione alle condizioni di temperatura e clorofilla presenti nelle due aree di studio al momento del campionamento. Le tecniche di *back-calculation* del giorno di schiusa e la definizione delle *back-trajectories* attraverso un approccio di tipo Lagrangiano sono state utilizzate al fine di stimare la collocazione spazio-temporale giornaliera della larva. Modelli statistici multivariati avanzati sono stati successivamente implementati al fine di mettere in relazione l'accrescimento medio giornaliero con le condizioni di temperatura e clorofilla (derivanti da satellite) presenti nei punti identificati per ogni larva dalla schiusa al momento del campionamento. I risultati hanno mostrato la presenza di *fast growers* nel Tirreno centrale relativamente ai primi tre giorni di vita dopo la schiusa, in relazione a condizioni di temperatura e concentrazioni di clorofilla più elevate se confrontate con le aree di deposizione dello Stretto di Sicilia. Questi stadi coincidono con il periodo in cui la larva si alimenta per mezzo del sacco vitellino di derivazione materna (alimentazione endogena). In questo contesto, le differenze riscontrate suggerirebbero un effetto legato alle differenti condizioni di temperatura presenti tra le due aree di studio. Tale evidenza è in accordo con i risultati emersi dal modello additivo generalizzato ad effetto misto (GAMM), il quale mette in luce una significativa relazione non lineare positiva della temperatura con la variabilità dell'accrescimento medio giornaliero. In particolare, un aumento di pendenza della curva è emersa per valori di temperatura superiori a 24.5 °C. Al contrario, la clorofilla non ha mostrato un effetto significativo correlato all'accrescimento medio giornaliero. Questi risultati aprono nuove prospettive circa l'effetto che i *cold filaments* identificati nel Canale di Sicilia possono imprimere sulle *performance* di crescita nei primi giorni di vita delle larve di questa specie termofila. In accordo con i risultati di questo studio, la risalita di acque fredde influenza negativamente l'accrescimento larvale, aumentando le probabilità di morte legate al perpetuarsi per un tempo maggiore in uno stadio critico e contraddistinto da alti tassi di mortalità naturale. Infine, questo studio ha evidenziato la presenza di condizioni ambientali idonee per la riproduzione e lo sviluppo larvale di queste specie in una delle zone collocate più a nord considerando l'area di distribuzione di questa specie in Mediterraneo, suggerendo che altri fattori, come la temperatura minima nel periodo più freddo dell'anno, potrebbero giocare un ruolo più importante nel limitare la distribuzione verso nord di questa specie.

*Sardinella aurita* rappresenta un interessante caso riguardante le relazioni che intercorrono tra le forzanti fisiche e gli effetti sul *life history traits* di questa specie a livello larvale. Tuttavia, ulteriori studi

indirizzati verso una stima quantitativa delle reclute e della biomassa del popolamento adulto in relazione al verificarsi di determinate forzanti fisiche, necessiterebbero di essere portati avanti allo scopo di fornire informazioni più dettagliate circa i meccanismi coinvolti. In questo contesto, i metodi di oceanografia operativa rappresentano un potente strumento da accoppiare alla raccolta di dati ittioplanctonici, in particolare a quelli relativi alla distribuzione spaziale e ai tratti biologici che coinvolgono i primi stadi del ciclo vitale dei popolamenti ittici. Considerando l'attuale disponibilità di grandi moli di dati di derivazione satellitare relativi alle condizioni ambientali e ricoprenti un elevato *range* spazio-temporale, tale approccio multidisciplinare potrebbe rappresentare un background oggi possibile sul quale basare modelli previsionali e studi di *stock assessment* finalizzati ad una gestione sostenibile delle risorse alieutiche.

## INTRODUCTION

Marine fish have always been one of the most important resources for humans needs. An important sector of the economy of several Countries is based on activities linked to this kind of resource. Fishery and aquaculture are only some business among a suite of employment linked to the exploitation of fish species.

Increasing of human populations, improving of technology and the commercial connections at global level have led human activities towards an exploitation always more profitable of the fishery resources. This effort has culminated in a development of the fishing activity, as well as an increasing impact of them on the fish populations. Use of always more efficient gear aimed to fish capture has led to the contraction of fish population due to overfishing (e.g. Lleonart and Maynou, 2003; Tudela, 2004; Colloca et al., 2013). Moreover the indiscriminate and environmentally-invasive use of the fishing methods (e.g. bottom trawling) unable to perform a selection of the target species, has dramatically impacted the habitat suitability, contributing with a negative added-value to the biomass recovery of commercially important fish species.

In this framework, population dynamics studies not addressed to an analysis of the connections at ecosystem level, have appeared not adequate to provide a comprehensive picture of the occurring phenomena. The relative failure of conventional fisheries management based on this kind of approach has been abundantly described (e.g. Garcia, 1992; Garcia et al., 1996; Garcia et al., 1997; Sutinen and Soboil, 2003).

Therefore, the concept of Ecosystem Management emerged in the last decades. It was formalized at global level in 1972 at Stockholm Conference on the Human Environment and strengthened by the Conference on Environment and Development (UNCED) and Convention on Biological Diversity (1992). Thereafter, it was consequently adopted by the FAO technical Consultation with the term of Ecosystem Approach to Fisheries (EAF) in 2002.

After that, fisheries management based on ecosystem approach has been object of several studies and international conventions (see Garcia and Cochrane, 2005, for a review). Studies aimed to figure out mechanism underlying of the relationship between fish populations and the ecosystem have carried out always more frequently. Understanding the environmental effects of the variability in abundance and

distribution of exploited fishery stocks became a key issue for management strategies and sustainability of the fisheries resources.

In this context, the comprehension of the dynamics involving fish population is not able to leave something out of the study of the fish reproduction processes. Recruitment of the new generations into the adult populations is directly correlated to the reproductive success and affect the fish biomass that will occur in the following years. Specifically, several studies highlighted the strong connection between recruitment and survival of the early life planktonic stages of fish, i.e. eggs and larvae (Cury & Roy, 1989; Bernal, 1991; Hunter and Alheit, 1995; Bakun, 1996; Patti et al. 2004). Larval fishes suffer prodigious mortality rates, eliminating 99% of the brood within a few days after first feeding (Hjort, 1914). Survivals of the early life stages is tuned and strongly dependent to environmental conditions occurring during the spawning periods. Understanding mechanism underlying the reproduction success in term of mortality rates of fish and eggs larvae is always one of the most challenge of the fisheries science.

For this purposes, ichthyoplanktonic-monitoring programs has been carried out in different part of the world with the aim to clarify ecological and biological features of fish eggs and larvae and relationship with the surrounding environment. For instance, a very long time series is represented by the ichthyoplanktonic surveys carried out from the 1949 by the CalCOFI multi-agency partnership in order to investigate the collapse of sardine population in California. In the Mediterranean Sea, one of the most important ichthyoplanktonic time series were collected in the framework of ANSIC (from 1997 to 2004) and BANSIC (form 2005 to nowadays) cruises carried out in the Italian side of the Strait of Sicily, thanks to the effort of the Italian National Research Council (CNR). Main goal of these cruises is the study of the early life history traits of the European anchovy (*Engraulis encrasicolus*) due to the commercial importance as fishery resource.

However, these surveys-series has made possible the collection of ichthyoplanktonic and environmental data regarding a multitude of species at eggs and larval stage present in the first layers of the water column during the summer time.

It has been highlighted that mesoscale oceanographic structures, including geostrophic and wind influenced current regimes (wind-induced mixing of the surface layer, upwelling) play an important role

in determining the patterns of abundance and distribution of larval fish populations due to the planktonic nature of this stages. This because they act as mechanisms of retention and concentration of fish larvae in recruiting areas, affecting environmental conditions and hence, growth and survival (Alemany et al., 2006; Paris et al., 2007; Falcini et. al., 2015).

Determination of the links between physical forcings, induced modifications of fundamental environmental parameters and growth of fish larvae could be crucial in order to understand how environment affect and tune the reproduction success in terms of survival of the early life stages, recruitment and biomass production.

The main goal of this doctoral dissertation is to clarify the key role of physical forcings in affecting the spatial distribution of planktonic stage of marine fish species that spawn in the summer period in the central Mediterranean Sea, shedding light on effects upon early life history traits, primarily growth performance and mortality rates at larval stage.

In the Chapter 1 a case study occurred in 2009 were used to examine the effects of particular environmental conditions on the structure of larval fish assemblages in two oligotrophic Mediterranean areas: the Southern Tyrrhenian Sea and the Strait of Sicily. For the first time, a framework of the larval fish assemblages on a large scale in central Mediterranean Sea is provided. Moreover, relationships with environmental variables, mainly temperature, salinity, fluorescence and zooplankton biomass, were discussed in light of surface hydrodynamic circulation and morphological features of the study areas.

In the chapters 2 and 3, a particular focus has been carried out on early life stages of *Sardinella aurita*. This species is one of the most exploited fish species over the Mediterranean fishery resource, especially in the Ionian and Levant FAO division fishing areas. Moreover, a gradual northwards expansion of this thermophilic species has been reported along different areas of the Mediterranean in correspondence to warming of the sea water, with expected effects on ecosystem and fisheries. In spite of the commercial and ecological importance of this species, a gap of knowledge on early life history traits exist in the Mediterranean Sea as well in other areas of its spatial distribution range. Therefore, the last part of this dissertation aimed at identify the main mesoscale oceanographic structure able to affect larval dispersion and survival of the early life stage of this species, taking into account variations of growth rate related to the surrounding environment.

Specifically, chapter 2 provide a picture of the spatial distribution patterns of the early life stages in the Strait of Sicily, allowing to identify for the first time the mains spawning and retention areas in this zone. Lagrangian simulations and satellite data (i.e., sea surface temperature, wind, and chlorophyll-a) were used to recognize the main oceanographic patterns that mark eggs and larvae transport processes. Moreover, a mechanistic explanation of the observed transport process by using a potential vorticity model is provided and the role of wind stress in generating cold filaments able to connect the mains recruiting areas is discussed.

Finally, in the chapter 3, larval growth performances were studies in relation to different environmental conditions taking into account two site of the central Mediterranean Sea located along a latitudinal gradient: Strait of Sicily (South) and central Tyrrhenian Sea (North). The potential effect of the temperature and food availability conditions is modelled in relation to otolith microstructures and an assessment of the environment suitability in the northern as well as in the south area is provided in light of the northwards expansion of this species in the Mediterranean Sea.

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### **CHAPTER 1: Different key roles of mesoscale oceanographic structures and ocean bathymetry in shaping larval fish distribution pattern: a case study in Sicilian waters in summer 2009**

#### **Abstract**

Fish larvae data collected in year 2009 were used to examine the effects of particular environmental conditions on the structure of larval assemblages in two oligotrophic Mediterranean areas (the Southern Tyrrhenian Sea and the Strait of Sicily). For this purpose, relationships with environmental variables (temperature, salinity and fluorescence), zooplankton biomass, water circulation and bathymetry are discussed. Hydrodynamic conditions resulted very different between two study areas. Southern Tyrrhenian Sea was characterized by moderate shallow circulation compared to the Strait of Sicily. In this framework, distribution pattern of larval density in the Tyrrhenian Sea was mainly driven by bathymetry, due to spawning behavior of adults fish. There, results defined four assemblages: two coastal assemblages dominated by pelagic and demersal families and two oceanic assemblages dominated by mesopelagic species more abundant in western offshore and less abundant in eastern offshore. The assemblage variations in the western side were related to the presence of an anti-cyclonic gyre in the northern side of the Gulf of Palermo, while in the eastern side the effect of circulation was not very strong and the environmental conditions rather than the dispersal of species determined the larval fish communities structure. Otherwise in the Strait of Sicily the currents were the main factor governing the concentration and the assemblages structure. In fact, the distribution of larvae was largely consistent with the branch of the Atlantic Ionian Stream (AIS). Moreover, very complex oceanographic structures (two cyclonic circulations in the western part of the study area and one anti-cyclonic circulation in the eastern part) caused the formation of uncommon spatial distribution of larval fish assemblages, only partially linked to bathymetry of the study area. Typically coastal larvae (pelagic families: Engraulidae and Clupeidae) were mostly concentrated in the offshore areas and off Capo Passero, where the presence of a thermo-haline front maintained their position in an area with favourable conditions for larval fish feeding and growth.

# Chapter 1

## *Larval Assemblages*

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### **Introduction**

The studies on ichthyoplankton distribution, mainly of commercially important species, play an important role in ecology and evolution of fish populations (Moser & Smith, 1993; Neilson & Perry, 1990), because the spatial distribution of early life stages can be the major determinant of recruitment success and consequently of the adult population sizes (Boehlert & Mundy, 1993; Govoni, 2005; Moser & Watson, 2006; Sinclair, 1988). The early life history of the fish populations depends on several factors that affect mainly the spawning biomass of fish adults (Basilone *et al.*, 2013; Giannoulaki *et al.*, 2013; Somarakis *et al.*, 2004) and the larval fish conditions (Riveiro *et al.*, 2011), mainly due to feeding success (Pepin *et al.*, 2014), to optimal habitat (Sabatés *et al.*, 2006, 2007; Valavanis *et al.*, 2008) and to predation (Litvak & Leggett, 1992; Steele & Forrester, 2002; Yin & Blaxter, 1987). Moreover, mesoscale oceanographic structures including geostrophic and wind influenced current regimes (wind-induced mixing of the surface layer, upwelling) play an important role in determining the patterns of abundance and distribution of larval fish populations. This because they act as mechanisms of retention and concentration of fish larvae in recruiting areas creating oceanic conditions favorable for the growth and survival (Alemany *et al.*, 2006; Falcini *et al.*, 2015; Paris *et al.*, 2007).

In the past, the influence of these factors on larval distribution was widely analyzed in the Mediterranean Sea, a semi-enclosed basin of which environmental characteristics are sensitive to both basin scale and local effects (*e.g.* winds, topography) and fluctuations of fish populations are not exclusively linked to fishing activity, but rather significantly affected by ocean conditions (Falcini *et al.*, 2015; Lloret *et al.*, 2000). Extensive work has mainly covered the coastal waters of the north-western part of the Mediterranean Sea (Alemany *et al.*, 2006; Alvarez *et al.*, 2012; García & Palomera, 1996; Olivar *et al.*, 2012; Olivar & Sabatés, 1997; Palomera & Olivar, 1996; Palomera & Sabatés, 1990; Sabatés, 1990, 2004; Sabatés & Olivar, 1996; Sabatés *et al.*, 2007) and to a lesser extent the central (Cuttitta *et al.*, 2003, 2004) and the eastern parts (Aegean Sea: Isari *et al.*, 2008; Somarakis *et al.*, 2011; Tsikliras & Koutrakis, 2011; Tsikliras *et al.*, 2014; eastern Ionian waters: Granata *et al.*, 2011; Tunisian waters: Koched *et al.*, 2013; Zarrad *et al.*, 2013). These studies carried out in the Mediterranean Sea showed that there was a

clear bathymetric separation of larval fish assemblages, due to different spawning behavior of adults: inshore assemblages have a different composition from offshore ones (Alemany *et al.*, 2006; Giordano *et al.*, 2014; Granata *et al.* 2011; Sabatés & Olivar, 1996). Generally, bathy and mesopelagic families were characteristic members of the oceanic group, while the inshore species consist of pelagic and demersal (Beldade *et al.*, 2006; Sabatés *et al.*, 2003; Tsikliras & Koutrakis, 2011). However, this trend can be disrupted by peculiar mesoscale oceanographic structures, because currents, fronts and gyre can reshape the distribution of fish larvae with advection and concentration (Agostini & Bakun, 2002; Falcini *et al.*, 2015; Sabatés *et al.*, 2013). On the other hand, the environmental parameters that can affect the distribution of fish larvae, such as temperature and food availability, are strictly dependent on local conditions and vary considerably at reduced spatial scale (García Lafuente *et al.*, 2002).

This is the case of spatial distribution of larval fish assemblages in two oligotrophic areas in the Mediterranean Sea with different and peculiar environmental conditions: southern Tyrrhenian Sea and Strait of Sicily. The southern Tyrrhenian Sea has high relevance as a probable nursery area for many commercially important pelagic and coastal fishes such as *Seriola dumerili*, *Xiphias gladius* and *Thunnus thynnus* and it is a habitat for mesopelagic fishes and squids, and plays a key role in deeper ecosystem energy flux (Bruno *et al.*, 2001; Giordano *et al.*, 2014; Granata *et al.*, 2011). The Strait of Sicily is one of the main fishing sites in the Mediterranean (García Lafuente *et al.*, 2002). It is a region characterized by a dominant hydrographic feature (the Atlantic Ionian Stream (AIS)) that has clear influence on the spawning strategy and the recruitment success of many fish species (García Lafuente *et al.*, 2002, 2005; Mazzola *et al.*, 2000, 2002).

In this paper, the different roles of hydrographic, physical, chemical and biological conditions in these two study areas during summer 2009 were showed, with the aim of determining what are the conditions in which the dynamics of transport and the chemical-physical and biological properties can be decisive in affecting the spatial composition of fish larvae in Mediterranean Sea.

### Material and Methods

#### *Ichthyoplankton and oceanographic sampling*

The two study areas are located in the southern side of the Tyrrhenian Sea and in the Strait of Sicily (Fig. 1).

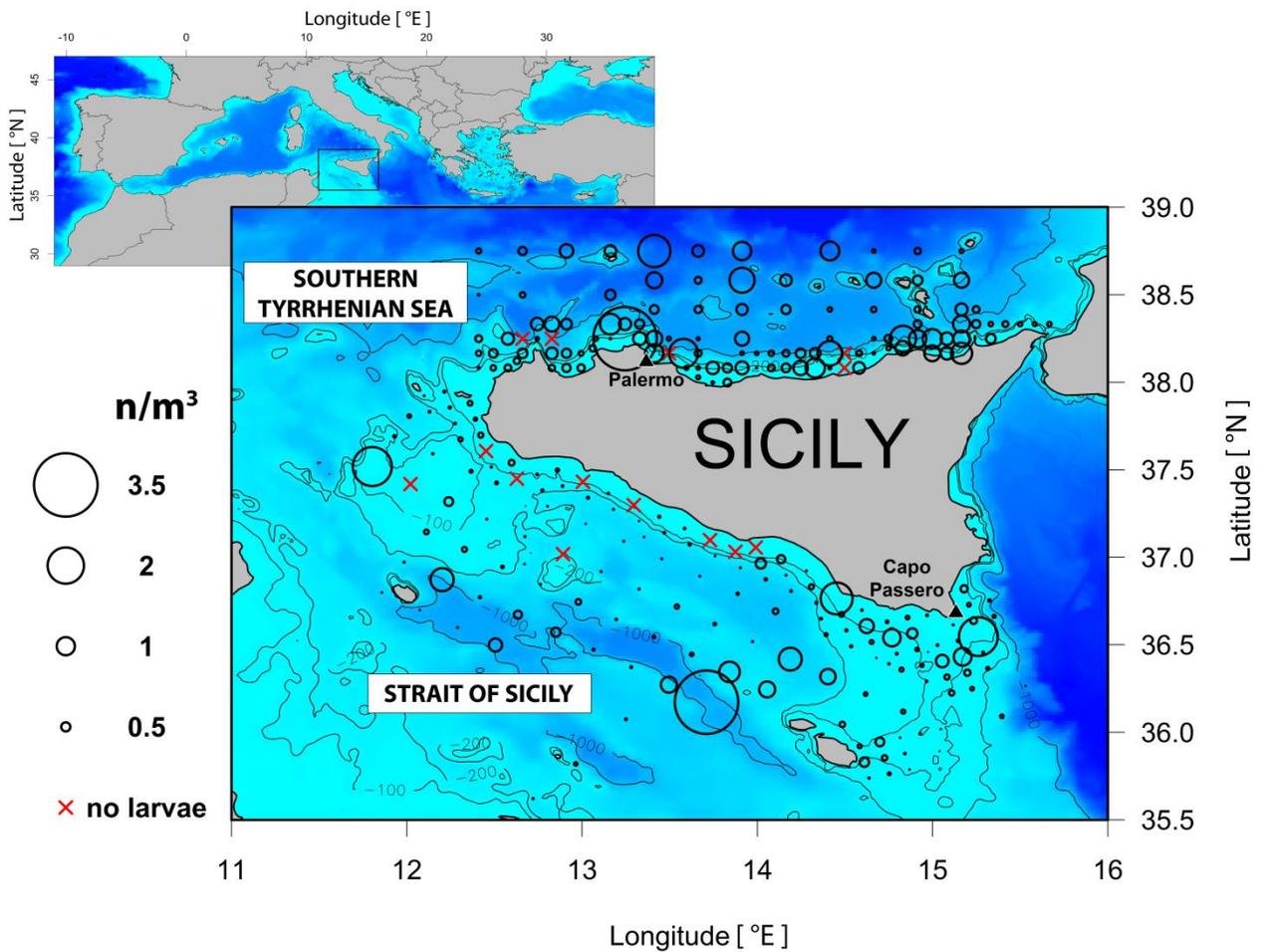


Fig. 1 Larval fish distribution in the study areas. The circle dimension is proportional to the total larval fish abundance.

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Oceanographic data and ichthyoplanktonic samples were collected during two oceanographic surveys on board of the O/V *Urania*: Bansic 2009 carried out in the period 04 - 21 July 2009 in the Strait of Sicily and MedSudMed 2009 carried out from 26 July to 3 August 2009 in the Southern Tyrrhenian Sea. The sampling was made on a station grid of 4×4 nautical miles within the 200 meters bathymetry and a grid of 12×12 nautical miles for the off-shore areas over the 200 meters bathymetry. In each station ichthyoplankton samples were collected by means of a Bongo40 net, which is composed by two coupled nets with the inlet mouth diameter of 40 cm and mesh size 200 µm. The plankton oblique tows were carried with a constant speed of 2 knots lasting from 12 to 15 minutes out to a depth of 100 m, wherever possible, because this layer characterizes the majority of the fish larvae in the study areas (Olivar *et al.*, 2001, 2014; Sabatés *et al.*, 2008). The larval fish collecting procedure was conducted during day and night, because, during the summer, fish pelagic larvae are more concentrated in the surface layer during the night and more dispersed along the column water during the day (Olivar *et al.*, 2001; Sabatés *et al.*, 2008), whereas the opposite occurs at least for most abundant mesopelagic species larvae (Olivar, 2014). The filtered water volume of each mouth was measured by a calibrated flow-meters (type G.O. 2030). The samples were stored in formaldeid at 4% buffered with borax. Fish larvae were sorted from the rest of the plankton and identified to the family taxonomic level. Taxonomic identification was based on Bertolini *et al.* (1956), Costa (1999), Moser and Ahlstrom (1996) and Tortonese (1970). The number of fish larvae from each sample was standardized to n/m<sup>3</sup> according to Perez Ruzafa *et al.* (2004).

In all the stations, continuous vertical profiles of temperature, salinity and fluorescence were obtained from the surface to the bottom by means of a multiparameter probe SEABIRD mod. 9/11 plus mounted on a General Oceanics rosette equipped with 24 Niskin Bottles. The probes were calibrated before and after the cruise at the NURC (NATO Undersea Research Centre) in La Spezia, Italy. Each parameter has been calculated as the average of the values for each meter of the layer from surface to 50 m depth, that corresponds about to the average thermocline calculated in the study areas.

The circulation features were evaluated by means of the altimeter products (Absolute Dynamic Topography) and geostrophic velocity field, produced by Ssalto/Duacs and distributed by Aviso, with support from Cnes (<http://www.aviso.oceanobs.com/duacs/>). The period selected to evaluate the

circulation pattern was 15 July–14 August 2009 for the Tyrrhenian Sea and 01-31 July 2009 for the stations in Strait of Sicily.

Since results of this study about the larval fish distribution in Strait of Sicily suggested a relationship with offshore wind-induced currents, the influence of wind on surface circulation was evaluated by means of satellite wind stress ( $\vec{\tau}$ ) and Ekman transport ( $\vec{m}$ ) from ocean surface 6-hourly wind data ( $\vec{U}_{wind}$ ), provided by the Cross-Calibrated Multi-Platform project (25 x 25 km, <http://podaac.jpl.nasa.gov>).

Wind stress was obtained as:

$$\vec{\tau} = \rho_{air} C_d |\vec{U}_{wind}| \vec{U}_{wind},$$

where  $\rho_{air}$  is the air density and the dimensionless friction coefficient  $C_d = 0.0012$  for  $0 < |\vec{U}_{wind}| < 11$  m/s and  $C_d = 0.00049$  for  $|\vec{U}_{wind}| \geq 11$  (Large & Pond, 1981; McClain & Firestone, 1993).

Ekman transport was calculated as:

$$\vec{M} = (\rho_{water} f)^{-1} \vec{\tau} \times \hat{k},$$

where  $\rho_{water}$  is the water density,  $f$  the Coriolis parameter, and  $\hat{k}$  is the vertical unit vector (Pickett & Paduan, 2003). Finally, the Ekman transport  $\vec{M}$  was spatially represented in daily maps corresponding to survey period (from 4 to 21 July 2009).

### Data analysis

A generalized additive model (GAM; Hastie & Tibshirani, 1986) for each study area was applied on total larval fish abundance and environmental parameters to define the factors that influenced larval fish concentration. Fluorescence and zooplankton weight of the Tyrrhenian Sea were transformed into natural logarithm in order to achieve uniform distribution for GAM application. The quasi-Poisson error distribution with the log link function was used and the natural cubic spline smoother was applied for smoothing the GAM fitting. The selection of the final model was performed by minimizing the

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Generalized Cross-Validation (GCV) and maximizing the level of explained deviance (0–100). All first-order interactions of the main effects were tested. Validation graphs (*e.g.* residuals versus fitted values, QQ-plots and residuals versus the original explanatory variables) were then observed to detect the existence of any pattern and possible model misspecification.

Afterwards, the stations were classified according to their species composition similarity by hierarchical clustering using the Bray-Curtis distance measure (Bray & Curtis, 1957) and Ward's linkage as grouping method (Ward, 1963). Only dominant families, defined as those with total density >0.5 individuals per m<sup>3</sup>, and the stations with presence of fish larvae were used in order to avoid problems, *e.g.* biasing or dominating the ordination. No scaling of the data was preventively carried out for the taxonomic dataset of the Tyrrhenian Sea, while the density values of the Strait of Sicily were transformed into square root, because some larval fish taxa exhibited a large variance and they could be decisive in determining the main sources of information. Analysis of similarities (ANOSIM) was performed to test the significance of differences in groupings separated in the cluster analysis (Clarke, 1993). Levels of occurrence, abundance of species and geographical distinctness among species and station groups were then used as subjective criteria to fine-tune the identification of clusters.

A Principal Coordinate Analysis (PCoA; Gower, 1966) was performed on Bray-Curtis dissimilarity of the larval fish assemblages to describe relationships among clusters and to show their general spatial pattern. The equilibrium circle (Legendre & Legendre, 1998) was used to select the families, that significantly contributed to the axes shown in the ordination graph. Specifically, they must have vectors outside of the equilibrium circle. The first and second dimensions of the PCoA were mapped to reveal gradients and patchiness.

A distance-based redundancy analysis (dbRDA), based on the Bray-Curtis dissimilarities and limited to 2 axes, was performed to explore which group of habitat variables best explained the spatial variation observed in larval fish assemblage structure (McArdle & Anderson, 2001). Environmental variables were standardized to obtain comparable scales (Clarke & Warwick, 1994). Distance based redundancy analysis (dbRDA) biplots were generated to visually display the direction and magnitude of the

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relationships between habitat factors and larval fish families (Legendre & Anderson, 1999). All the statistical analysis were carried out using statistical software R (R 3.0.1; R Core Team, 2013).

### **Results**

#### *Larval fish abundance*

In the 109 stations in the Tyrrhenian Sea, total fish larvae density ranged between  $\sim 0.027$  n/m<sup>3</sup> and  $\sim 1.941$  n/m<sup>3</sup> (mean  $0.296$  n/m<sup>3</sup>; standard deviation  $0.243$ ). The station with the most concentrated density was in the western coastal side of the study area in the gulf of Palermo. In general, ichthyoplankton showed a patchwork distribution with no difference between offshore and coastal area (KW=0.791; p-value>0.05) and with higher concentrations of larvae in the eastern coastal side compared to the western coastal side (KW=5.237; p-value<0.05) (Fig. 1).

In the 150 stations in the Strait of Sicily total fish larvae density ranged between  $\sim 0.011$  n/m<sup>3</sup> and  $\sim 3.572$  n/m<sup>3</sup> (mean  $0.433$  n/m<sup>3</sup>; standard deviation  $0.561$ ). Largest abundance values were found in the eastern part of the study area (KW=5.868; p-value<0.05), over the continental shelf area separating Sicily coasts from Maltese Islands (Fig. 1).

The generalized additive model for the Tyrrhenian Sea indicated the zooplankton weight, the temperature and the natural logarithm of fluorescence as the most important factors to explain the variability of larval fish abundance. The explained deviance by the model was the 34.2 % of the total deviance (Table 1).

The relationship between larval fish abundance and zooplankton weight and temperature was linearly increasing, indicating a positive effect on abundance for zooplankton values higher than about 4.40 g and temperature values higher than about 20.5 °C. The effect of fluorescence values was increasing until 0.04 mg L<sup>-1</sup> and almost absent for higher values (Fig. 2a).

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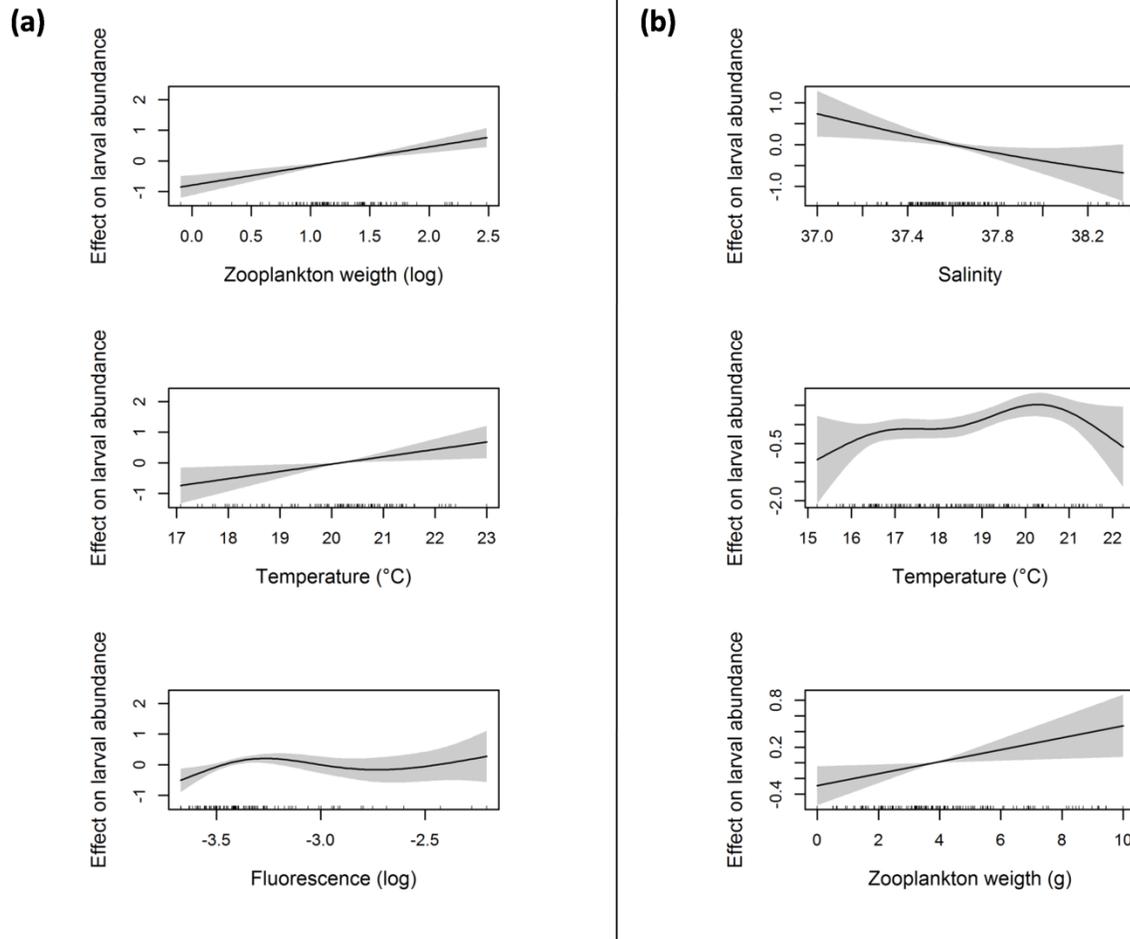


Fig. 2 (a) Plots of the smoothing response of the Generalized Additive Model for larvae abundance in the Tyrrhenian Sea. (b) Plots of the smoothing response of the Generalized Additive Model for larvae abundance in the Strait of Sicily. Black thick line indicates the value of the GAM smoothing response and grey area represents the 95% confidence intervals.

The selected parameters for the best model to explain the larval fish abundance in the Strait of Sicily were salinity, zooplankton weight and temperature. The explained deviance by the model was the 42.5 % of the total deviance (Table 1).

Table 1. Analysis of deviance for GAM covariates of the final model fitted on total larval fish abundance.

<b>Tyrrhenian Sea</b>			
<b>Selected variables</b>	<b>Degree of freedom</b>	<b>F</b>	<b>p-value</b>
Zooplankton	1	23.71	4.15e-06
Temperature	1	6.50	0.0124
Fluorescence	3.19	3.03	0.0251
<b>Strait of Sicily</b>			
<b>Selected variables</b>	<b>Degree of freedom</b>	<b>F</b>	<b>p-value</b>
Salinity	1.28	6.52	0.0046
Zooplankton	1	3.07	0.0001
Temperature	3.54	3.66	0.0078

The higher larval fish abundance was associated with the lowest salinity values (<37.6) and with optimal temperature in the range 19-21°C. The effect of the zooplankton on larval fish abundance was linearly increasing and positive for zooplankton values higher than about 4 g (Fig. 2b).

### *Larval fish composition*

A total of 2,270 fish larvae from 36 families in the southern Tyrrhenian Sea and 2,588 fish larvae from 34 families in the Strait of Sicily were identified (Table 2). The three most abundant families accounted for 64.89 % of the total catch in the Tyrrhenian Sea. They were Gonostomatidae (37.36%), Myctophidae (17.05 %) and Clupeidae (10.48 %). Except Phosichthyidae (9.12 %), Engraulidae (5.81 %) and Gobidae (4.85 %), all other 30 families comprised less than 2 % of the total number of larvae collected. In the Strait of Sicily, the most abundance families were Engraulidae (43.55 %) and Clupeidae (21.52 %), and except Gonostomatidae (7.53 %), Gobidae (5.91 %), Myctophidae (4.79%), Labridae (2.32 %) and Sparidae (2.20 %), all other families comprised less than 2 % of the total larvae.

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Table 2. Larval fish families recorded in Tyrrhenian Sea and the Strait of Sicily. Ecological group (B: bathypelagic fish, P: pelagic fish, M: mesopelagic fish, D: demersal fish), abundance (number of fish larvae for each family) and percentage of total abundance (number of fish larvae for each family/ total number of fish larvae) are reported.

<b>Family</b>	<b>Ecological group</b>	<b>Abundance Tyrrhenian Sea</b>	<b>Percentage Tyrrhenian Sea</b>	<b>Abundance Strait of Sicily</b>	<b>Percentage Strait of Sicily</b>
Ammodytidae	D	14	0.617	2	0.077
Apogonidae	D	3	0.132	1	0.039
Blennidae	D	1	0.044	7	0.270
Bothidae	D	24	1.057	35	1.352
Bramidae	P	2	0.088	1	0.039
Callionymidae	D	5	0.220	20	0.773
Carangidae	P	10	0.441	31	1.198
Centracanthidae	P	7	0.308	17	0.657
Centriscidae	D	1	0.044	1	0.039
Cepolidae	D	6	0.264	1	0.039
Clupeidae	P	238	10.485	557	21.522
Congridae	D	2	0.088	20	0.773
Coproidae	D	1	0.044	0	0.000
Cynoglossidae	D	1	0.044	0	0.000
Engraulidae	P	132	5.815	1127	43.547
Evermannelidae	B	0	0.000	5	0.193
Gadidae	D	4	0.176	37	1.430
Gobidae	D	110	4.846	153	5.912
Gonostomatidae	B	848	37.357	195	7.535
Labridae	D	25	1.101	60	2.318
Myctophidae	M	387	17.048	124	4.791

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Ophichthidae	D	1	0.044	2	0.077
Ophidiidae	D	1	0.044	0	0.000
Paralepidae	M	113	4.978	14	0.541
Phosichthyidae	B	207	9.119	23	0.889
Pomacentridae	P	32	1.410	14	0.541
Scombridae	P	18	0.793	22	0.850
Scophthalmidae	D	0	0.000	2	0.077
Scorpaenidae	D	3	0.132	12	0.464
Serranidae	D	25	1.101	27	1.043
Soleidae	D	0	0.000	3	0.116
Sparidae	D	34	1.498	57	2.202
Sternoptychidae	B	1	0.044	1	0.039
Stomiatidae	P	1	0.044	0	0.000
Stomiidae	B	1	0.044	0	0.000
Synodontidae	D	4	0.176	0	0.000
Trachinidae	D	1	0.044	9	0.348
Trichiuridae	M	1	0.044	1	0.039
Triglidae	D	0	0.000	5	0.193
Tunnidae	P	6	0.264	2	0.077
<b>Total</b>		<b>2270</b>		<b>2588</b>	

Four larval fish assemblages were defined by the Bray–Curtis dissimilarity index for sample from Tyrrhenian Sea and five assemblages from Strait of Sicily, as suggested by their respective dendrograms (Fig.3 a and b).

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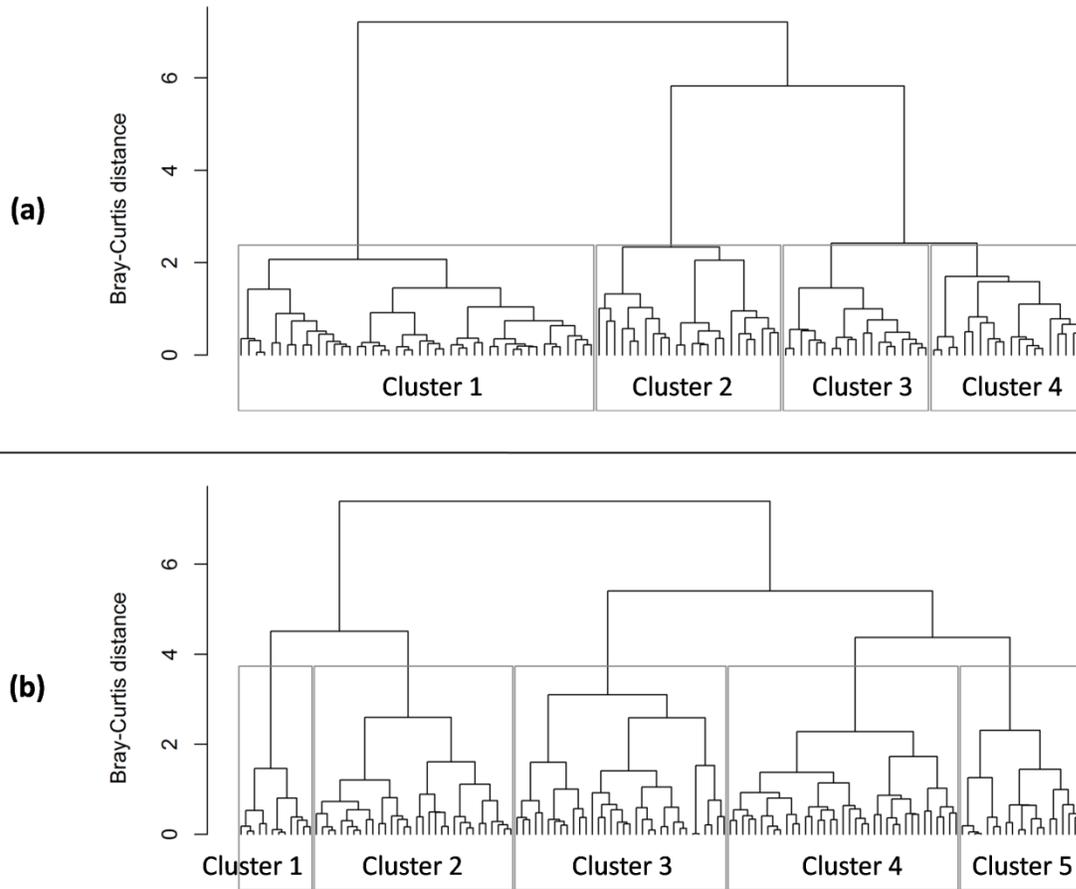


Fig. 3 Dendrograms generated by hierarchical cluster analyses with Bray-Curtis distance and Ward's grouping method applied on larval families composition in the southern Tyrrhenian Sea (a) and Strait of Sicily (b).

The clusters differed substantially in family composition and abundance, presenting distinct assemblage structures in both sampling areas. The ANOSIM analysis showed significant dissimilarity between families assemblages obtained from cluster analysis in Tyrrhenian Sea (Global  $R = 0.6625$ ;  $p < 0.001$ ) and in the Strait of Sicily (Global  $R = 0.5523$ ;  $p < 0.001$ ). The families that significantly contributed to the axes shown in the ordination graph of PCoA applied on data from Tyrrhenian Sea were Gonostomatidae, Myctophidae, Gobidae, Engraulidae and Clupeidae. The first dimension of the PCoA well discriminated two groups: the lower values characterized clusters 1 and 2 and families

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Gonostomatidae and Myctophidae and the higher values characterized clusters 3 and 4 and families Engraulidae, Gobiidae and Clupeidae (Fig. 4a) . This dimension showed a strong coastal–offshore gradient, indicating that the first assemblage (clusters 1 and 2 with mesopelagic families) was mainly present in the offshore area unlike families belonging to the second assemblage (clusters 3 and 4 with neritic families) located in coastal areas (Fig. 4b). The second dimension of the PCoA discriminated larvae of clusters 1 and 3 with small pelagic families from larvae of cluster 2 with mesopelagic families (Fig. 4a). This dimension showed different values in specific areas. In particular, higher values (clusters 1 and 3 with neritic families) were found in the Gulf of Palermo and in the easternmost part of the study area (Fig. 4c). Otherwise, negative values (cluster 2, mesopelagic fish larvae), were present in the offshore zone and in central-east coastal zone with exception of area from 14.75° and 15° of longitude (Fig. 4c).

The families that significantly contributed to the PCoA axes defined by data from Strait of Sicily were Clupeidae, Engraulidae, Gobiidae, Labridae, Bothidae, Myctophidae. The first dimension of the PCoA discriminated two groups: the lower values characterized clusters 1 and 4 and families Clupeidae and Engraulidae and the higher values clusters 2 and 5 and families Gonostomatidae and Myctophidae (Fig. 4d). This dimension showed the presence of the second assemblage (clusters 2 and 5 with mesopelagic families) in the western coastal area up to 14.5° of longitude, while the first assemblage (clusters 1 and 4 with small pelagic families) was dominant in the eastern coastal area and offshore (Fig. 4e). The second dimension of the PCoA discriminated larvae from cluster 5 with families Bothidae, Labridae and Gobiidae (lower values) from larvae from clusters 2 and 4 with families Clupeidae and Gonostomatidae (higher values) (Fig. 4d). This dimension showed lower values in the central area (from 13° to 15° of longitude) and higher values in the remaining areas (Fig. 4f).

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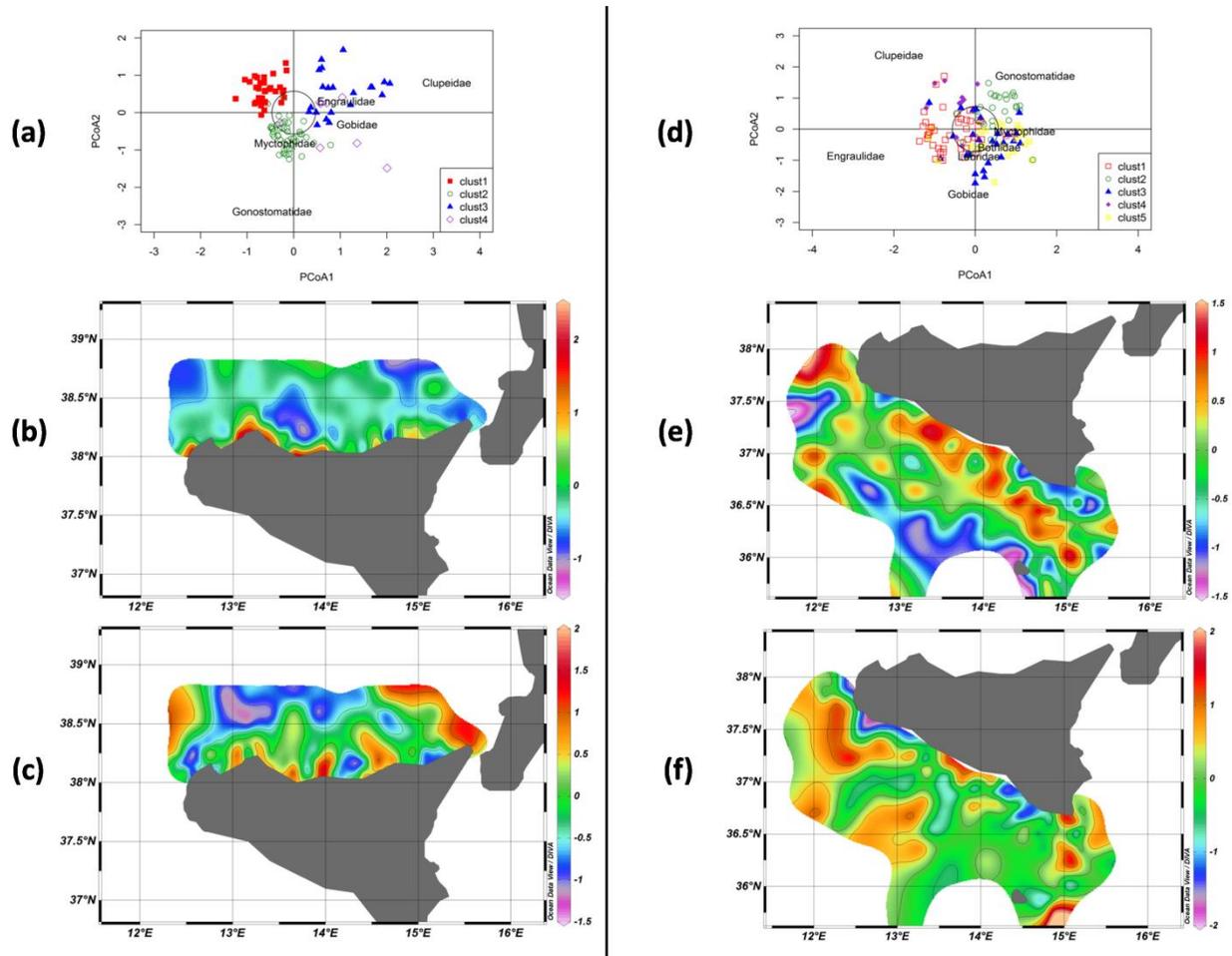


Fig. 4 (a) Bivariate plot of first and second principal coordinates on larval fish composition in the Tyrrhenian Sea. The equilibrium circle was added to a PCoA ordination diagram; (b) Geographical distribution of the first principal coordinates on larval fish composition in the Tyrrhenian Sea; (c) Geographical distribution of the second principal coordinates on larval fish composition in the Tyrrhenian Sea; (d) Bivariate plot of first and second principal coordinates on larval fish composition in the Strait of Sicily. The equilibrium circle was added to a PCoA ordination diagram; (e) Geographical distribution of the first principal coordinates on larval fish composition in the Strait of Sicily; (f) Geographical distribution of the second principal coordinates on larval fish composition in the Strait of Sicily.

### Environmental parameters

The surface waters dynamics evidenced the presence of an anti-cyclonic eddy in the Tyrrhenian Sea located in the area from longitude 13°E to 14.5°E (Fig. 5a). This structure involved a sub-surface section of the water column until about 300 m of depth (Fig. 5b) and it was particularly evidenced by the isopycnal line 29  $\text{Kg m}^{-3}$  that in the middle sank from 200 m to 300 m.

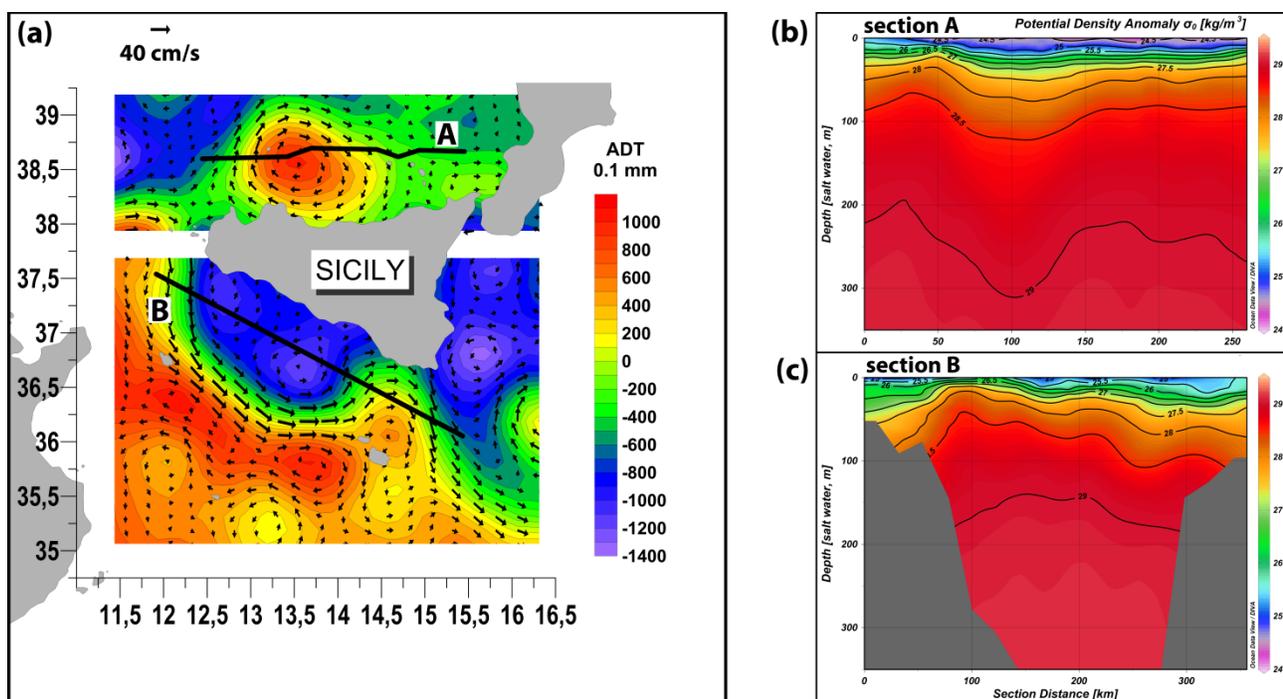


Fig. 5 (a) Absolute Dynamic Topography (ADT) and Mean geostrophic velocity field by Aviso; (b) Vertical section of density profiles across the transect A (Fig. 5a) in the upper 350 meters; (c) Vertical section of density profiles across the transect B (Fig. 5a) in the upper 350 meters.

The areal distribution of the parameters (temperature, salinity, fluorescence and zooplankton weight) showed the variability of the sub-surface layer. The isosurface of temperature (Fig. 6a) showed the presence of colder water in the western area of the study area and simultaneously an increase of temperature in

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the central area due essentially to the presence of anti-cyclonic eddy that generated a flexion of isotherms (not shown), such as of isopycnal lines (Fig. 5b). The salinity (Fig. 6b), fluorescence (Fig. 6c) and zooplankton (Fig. 6d) minimum values were in correspondence with this eddy. The zooplankton isosurface (Fig. 6d) showed higher values in the entire area further to the west and in the coastal eastern side.

### *Strait of Sicily*

Geostrophic velocity field showed that the surface circulation was mainly affected by the eastward flow of AIS, which was characterized by its typical meandering path that borders the eastern coast of Sicily and reached maximum values of  $\sim 44 \text{ m s}^{-1}$  in the core (Fig. 5a).

Moreover satellite images (Fig. 5a) and density interfaces (Fig. 5c) showed the presence of two cyclonic structures in western side of the Strait of Sicily and a modest and superficial anti-cyclonic structure in eastern part. Specifically, the isopycnal 28.5 was about 50 m depth in the western part of Sicily and sank to about 120 m depth in the eastern part (Fig. 5c). Analysis of temperature and salinity data in the layer 0-50 m evidenced colder (16-18 °C) and saltier (37.75-38 PSU) water in coastal zone, especially in the Adventure Bank (western part of the study area) (Fig. 6a and Fig. 6b). The Bank was also characterized by higher fluorescence (Fig. 6c) and zooplankton (Fig. 6d) values. This may indicate the presence of wide coastal upwelling phenomena, generated by wind. Daily Ekman transport maps ( $\bar{m}$ ) showed a clear transport of water from coastal to offshore zone for most of the days during the survey (Fig. 7). Indeed moderate ( $4 < \bar{m} < 8 \text{ m}^2 \text{ s}^{-1}$ ) and intense ( $\bar{m} > 8 \text{ m}^2 \text{ s}^{-1}$ ) values of cross-shore transport were evident in 11 over 18 analyzed days while in the other days the transport is weak or absent, especially in the coastal zone.

Moreover, a marked thermo-haline front was present in the easternmost part, beyond 15° of longitude and high zooplankton values were found in the western area adjacent to this front (from 13.5 to 14.5° of longitude; Fig. 6d).

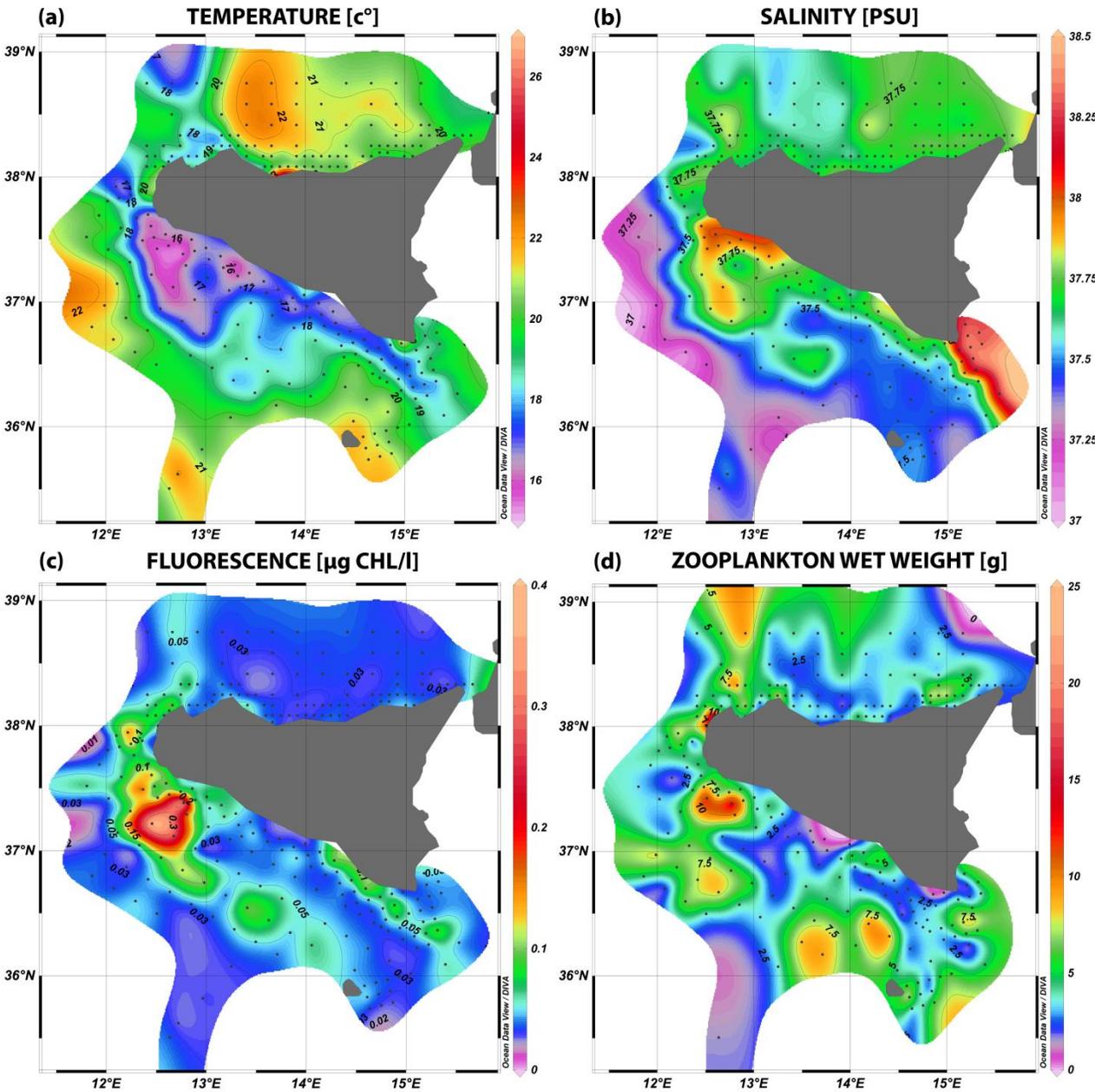


Fig. 6 Horizontal distribution pattern of mean values of temperature (a), salinity (b) and fluorescence (c) salinity in the layer 0-50 m and of total zooplankton weight (d).

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### *Correlation between larval fish assemblages and environmental variables*

The dbRDA provided an ordination that was constrained by some environmental variables. Results of the routine for the Tyrrhenian Sea showed that only the first two axes were significant which explained 77.20% of the overall variability. All environmental parameters, except the salinity, were significant. The biplot of the first two dbRDA axes (Fig. 8a) revealed a separation among clusters based on the environmental parameters. The first RDA axis revealed a separation between cluster 1, negatively correlated to temperature, zooplankton weight and fluorescence from cluster 2 positively correlated to the same parameters. The second axis identified neritic families (cluster 4) positively correlated with fluorescence and zooplankton weight and negatively correlated with temperature.

Results of the dbRDA for the Strait of Sicily showed that the first two axes were significant explaining the 63.41% of the overall variability. All parameters were significant. The first RDA axis revealed a separation between the first assemblage with small pelagic larvae (clusters 1 and 4) and the second assemblages (clusters 2 and 5). The second RDA axis separated clusters 3 and 5, positively correlated with zooplankton weight and salinity, from clusters 2 and 4, positively correlated with fluorescence and temperature (Fig. 8b).

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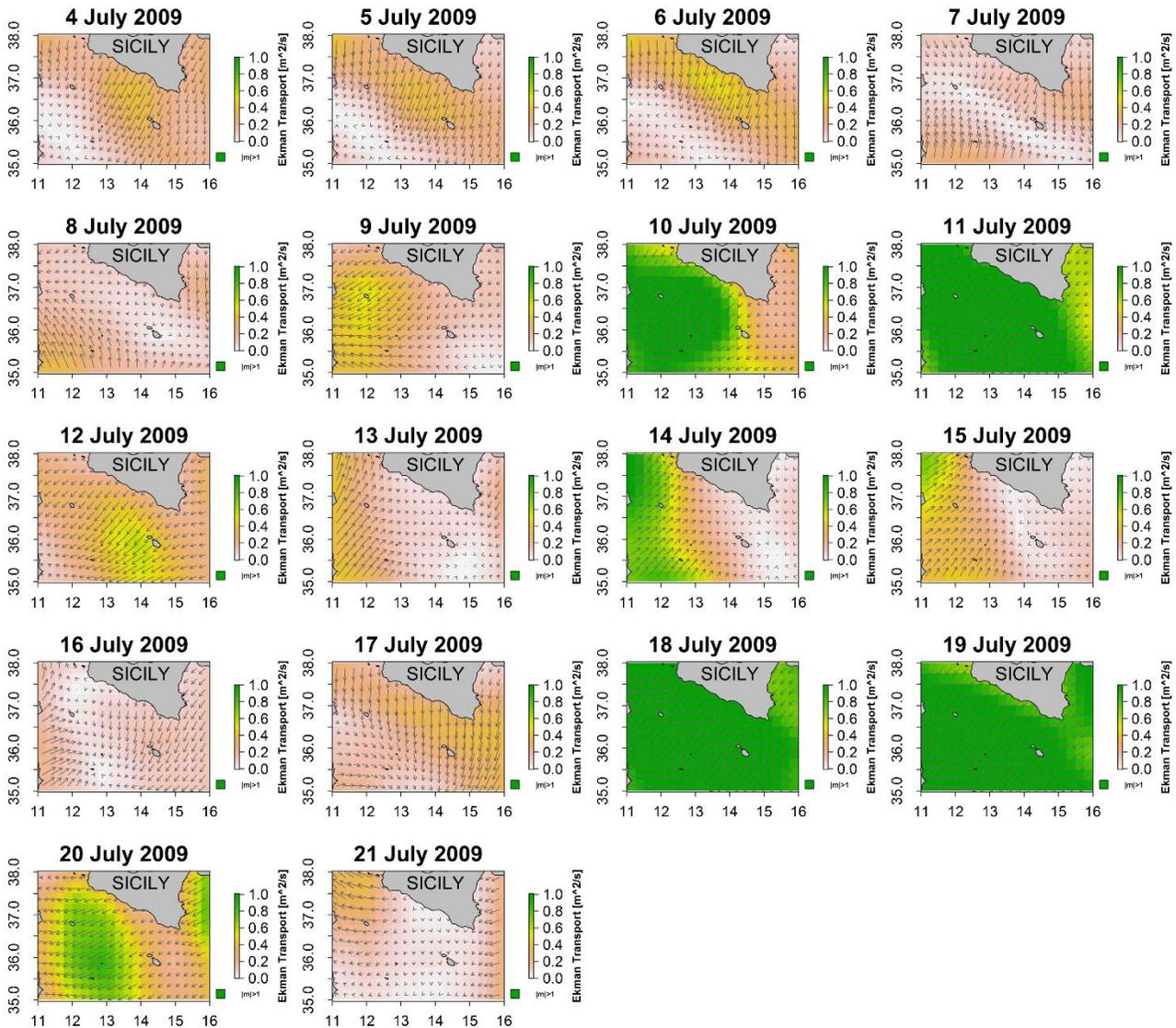


Fig. 7 Daily Ekman transport ( $\text{m}^2 \text{s}^{-1}$ ) during the cruise (Bansic 09) in Strait of Sicily. Arrows and colors indicate the direction and the intensity of the transport respectively.

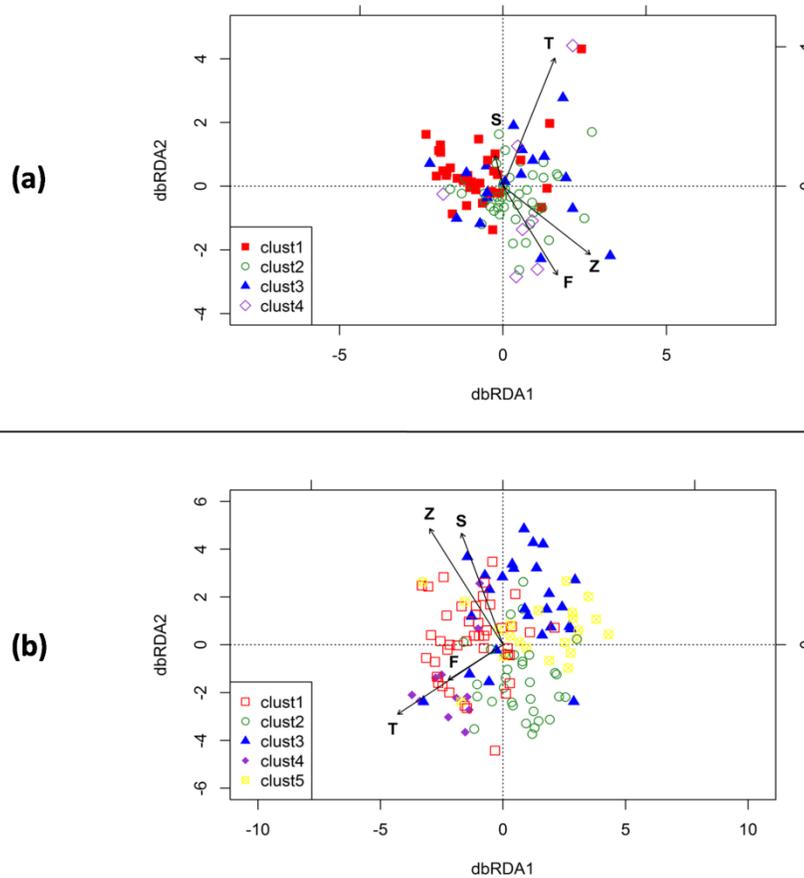


Fig. 8 Db-RDA ordination graph for the first two axes for the larval fish composition using Bray-Curtis distance and the CTD parameters (T: temperature; S: salinity; F: fluorescence; Z: zooplankton weight) as a constraining variable for the southern Tyrrhenian Sea (a) and for the Strait of Sicily (b).

### Discussions

This study aims to examine the responses of larval fish assemblages to different hydrodynamic conditions, in order to clarify the role that mesoscale oceanographic structures, such as gyres, fronts and currents, can play compared to different chemical, physical and biological conditions. These factors can

contribute to the survival and distribution of fishes at early development stages and consequently to the recruitment of marine fish populations.

The southern Tyrrhenian Sea is a typically oligotrophic area but little is known about the effects of physical forcings on the biology and ecology of fish populations (Azzaro *et al.*, 2003; Carrada *et al.*, 1992; De Domenico, 1979; Giordano *et al.* 2014). Open southern Tyrrhenian Sea is considered as an important spawning area for the swordfish (*Xiphias gladius*; Romeo *et al.*, 2009) and the Atlantic blue fin tuna (Sinopoli *et al.*, 2004). Moreover, a previous study showed that it is a relevant nursery area for the short-finned squid *Illex coindetii* (Perdichizzi *et al.*, 2011), which represents an important prey for several adults fish species.

Our results in Tyrrhenian Sea generally permitted the identification of two main larval fish assemblages, separating a group of shallower stations where most larvae belonged to neritic families and another group of deeper stations where mesopelagic families were dominant. This bathymetric separation confirmed a study on ichthyoplankton composition carried out in June 2006 that covered only the eastern coastal side of southern Tyrrhenian Sea, from Cape Cefalù to Cape Rasocolmo. In fact, this study showed that in this region three main assemblages can be distinguished: shore fish larvae (Labridae, Serranidae) which dominate in coastal areas, larvae found over the continental shelf (Sparidae, Scorpaenidae, Bothidae, Mullidae, Engraulidae, Gobiidae, Trachinidae, Blennidae) and larvae of meso- and bathypelagic species, which mainly over slope and open waters (Gonostomatidae, Myctophidae, Sternoptychidae). This inshore–offshore gradient was explained with respect to the hydrographic features and structures determined by the surface circulation path (Giordano *et al.*, 2014). Moreover, these results were very similar in terms of species composition and distribution to what found in nearby areas in the Mediterranean Sea (Alemany *et al.*, 2006; Sabatés & Olivar, 1996). However, mesopelagic larvae (*e.g.* Myctophidae), which presented an irregular distribution, were abundant not only offshore but also in coastal stations. This situation could be linked to larval fish transport by oceanographic structures characterizing the southern Tyrrhenian Sea and affecting strictly the habitat (García Lafuente *et al.*, 2002). There, the main feature observed was the presence of an anti-cyclonic gyre in the northern side of the Gulf of Palermo, which was characterized by warmer and less salty water, with low values of linked-

productivity parameters (fluorescence and zooplankton weight) in the core. This structure generated a near-coast accumulation area, exhibiting the maximum larval fish concentration. Specifically, the anti-cyclonic eddy could have acted as mechanisms of retention and concentration of different larval fish taxa, especially belonging to Clupeidae, Engraulidae and Gobiidae families and facilitated the transport inshore of mesopelagic larvae. In fact, it was shown that the dynamics of anticyclonic flow patterns imply convergence and downwelling (Bakun, 1996), with associated opportunities for concentrating small organisms in convergent frontal formations (Agostini & Bakun, 2002). The occurrence of mesopelagic species in neritic zones, where the spawning is not present, was observed also in other areas of the Mediterranean Sea, particularly in the Balearic Sea (Alemany, 1997), in the northern Aegean Sea (Koutrakis *et al.*, 2004; Tsikliras *et al.*, 2009) and in Costa Brava (Palomera & Olivar, 1996). Conversely, the eastern side of Tyrrhenian Sea was an area with apparently more stable hydrodynamic conditions. In this area, the larval fish assemblages were more regular, corresponding to the absence of relevant mesoscale oceanographic forcings. The environmental conditions rather than the dispersal of species could have partially determined the structure and dynamics of the eastern communities. In fact, larvae belonged to demersal and pelagic families were mainly concentrated in the coastal part, characterized by higher surface temperature values and mesopelagic larvae could be found mainly offshore as well as total zooplankton, with lower density values compared to the western offshore areas.

A different situation was found in the Strait of Sicily, which was also classified as an oligotrophic area (Malanotte Rizzoli *et al.*, 1997; Van Wambeke *et al.*, 2002). It was characterized by a very complex sea circulation that can have strongly influenced the spawning strategy and the recruitment success of many fish species (Cuttitta *et al.*, 2003, 2015; Fortibuoni *et al.*, 2010; Garofalo *et al.*, 2010). In particular, many studies (Basilone *et al.*, 2013; Cuttitta *et al.*, 2015; Falcini *et al.*, 2015; García Lafuente *et al.*, 2002) have focused on the influence of oceanographic factors on the ecology and reproductive biology of the European anchovy (*Engraulis encrasicolus*), which is the target species of fisheries along the southern coast of Sicily, representing one of major fish resource in terms of economic importance (Patti *et al.*, 2005, 2012). In this study, the influence of the circulation pattern in the Strait of Sicily on the distribution of fish larvae was very clear and it can be considered the main factor governing the larval fish retention and shaping the observed assemblages. In fact, the distribution of total larvae was largely consistent with

the branch of the AIS, running roughly parallel to the southern Sicilian coast. AIS was able to advect the larvae towards a retention area at the south-easternmost corner of Sicily (off Capo Passero). Here, the presence of a thermo-haline front, as evidenced by temperature and salinity fields, allowed the larvae to maintain their relative position in an area with enhanced trophic condition, so probably providing more favourable conditions for larval fish feeding and growth (García Lafuente *et al.*, 2002, 2005; Mazzola *et al.*, 2002). As support of this finding, our results showed that this area was characterized by the mixing of all clusters. This happened because AIS transport and the thermo-haline front conveyed there the larvae belonged to families different from ecological point of view (demersal and small pelagic together with meso and bathypelagic fish larvae). In addition, the spatial extension of AIS path may have had important consequences on the predominant hydrological phenomena occurring in the region, such as the offshore extension of coastal upwelling (García Lafuente *et al.*, 2005). In summer 2009, the flow of the stream moved further offshore (Bonanno *et al.*, 2014) and so the study area showed a broader upwelling extension centred in the western zone on Adventure Bank and expanded eastward, modifying surface temperature, salinity and linked-productivity parameters (fluorescence and zooplankton weight). This feature was confirmed by the analysis of Ekman transport, which showed that strong and persistent transport of water induced by Mistral (NW) and Westerly winds and directed offshore was detected during the survey. These winds could have played a key role in shaping larval fish distribution pattern, because they could have modulated the capacity of advection of fish larvae offshore, similarly to what observed by Falcini *et al.* (2015) for the anchovy larvae in year 2004. In fact, in correspondence with these processes, typically neritic larvae (pelagic families: Engraulidae and Clupeidae) were found in the offshore stations.

Therefore, in contrast to the findings for the southern Tyrrhenian Sea, complex mesoscale oceanographic structures (*e.g.* AIS pathway, coastal upwelling, gyres and fronts), that interacted synergistically, were able to drive the mainly patterns of distribution of fish larvae in the Strait of Sicily. This evidence was supported also by the analysis of significance of environmental parameters affecting larval fish distribution. Indeed, only salinity and zooplankton weight were found to determine the taxonomic composition, discriminating small pelagic fish larvae species from larvae belonging to Gonostomatidae family. The association between salinity and zooplankton is mostly linked to the features of AIS surface

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currents, characterized by relatively low salinity values and able to transport zooplankton along its path off southern Sicilian coasts (Patti *et al.*, 2005).

### **Conclusions and published paper**

This case study evidenced the need to assess specific environmental conditions driving the dynamics of development of fish larvae and consequently the recruitment success of marine populations in a framework of efficient monitoring plans. The results underlined the different roles of mesoscale oceanographic structures compared with chemical, physical and biological parameters in shaping the spatial pattern of larval fish distribution in the central Mediterranean Sea in a case study of Summer 2009. In the Southern Tyrrhenian Sea more stable hydrodynamic conditions were observed. In this framework, although the anti-cyclonic eddy in the central part of the study area was able to transport part of mesopelagic larvae (*e.g.* mainly Myctophyidae) in a restricted portion of the northern Sicilian coast, bathymetry and environmental conditions played a key role in determining larval fish distribution. Conversely, in the Strait of Sicily the distribution of early life stages of fish marine species was highly dependent on marked hydrodynamic features (current speed up to ~ 44 cm/s). In this context, larval fish distribution showed complex spatial patterns, not associated with bottom depth as found in other similar studies in Mediterranean (Alemany *et al.*, 2006; Giordano *et al.*, 2014; Granata *et al.* 2011; Sabatés & Olivar, 1996) but with mesoscale oceanographic structures (*e.g.* surface currents, gyres and fronts), as reported in different cases by Bakun (2006), Basilone *et al.* (2013), Palomera (1992) and Sabatés *et al.* (2001). These structures conveyed the larvae in retention areas with conditions favorable for their survival and growth (Cushing, 1990). Additionally, it was important to underline the effect of Mistral (NW) and Westerly winds in modulating the process linked to upwelling and the transport of neritic fish larvae offshore, as described by Falcini *et al.* (2015) about dispersion of anchovy larvae in year 2004.

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#### **CHAPTER 2: Early life stage of *Sardinella aurita* in the Sicily Channel (Mediterranean Sea): the key role of physical forcings and implications for fisheries management**

##### **Abstract**

Multidisciplinary studies are recently aiming to define diagnostic tools for fishery sustainability by coupling ichthyoplanktonic datasets, physical and bio-geochemical oceanographic measurements, and ocean modelling. The main goal of these efforts is the understanding of those processes that control dispersion and fate of fish larvae and eggs, and thus tuning the inter-annual variability of biomass of fish species. Here we analyzed eggs and larvae distribution, as well as biological features, of *Sardinella aurita* in the north-eastern sector of the Sicily Channel (Mediterranean Sea) from ichthyoplanktonic data collected during the 2010 and 2011 summer cruises. We make use of Lagrangian simulations and satellite data (i.e., sea surface temperature, wind, and chlorophyll-a) to recognize the main oceanographic patterns that mark eggs and larvae transport processes. We finally provide a mechanistic explanation of the observed transport process by using a potential vorticity (PV) model that takes into account the role of wind stress in generating cold filaments that are able to connect the main recruiting areas: water off Malta and Capo Passero. Our results show that the strong offshore transport towards Malta occurring in 2010 was likely due to a persistent Mistral wind forcing that generated a high-PV cold filaments. Such a pattern was not found in the 2011 analysis, which indeed showed an along-shore transport towards the recruiting area of Capo Passero. Our work describes, for the first time, the spatial distribution of the early life stage of *Sardinella aurita* in the northern part of the Sicily Channel and provides useful insights regarding operational oceanography for sustainable fishery.

#### **Introduction**

Small pelagic fish are fundamental elements of the coastal marine ecosystem due to their role in energy transfer, from primary and secondary producers to top predator of the food web (Cury et al., 2000; Bănanu et al., 2013). Representing about 48% of the total reported landings (FAO-GFCM, 2015), *Engraulis encrasicolus*, *Sardina pilchardus*, *Sprattus sprattus* and *Sardinella aurita* constitute one of the most important fishery resource in the Mediterranean Sea.

Typically, biomass of these species is characterized by large inter-annual fluctuations (Lloret et al., 2001 and 2004, Patti et al., 2004). Assessing and understanding the root causes of this harvested fish population variability is one of the challenges for the sustainable management of fishery resource and marine ecosystems. Previous studies attributed biomass fish variations of small pelagic fish to environmental factors that are able to change the natural mortality rate of the early life stage, affecting the recruitment of the new generation in the adult fish population (Cury & Roy, 1989; Bernal, 1991; Hunter and Alheit, 1995; Bakun, 1996; Patti et al. 2004). This processes, paired with life history traits (i.e., short lifespan and large fecundity) (Bakun, 1996), makes the population size of small pelagic fish strongly dependent on the abiotic environment.

In this framework, oceanographic processes play a key role in shaping the spatial distribution of the planktonic fish stages, controlling the conveyance from the spawning areas to more or less suitable retention zones (Shelton and Hutchings, 1990; Pepin et al., 2003; Petitgas et al., 2006). In particular, “food” concentration and availability is often modulated by mesoscale oceanographic structures that have a crucial effect on the fate of several fish species during their larval and juvenile stages (McNamara and Houston, 1987; Cushing, 1990). Therefore, the link among ocean currents, atmospheric forcing, spatial distribution of early life stages, and other environmental parameters is crucial for the sustainable management of fishery resources (Chavez et al., 2003; Pörtner and Knust, 2007).

Scientific advances have been addressed to define the dynamics of transport of eggs and larvae and the effects on recruitments of important commercial species in the Mediterranean sea, i.e. *Engraulis encrasicolus* (Garcia and Palomera, 1996; Agostini and Bakun, 2002; Lafuente et al., 2002; Cuttitta et.

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al., 2003, 2006; Zarrad et.al, 2006; Somarakis and Nikolioudakis, 2007; Sabatés et al., 2007; Sabatés et al., 2013) and *Sardina pilchardus* (Olivar et. al., 2001, 2003; Santos et. al., 2004; Alemany et. al., 2006; Tugores, 2011). However, *Sardinella aurita* represent one of the most abundant coastal fish species in the summer larval fish assemblage of the central as well as of other areas of the Mediterranean Sea (Somarakis et al., 2002; Sabatés et al., 2006; Isari et al., 2008; Olivar et al., 2010; Zarrad et al., 2013; Cuttitta et al., 2007 and 2016). Moreover, is one of the most exploited fish species over the Mediterranean fishery resource, especially in the Ionian and Levant FAO division fishing areas (FAO-GFCM, 2015). Last but not least an increasing abundance and gradual northward expansion of this termophilic species has been reported along different areas of the Mediterranean as effect of the global warming (Sabatés et al. 2006, Tsikliras, 2008, Sinovčić et al., 2004), with expected effects on ecosystem and fisheries.

Nevertheless, knowledge on spatio-temporal distribution of early life stages of round sardinella (*Sardinella aurita*, Valenciennes, 1846), are very poor and localized in the northwestern basin (e.g. Maynou et. al., 2008; Sabatés et al., 2009; Sabatés et al., 2013). Studies investigating on spatial distribution (Zarrad et al., 2012) or on larval biological features of this species (Cuttitta et al., 2000 and 2007) in the central Mediterranean sea were performed without, however, providing insights on the main dynamics transport in relation to the abiotic environment, which, by controlling the fate of larvae, could play a key role affecting mortality rates as well as recruitment and fluctuations of the adults population.

We here aim to identify the main oceanographic process that rules the offshore transport of eggs and larvae of *Sardinella aurita*, as observed on the south Sicilian coast (central Mediterranean Sea). Here, wind forcing (the Mistral, in particular) forms and enhances the coastal current flowing southeastward along Sicilian coast due to up-welling effects (Pratt and Whitehead, 2007): blowing wind along the coastline creates offshore Ekman transport at the sea surface; consequently, water that is moved offshore is then replaced by deeper fluid that upwells and leads to colder surface temperatures along the coast; the resultant sloping interface implies a cross-shelf pressure gradient that triggers a geostrophic, along-shore flow (Falcini et al., 2015). This wind-induced effect, moreover, is at the base of the formation of cold filaments that propagates offshore from the eastern Sicilian tip (i.e., Capo Passero; Fig. 1) (Bignami et al., 2008). These kind of jets are often related to instabilities formed at an upwelling front (Flament et

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al., 1985, Washburn and Armi, 1988, Wang et al., 1988, Strub et al., 1991 and Haynes et al., 1993), in particular, when a short-term wind bursts hit restricted areas of the near-shore sea surface (Bignami et al., 2008).

We test this scenario by simulating Lagrangian transport of *Sardinella aurita* eggs and larvae within the Sicily Channel during the summer spawning period of 2010 and 2011. By pairing this analysis with biological and environmental data we try to depict the dynamic connection between spawning and recruiting areas and, in particular, the role of wind forcing in delivering *Sardinella aurita* larvae offshore. We finally confirm our hypothesis regarding the observed and simulated patterns by giving a physical interpretation of those Lagrangian dynamics.

Our study contributes to an improved understanding of the spatial and temporal dynamics affecting planktonic stage of fish, providing essential information in the framework of an ecosystem approach addressed to a sustainable management of the fishery resources.

## Data and Methods

### *The biological dataset*

Ichthyoplanktonic data were collected during two cruises carried out from 25 June to 14 July 2010 (Bansic 2010) and from 8 to 27 July 2011 (Bansic 2011) on board the R/V *Urania*, in correspondence with the main reproductive activity of this species (Tsikliras and Antonopoulou, 2006). 190 and 131 stations were sampled in Bansic 10 and Bansic 11, respectively (Fig. 1). The systematic sampling is constituted by a regular grid of stations ( $1/10^\circ \times 1/10^\circ$  along the continental shelf, and  $1/5^\circ \times 1/5^\circ$  further offshore). Planktonic sampling was conducted by using vertical CalVET (one mouth of 25 cm inlet diameter, 150  $\mu\text{m}$  mesh) and oblique Bongo 40 net (two mouth of 40 cm inlet diameter, 200  $\mu\text{m}$  mesh, towed at 2 knots). The nets were hauled from within 5 m from the bottom to the surface, or from 100 m to the surface in deep stations. In each mouth, calibrated flow-meters were mounted in order to calculate the volume of filtered water ( $\text{m}^3$ ). To preserve planktonic samples, borax-buffered solution of 4% formaldehyde and seawater (for CalVET and mouth 1-Bongo 40 samples) and solution of 70% ethanol

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(for mouth 2-Bongo 40 samples) were used. In order to identify eggs and larvae of *Sardinella aurita*, all samples were observed under a microscope in a land-based laboratory and fish eggs and larvae were sorted from the rest of the plankton and identified according to Whitehead et al. (1988).

The number of *Sardinella aurita* eggs and larvae, collected at each station, was normalized as

$Y_i = \frac{(10 \times d_i \times x_i)}{v_i}$ , where  $Y_i$  is the number of larvae/eggs of each species under 10 m<sup>2</sup> of sea at station  $i$ ,

$x_i$  is the number of larvae/eggs taken at station  $i$ ,  $v_i$  is the volume of water filtered in m<sup>3</sup> and  $d_i$  is the maximum depth reached by net.

Eggs and larvae were preserved in formaldehyde and were used for the determination of the stage of development. In particular, we assigned to each egg a stage number from 1 (stage after fecundation, with a single cell) to 11 (stage pre-hatching) by following the technique proposed by Gamulin and Hure (1955) and Whitehead (1988). We considered stage from 1 to 4 as “early stage”, from 5 to 8 “middle stage”, and from 9 to 11 “late stage”. *Sardinella aurita* larvae were photographed using binocular stereo microscope with integrated camera and total length (TL, mm) were obtained from the analysis of image performed with suitably calibrated software (Image Pro Plus 6.0, Image Cybernetics, RoperIndustries, SilverSpring, MD, USA).

#### ***The remote sensing dataset***

We paired the biological dataset with daily sea surface temperature (SST) and chlorophyll-a concentration (Chl-a) data from remote sensing (Table 1), and we evaluated cruise-averaged spatial maps (for each environmental parameter). This allowed us to first recognize the main hydrographic features that occurred at sea surface of the Sicily Channel and the relations between *Sardinella aurita* eggs and larvae distributions and environmental parameters. Moreover, daily SST data of a period preceding two surveys (June-July 2010 and 2011) allowed us to identify cold filaments in the spawning areas that could affect the adults spawning due to the influence of water temperature (Ben Tuvia, 1960; Palomera and Sabates, 1990; Ettahiri et al., 2003; Tsikliras, 2008). We considered SST values recorded in the identified spawning area (14° - 15.5° of longitude and 35.4° – 37° of latitude) on the continental shelf (between 0

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and 200 depth meters) for each day from 1<sup>st</sup> June to 27<sup>th</sup> July, i.e. from the begin of the spawning period (Tsikliras and Antonopoulou, 2006) to the end-day of the 2011 survey. Moreover, we analyzed wind stress ( $\vec{\tau}$ ) and Ekman transport ( $\vec{m}$ ) from remote sensing. These quantities are derived from ocean surface 6-hourly wind data ( $\vec{U}_{wind}$ ), provided by the Cross-Calibrated Multi-Platform project (Table 1).

Wind stress is obtained as:

$$\vec{\tau} = \rho_{air} C_d |\vec{U}_{wind}| \vec{U}_{wind}, \quad (1)$$

where  $\rho_{air}$  is the air density and the dimensionless friction coefficient  $C_d = 0.0012$  for  $0 < |\vec{U}_{wind}| < 11$  m s<sup>-1</sup> and  $C_d = 0.00049$  for  $|\vec{U}_{wind}| \geq 11$  m s<sup>-1</sup> (Large and Pond, 1981; McClain and Firestone, 1993).

Ekman transport is then calculated as (Pickett and Paduan, 2003)

$$\vec{M} = (\rho_{water} f)^{-1} \vec{\tau} \times \hat{k}, \quad (2)$$

where  $\rho_{water}$  is the water density,  $f$  the Coriolis parameter, and  $\hat{k}$  is the vertical unit vector.

Table 1. Satellite products that are used in this work.  $\Delta t$  and  $\Delta x$  indicate temporal and spatial resolutions, respectively. SST: sea surface temperature Pathfinder V5.2 (PFV52) AVHRR data interpolated through an Optimal Interpolation algorithm (Pisano et al., 2015); Chl-a: sea surface chlorophyll concentration computed by applying the MedOC4 algorithm (Volpe et al., 2007) to the ESA-CCI remote sensing reflectance (Rrs) spectra (ESA-CCI Rrs results from the merging of SeaWiFS, MODIS-Aqua and MERIS sensors); Ocean Wind: Cross-Calibrated, Multi-Platform Ocean Surface Wind Velocity Product (multi-sensor, made of SeaWinds su QuikSCAT e ADEOS-II, AMSR-E, TRMM TMI, SSM/I).

Parameter	$\Delta t$	$\Delta x$	Data source
SST – Pathfinder V5.2 (PFV52) AVHRR L4 data	daily	4 × 4 km	<a href="http://www.myocean.eu.org/">http://www.myocean.eu.org/</a>
Chl – ESA-CCI-L4 data	daily	4 × 4 km	<a href="http://www.myocean.eu.org/">http://www.myocean.eu.org/</a>
Ocean Wind	6 hours	25 × 25 km	<a href="http://podaac.jpl.nasa.gov">http://podaac.jpl.nasa.gov</a>

#### *The Lagrangian simulations*

Modern Lagrangian modelling techniques have been developed recently to simulate tracer trajectories from Eulerian velocity fields. Palatella et al. (2014) introduced a Lagrangian approach (LaCasce, 2008) as a first step towards a better understanding of the relationship between anchovy population and sea surface dynamics. This type of study is focused, in particular, on the Lagrangian connectivity (Cowen et al., 2000) between spawning and nursery areas. More specifically, the approach seeks to estimate the amount of larvae coming from a certain spawning region that are able to reach a particular nursery region. However, there are two main issues related to the simulation of transport and mixing of particles in an ocean circulation model: (i) the lack of resolution of meso- and submeso-scale horizontal motions and (ii) the underestimation of the vertical mixing in the upper layer. In our Lagrangian approach these two aspects are treated by adopting a kinematic Lagrangian modelling strategy.

Conservative chaotic flows are exploited to generate trajectories that accurately simulate (at least at a low order moment level) the typical small-scale turbulent motions affecting the dispersion of a given tracer distribution (Lacorata et al., 2008, 2014; Palatella et al., 2014). At this scope the kinematic velocity fields are composed by 3D time oscillating convective cells of various length sizes and with a given spatio-temporal scaling relationship (e.g. Kolmogorov's scaling). Anomalous behaviors due to unrealistic “sweeping effect”, i.e., a known drawback affecting kinematic simulations of turbulence, are ruled out by adopting the quasi-Lagrangian coordinates technique (Lacorata et al, 2008).

We here used the model provided by the Mediterranean Forecasting System (MFS; Tonani et al., 2008) as Eulerian input. Its domain covers the entire Mediterranean basin; the horizontal is 1/16 x 1/16 degree (~ 6.5 km); the model has 72 vertical layers, ranging from 1.5 m to 5000 m depth; wind forcing is provided by ECMWF data every six hours. The daily re-analysis velocity fields from the MFS model are used for the large-scale circulation while a 3D convective cell field are added to the main model in order to compensate the lack of effective mesoscale turbulent dispersion and vertical migration in the mixed layer, as discussed above. Such a pioneering approach represents a novelty, since it uses a kinematic model for mesoscale turbulent dispersion as coupled to a large-scale ocean circulation model (see details

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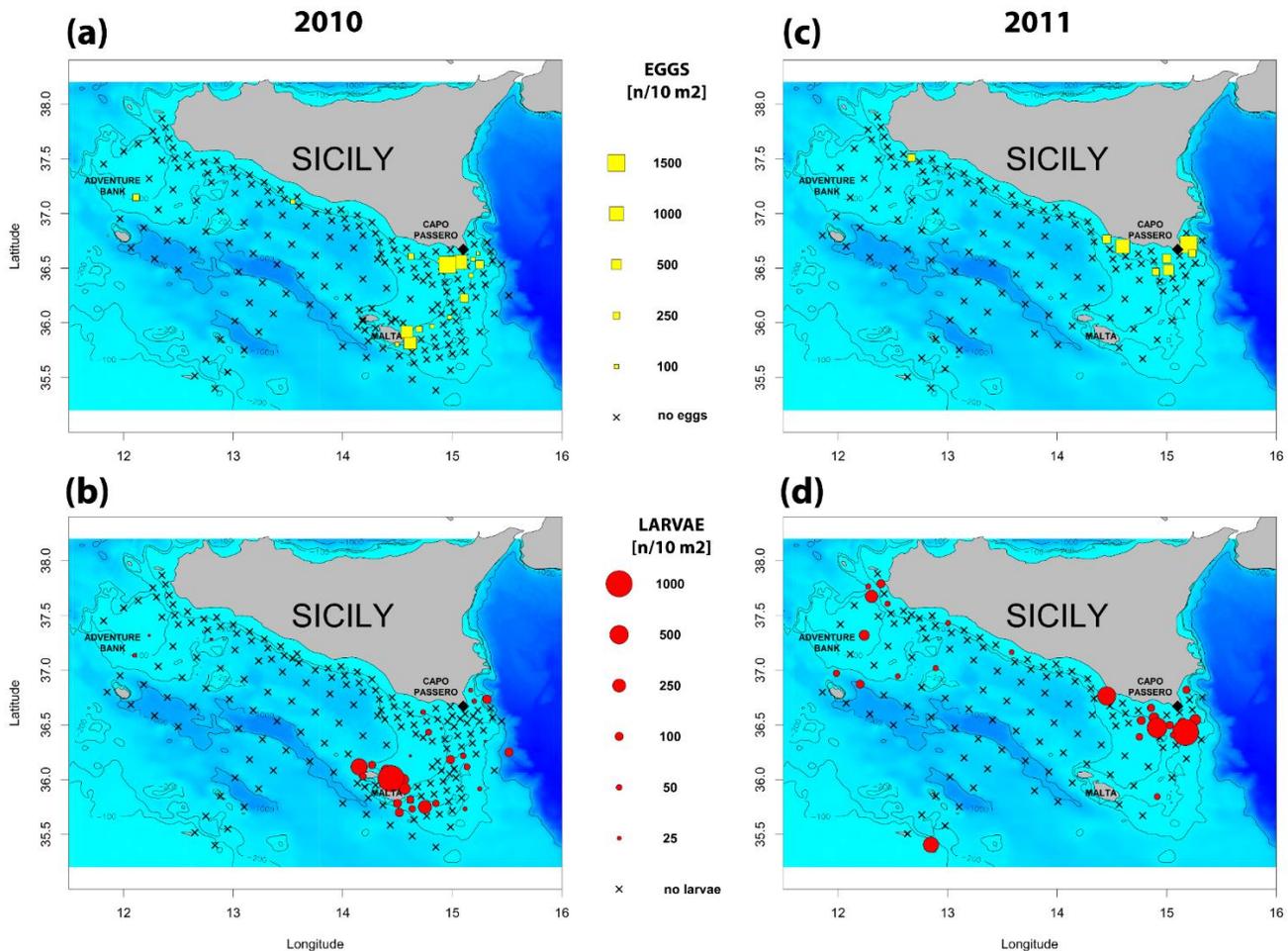
on the kinematic model set up, the 3D vertical mixing model, and the 2D mesoscale turbulence model in Palatella et al., 2014 and Lacorata et al., 2014).

To strengthen our analysis we also consider a second Eulerian field, i.e., the version 2.0 of the Geostrophic fields, provided by GlobCurrent project (Chapron et al., 2015; Johannessen et al., 2016), which consist of L4 satellite retrieved geostrophic, daily currents at 0.125° space resolution. The boundary conditions of our simulations are open (relatively to the Sicily Channel sub-domain), with rebound conditions of the Lagrangian particles against the coasts (an accurate modelling of the circulation in proximity of coastal boundaries is outside the capabilities of the Ocean model we used). The period covered by the simulation goes from June 1<sup>st</sup> to September 30<sup>th</sup>, for both 2010 and 2011. We assumed a source of passive neutrally buoyant particles emitting at constant rate in the period from June 1<sup>st</sup> to September 15<sup>th</sup>. The total number of numerical trajectories analyzed in each run is 25600.

## **Results**

### *Spatial distribution pattern of eggs and larvae*

Most of the eggs and larvae of *Sardinella aurita* were found between coastline and the 200 m isobaths. In both 2010 and 2011, an overall high concentration was found in the easternmost part of the study area (Fig. 1). The high abundance of planktonic stage in this coastal area, off Capo Passero, allowed us to identify this zone as the most important spawning and retention area of the south Sicilian coast. Conversely, very low observations were detected on the Adventure bank, north-westernmost of the study area (Fig. 1).

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**Figure 1.** Map of the study area (i.e., Sicily Channel) showing the sampling stations. Bathymetry is indicated by contours and background colors, from cyan (shallower) to blue (deeper). The isobaths of 100, 200 and 1000 m are shown. Red circles and yellow squares represent larval (upper panels) and eggs (lower panels) density of *Sardinella aurita* during 2010 (left) and 2011(right) surveys, respectively. The main points of interest are indicated

However, different patterns were identified between the two years. In 2010, 194 eggs ( $36.65 \text{ eggs}/10\text{m}^2$ ) and 344 larvae ( $22.83 \text{ larvae}/10\text{m}^2$ ) of *Sardinella aurita* were found in the 10% and 17% of the stations sampled, respectively (Figs. 1a and 1b). A similar abundance of early, middle, and late egg stages were found in the area off Capo Passero (early stage: 36%; middle stage: 38%; late stage: 26%). In the region

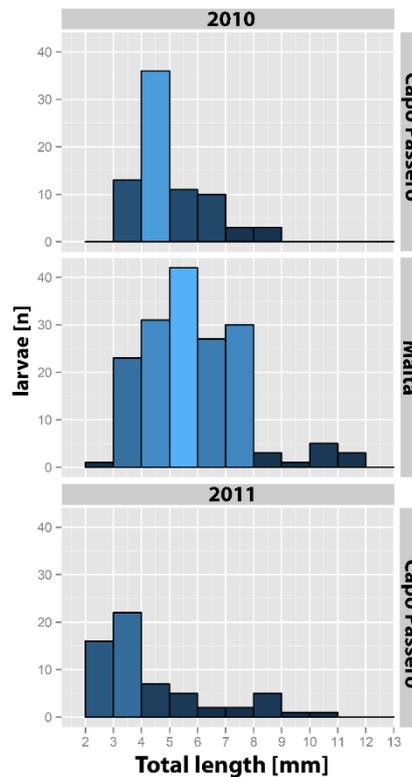
### *Sardinella aurita*: physical forcings

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close to Malta, we observed a predominance of middle stages, although early and late stage were also observed (i.e., early stage: 20%; middle stage: 53%; late stage: 27%).

Regarding frequency distribution of larval size classes (Fig. 2), in 2010 we found differences among zones. In Capo Passero, larval length ranged 3 and 9 mm, with class 3-4 mm as the most frequent. In Malta, we observed a wider range of length (2–12 mm) and size classes from 3 to 8 mm were characterized by the higher frequencies, although larvae longer than 9 mm were also detected exclusively in this zone.

Compared with the previous year, in 2011 we found lower density of both planktonic stages. In particular, 94 eggs (14.13 eggs/10m<sup>2</sup>) and 66 larvae (9.38 larvae/10m<sup>2</sup>) were present in the 6% and 19% of the sampled stations, respectively (Figs. 1c and d). No eggs and larvae were found in the Maltese waters, while most of them were identified off Capo Passero (Fig. 1c and 1d). Regarding eggs stages analysis, we observed a distinct dominance of late stage eggs off Capo Passero (early stage: 7%; middle stage: 31%; late stage: 62%). Frequency distribution of size classes (Fig. 2) shows that Capo Passero gathered larvae that range from 2 to 11 mm, with larvae shorter than 3 mm in total length as the most frequent in this area.

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**Figure 2.** Frequency distribution of total lengths (TLs) measured off Adventure Bank, Capo Passero, and Malta during the two Bansic 2010 and 2011 cruises.

These evidences set some hypotheses regarding the joint action between *in situ* spawning and eggs/larvae advection due to mesoscale, coastal oceanographic structures. In 2010, the presence of i) several stages of *Sardinella aurita* eggs off Capo Passero; ii) small size classes of larvae off Capo Passero; iii) late stages of larvae (>9 mm) off Malta, suggest an offshore advection of eggs and larvae from Capo Passero to Malta, reasonably due to wind-induced effects. Such a mechanism seems not to be active in 2011, where i) the dominance of late eggs stages; ii) the presence of a wide range of larval size classes; iii) the presence of late stage of larvae in Capo Passero, indicate eggs and larval development *in loco*, linked to the lack of durable oceanographic structures that would remove early life stages of *Sardinella aurita* from the spawning area (i.e., off Capo Passero). We therefore pursue the idea that advection towards Malta, occurring in 2010, likely removed *Sardinella aurita* larvae far from the conveyor belt that flow

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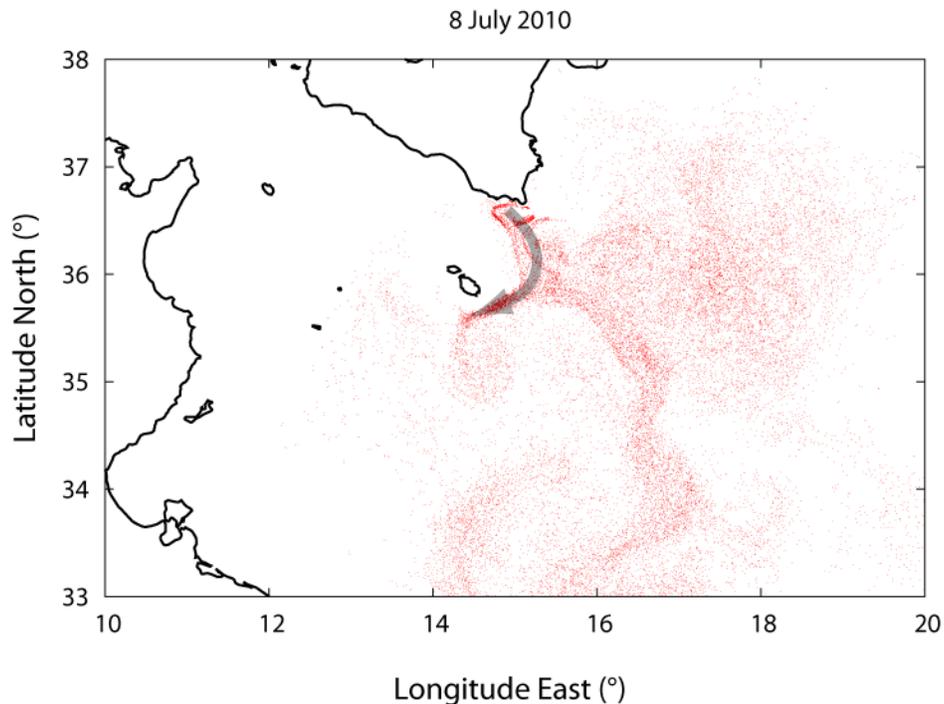
south-eastward, towards the recruitment area of Capo Passero (Agostini and Bakun, 2002; Cuttitta et al., 2006; Falcini et al., 2015).

#### ***Results from Lagrangian Simulations***

The hypothesis of a more intense offshore transport of *Sardinella aurita* occurred from the recruiting area of Capo Passero during the summer 2010 with respect to the 2011 is here explored by means of Lagrangian runs (Fig. 3). These simulations confirm the presence of a narrow filament that dynamically connects the spawning/recruiting area off Capo Passero with Malta. During the middle of June 2010 we notice a strong southward advection of particles that are thus delivered to eastern sector of Malta in a few days (i.e., ~ 50 km in 5 days that corresponds to a surface current of ~10 cm/s) by a particular branch of the Lagrangian trajectories. We observed two events: the first characterizing a brief period between June 10<sup>th</sup> - 13<sup>th</sup>; the second marked by several events occurring between June 20<sup>th</sup> and July 8<sup>th</sup>. The 2011 shows a similar pattern, although there is no evidence of a Lagrangian preferential path that connects Capo Passero to the eastern side of Malta and, for the whole July, the southward advection is much weaker with respect to the 2010 case.

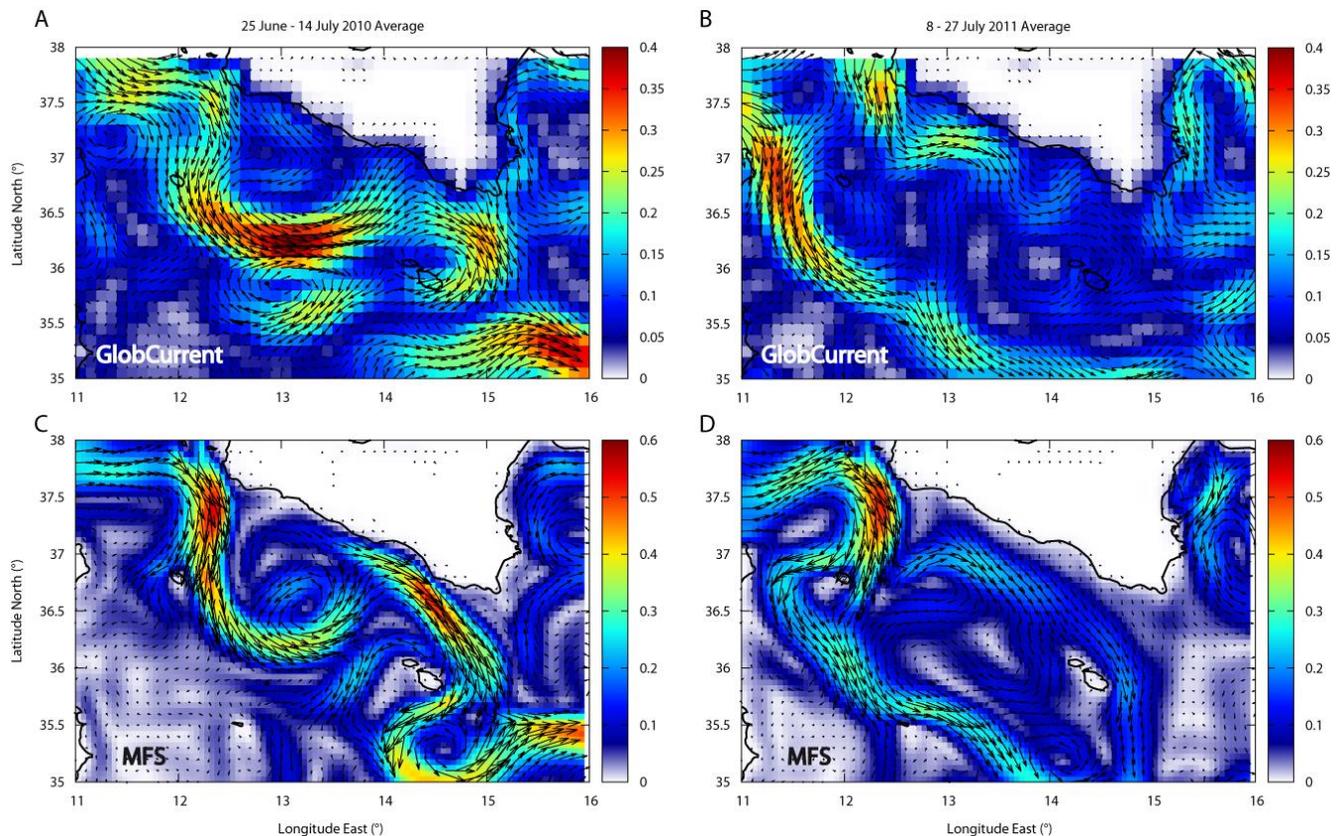
## *Sardinella aurita*: physical forcings

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**Figure 3.** Lagrangian run snapshot on 8 July 2010 showing the net transport (grey arrow) of *Sardinella Aurita* larvae (red dots) from Capo Passero to Malta that occurs along the cold filament forming off Capo Passero.

The comparison between these two scenarios is further stressed by two distinct Eulerian velocity fields, i.e., the MFS fields (Tonani et al., 2008) and the GlobCurrent fields (Chapron, 2015; Johannessen et al., 2016), averaged through the two oceanographic surveys (Fig. 4). The 2011 shows a much weaker velocity field – and the absence of the cold filament – that did not deliver larvae from Capo Passero to offshore.

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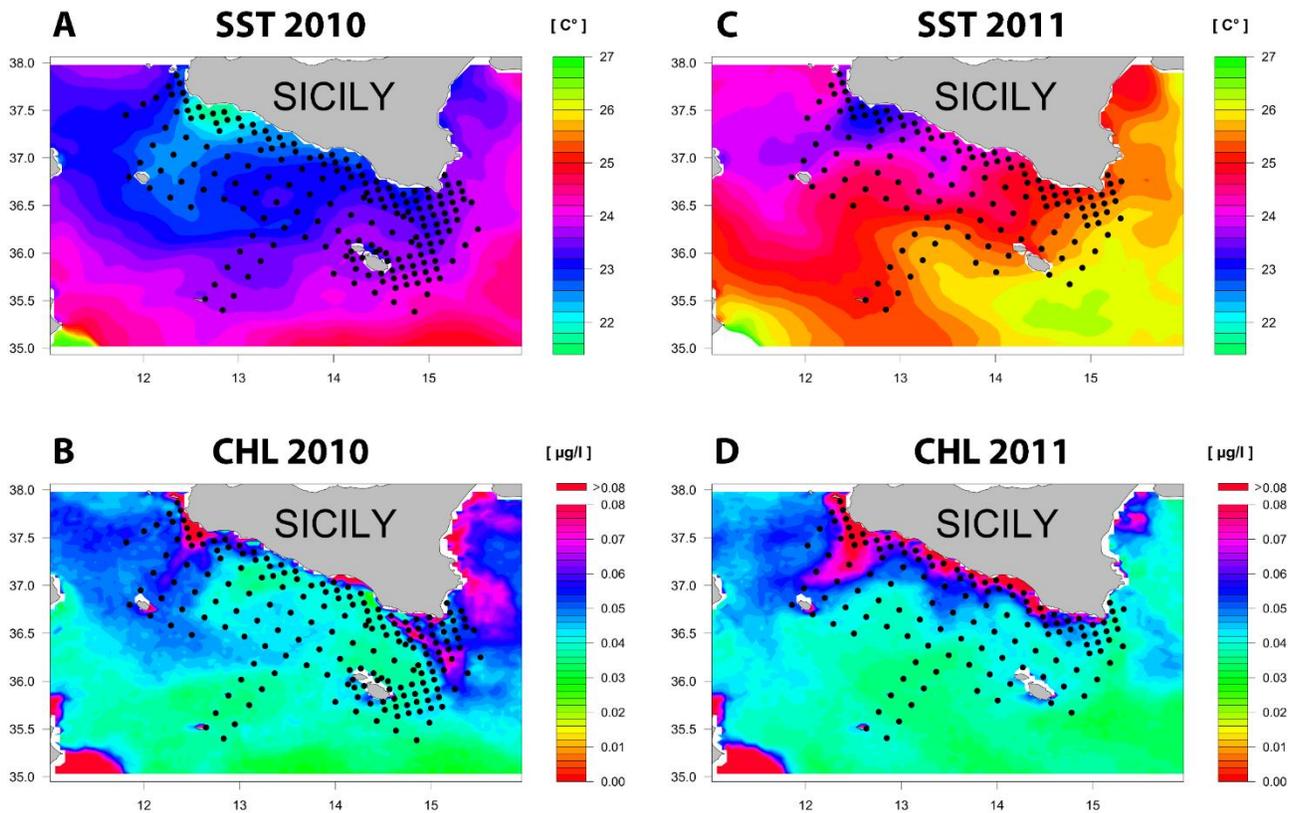
**Figure 4.** Cruise averaged Eulerian velocity fields for the two Bansic cruises: 25 June to 14 July 2010 (A and C); 8 to 27 July 2011 (B and D). The two Eulerian velocity fields are from GlobCurrent (A and B) and MFS (C and D). Although the two fields show some differences, both of them confirm a strong advection from the southeast Sicilian coast to Malta in 2010.

*Results from the satellite datasets*

SST and Chl-a concentration satellite patterns support the evidence of two different oceanographic conditions in the two study years (Fig. 5). In the 2010 the Sicily Channel was characterized by a colder surface water and a higher Chl-a concentration (mean SST=23.59 °C; mean Chl-a= 0.044 µg/l) compared to the 2011 (mean SST=25.08 °C; mean Chl-a = 0.042 µg/l). In particular, in 2010 satellite maps evidenced a cold and Chl-rich structure that protrudes offshore from Capo Passero (Figs. 5a and b). This structure is characterized by a SST  $\approx$  23.20 °C and a Chl-a  $\approx$  0.07 µg/l, and traces a curved path (i.e., a

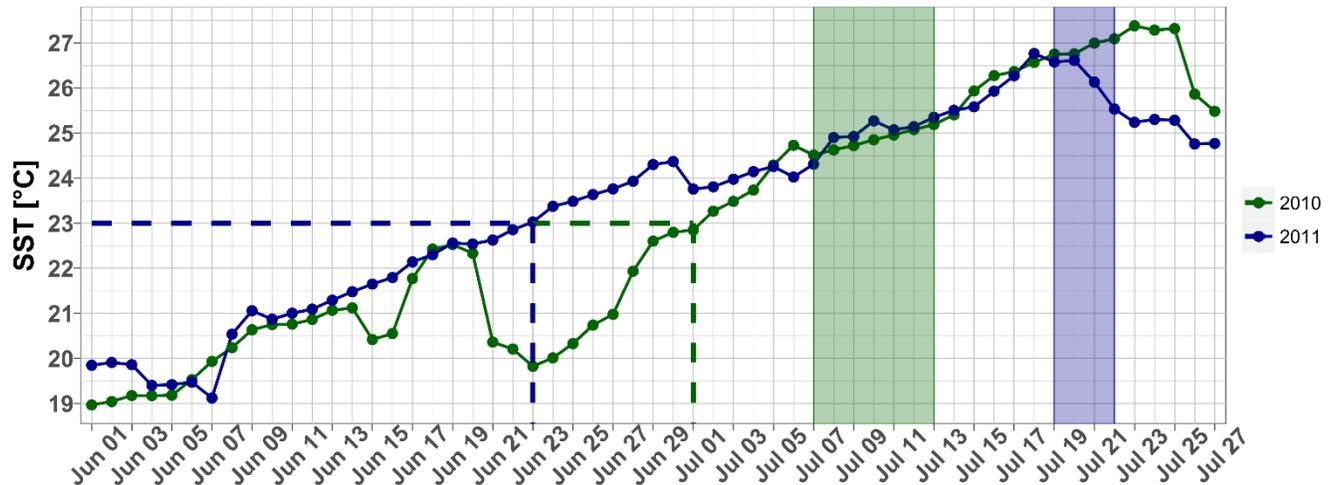
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filament). The 2011 does not show a similar pattern (Fig. 5c, d). Very low values of Chl-a concentration were found in the offshore zone in both years ( $<0.03 \mu\text{g/l}$ ).



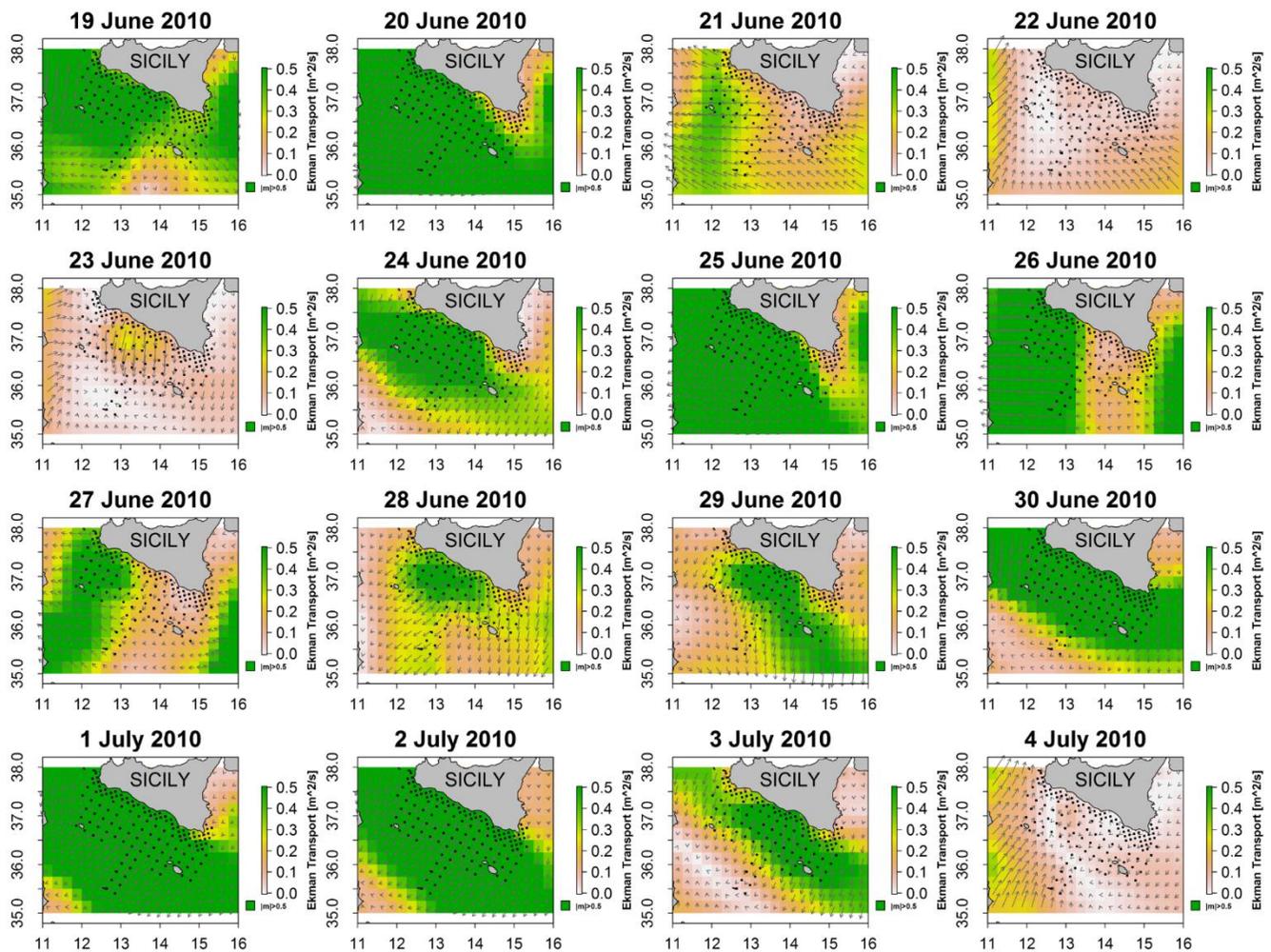
**Figure 5.** Cruise averaged Sea Surface Temperature (A, C) and Chlorophyll concentration (B, D) for the two Bansic 2010 and 2011 cruises.

We further analyzed SST time series averaged over the spawning area, from 1<sup>st</sup> June to July 27<sup>th</sup> of both 2010 and 2011 (Fig. 6). The 2010 shows the presence of cold events: a weaker one, around June 13<sup>th</sup>, and a colder and persistent one, marked by the colder pick between 21<sup>th</sup> and 25<sup>th</sup> of June, in accordance with the corresponding lagrangian simulation. On the contrary, no intense phenomena were observed in 2011 (Fig. 6).

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**Figure 6.** Mean daily SST in the spawning area (14–15.5 W; 35.4–37 N) of *Sardinella aurita*, from June 1<sup>th</sup> to 27<sup>th</sup> July. Dashed line highlight when threshold temperature of 23°C (onset of the spawning period) arise in the years 2010 and 2011. Green and blue rectangles show the sampling period in the spawning area in 2010 and 2011 respectively.

In seeking to understand the role of upwelling in the formation of such a cold and Chl-rich filament, we find a comforting agreement with Ekman transport maps resulted from wind speed patterns (Fig. 7). Between the 19<sup>th</sup> of June and the 4<sup>th</sup> of July 2010 a significant Ekman transport likely induced the formation of an upwelling and coastal current, that could be reasonably at the base of the arising of cold waters in the upper layers (Fig. 7). Although the 2011 is also characterized by strong wind events, it does not record the same persistency that we observe in 2010, when the Mistral wind blew steadily for 8 days. The different wind pattern that we observed for the two years is better investigated in the next section.

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**Figure 7.** Daily Ekman transport calculated from wind data from June 19<sup>th</sup> to July 4<sup>th</sup>. This period, temporally overlapped with ichthyoplanktonic data collection, showed the higher value of Ekman transport generated by Mistral winds.

*The surface cold filament model*

Based on the first results, we rationally hypothesize that the particularly strong Mistral wind pattern, and the front instabilities related to the coastal Ekman layer, occurred during the summer spawning in 2010, triggered the southward transport of *Sardinella aurita* larvae and eggs from Capo Passero, along the chlorophyll and cold reach filament. To diagnose this pattern, and to provide a mechanistic explanation regarding the link between the wind field and the onset of the cross-shore transport, we use a surface

cold filament model (Bignami et al., 2008). Wind forcing can directly produces shelf-blocked jets that are subsequently driven offshore by the general circulation (Crépon and Richez, 1982; McCreary et al., 1989; Salusti, 1998). The model describes the potential of these cold filaments and jets to propagate offshore and to maintain their coherent structure based on their potential vorticity (PV). These jets, indeed, are generated by a strong PV input due to upwelling and/or the funneling of strong, cold, and short-term wind bursts that blow over a restricted, shallow area of the sea surface near the coast (Holland, 1967). This PV ( $\Pi$ ) increase, due to the wind stress ( $\vec{\tau}$ ), is described by

$$\frac{d\Pi}{dt} = \frac{1}{\rho h} (\nabla \times \vec{\tau})_z, \quad (3)$$

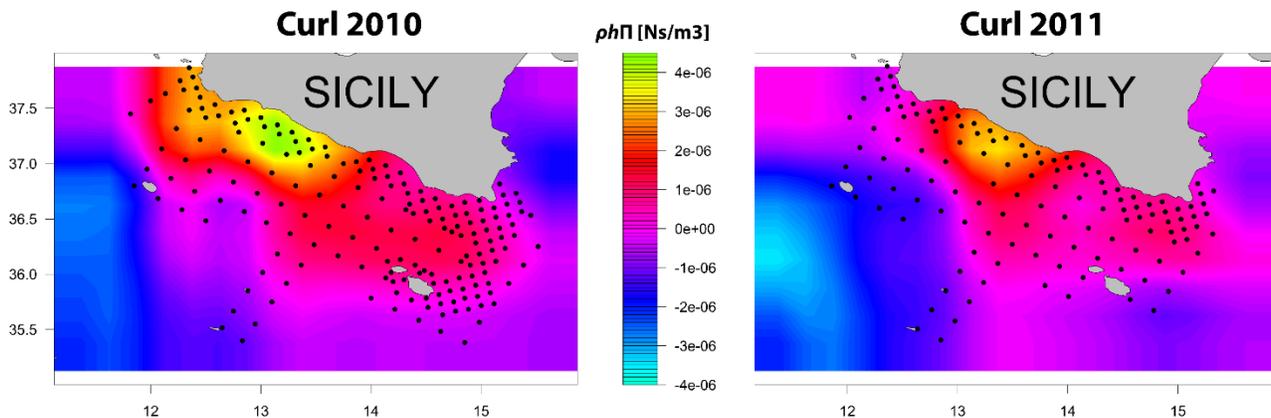
where  $\rho$  is the water density,  $h$  is the cold water thickness, and the subscript  $z$  indicates the third component (i.e., the vertical one) of the curl.

Equation (3) can be integrated in order to estimate, and to compare, the amount of PV accumulated on the shelf area during the two summer spawning periods in 2010 and 2011:

$$\Pi = \frac{1}{\rho h} \int_0^t \left( \frac{\partial \tau_y}{\partial x} - \frac{\partial \tau_x}{\partial y} \right) dt \quad (4)$$

Figure 8 shows the temporal integral of the curl of wind stress in Eq. (4) and fully confirms our hypothesis. Based on the surface cold filament model, the higher PV (i.e., higher  $(\nabla \times \vec{\tau})_z$ ) we observe in 2010 – with respect to the 2011 – marks the strong role of the wind stress in “loading” PV to the coastal water. Once the high PV is set such a strong and localized input does not remain confined to the coastal zone, but propagates offshore as a filament (Bignami et al., 2008).

We therefore point out that Ekman transport due to Mistral wind was particularly active for both years, thus generating a cold coastal current that efficiently transported eggs and larvae to Capo Passero from the whole Sicilian coast. However, only the 2010 case was characterized by a high PV input able to trigger the filament that delivered eggs and larvae offshore (i.e., around Malta).

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**Figure 8.** Integral of the curl of wind stress  $\rho h \Pi$  ( $\text{Ns/m}^3$ ) (see Eq. 4) performed throughout the Bansic 2010 (25 June to 14 July) and Bansic 2011 (8 to 27 July) cruises showing the more intense potential vorticity increase that occurred in 2010. Such a potential vorticity input led to the offshore propagation of the cold filament.

## Discussions

The presence of planktonic stage of *Sardinella aurita* in the south-eastern Italian side of the Sicily Channel allowed us to identify for the first time the main spawning areas in this study area: coastal waters off Capo Passero and Malta.

Marked difference in abundance and spatial distribution of eggs and larvae emerged among study years (i.e., 2010 and 2011) as well as among spawning and recruitment areas.

According to Cuttitta et al. (2007), we found higher density of eggs and larvae of *Sardinella aurita* in the “colder” year (i.e., 2010), in spite of tropical origin of this species. The overall dissimilarity among years could be linked to a match/mismatch between the peak of spawning and the sampling period, due to different temperature conditions. Previous studies conducted in the northwestern Mediterranean indicated that the spawning time of this species took place at temperatures higher than  $23^\circ\text{C}$  (Navarro, 1932, Andreu & Rodriguez-Roda 1951, Palomera and Sabatés 1990). In our study area, this threshold arises later in 2010, compared with 2011 (Fig. 6), due to cold filaments events able to decrease temperatures of the upper water layers. The overall higher density of early life stage of *Sardinella aurita*,

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which is found in 2010, could be hence the consequence of matching between delayed peaks of spawning paired with an earlier sampling cruise. On the other side, lower density found in the summer 2011 could be the result of mismatch between the peak of spawning and the sampling period due to the anticipated reproductive events (Fig. 6).

However, this picture not offer a sufficient description of the differences identified between the spawning and recruitment areas. Our analysis highlighted the crucial role of the physical forcing and the environmental-derived conditions in shaping the spatial distribution of the early life stage of this species. In 2010, the stronger action of Mistral winds gave rise to a net offshore water movement as a consequence of Ekman transport (Poulain and Zambianchi, 2007). Surface waters were replaced by deeper waters, creating nutrient-enriched and cold coastal currents that affected the spawning area of Capo Passero (Bignami et al., 2008). That intense wind-induced phenomena (i.e., PV inputs due to wind bursts blowing over shallow, coastal areas) have triggered a cross-shore transport of *Sardinella aurita* larvae from Capo Passero to Malta, as confirmed by lagrangian simulations and cold filaments model. In spite of the existence of eggs in both zone, in agreement with the above depicted scenario we observe high larval concentrations off Malta coupled with a low density of larvae in front of Capo Passero in 2010. The absence of bigger size classes in Capo Passero and opposite findings off Malta is also consistent with our hydrodynamic representation, due to the advection and the concurrently growth of larvae during the pathways. We detect cold filament events characterized by a mean cross-shore jet velocity of ~20 cm/s. By considering the age-length relationship (growth rate 1,2 mm/day, Ditty et al., 1994), the hypothesis of larvae ranged between 9 and 12 mm (i.e. 5-8 days) found in Malta and originated from eggs spawned in Capo Passero, is in agreement with the distances and the necessary advection period (~8-10 days). In these conditions, Malta can be identified as an important recruitment area, aggregating larvae spawned *in situ* with bigger ones removed from the south-eastward conveyor belt (i.e Cape Passero, see also Lafuente et al., 2002; Falcini et al., 2015 for the case of anchovy) and advected from the Sicilian coast, although it is not excluded that a fraction of larvae were forced also in direction of the offshore waters.

A different scenario emerged in 2011 showed warmer and nutrient-depleted surface waters in relation to inconsistent intensity and direction of wind and weakened coastal upwelling. Higher density of later stage

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of eggs and a wider range of larval size in Capo Passero is consistent with the reduced hydrodynamic conditions detected in relation to the lower blowing of Mistral winds and the consequent weakening of the upwelling system and cold filaments formation. In this case, geostrophic and along-coast currents play a key role driving the fate of the early life stage. In these conditions, Capo Passero assumes an important function of recruitment area for eggs developed *in situ* and larvae gathered from north-west as a consequence of along-shore advection (Lafuente et al., 2002, Falcini et al., 2015).

The occurrence of the one or the other scenario could assume great relevance in terms of growth and mortality rates due to larval development in environments characterized by different temperatures and food availability conditions. Enhanced food conditions could play a key role, affecting on the one hand the reproductive potential of the adults fish (e.g. Roy et al., 1989; Quaatay and Maravelias, 1999), which, being zooplankton feeders, are strictly linked to areas characterized by high primary productivity (Ben Tuvia, 1960; Sabatés et al., 2009). In addition, food availability could be able to reduce mortality rates linked to starvation, affecting the number of exogenous feeder larvae (longer than 4 mm) (Ditty et al., 1994). On the other hand, lower temperature could be crucial, decreasing the metabolic rate, limiting the energetic consumption and limiting foraging events (Blaxter, 1982; Houde, 1989). The explanation of how food availability and temperature could tune the survival of early life stage in this species is unknown and additional studies are needed. However, our study shed light on main mesoscale oceanographic dynamics occurring in Sicily Channel, which affect the surrounding environment of larvae, control fate and dispersion and hence tune mortality rates of the early life stages. Affecting the strength of recruitments, dynamics of the upper layer in this zone could be a crucial aspect to take into account in the framework of populations dynamics and fishery management of this species, as well as other species that here spawn planktonic stages.

### **Conclusions and published paper**

Dynamics of marine surface layer plays a fundamental and, for many aspects, unpredictable role as far as the life and the evolution of pelagic species are concerned. In the early life stage, fish larvae move as

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passively advected by the currents. Their fate is strictly related to their Lagrangian pathways across the sea and to the selection rules that may strongly affect their population. A systematic study of the dynamical evolution of marine species can only be assessed by means of accurate modeling of velocity fields and Lagrangian transport, as well as by a deep understanding of the physical processes that rule larval fate and dispersion.

We adopted this approach on ichthyoplanktonic dataset of *Sardinella aurita* collected in two years characterized by opposite environmental conditions and we pointed out how the occurrence of Mistral winds in the summer can modify the fate of the planktonic stage of this species. We can conclude that, affecting temperature, food availability and dispersion of the early life stage, wind is a crucial aspect to take into account in order to assess the recruitment and the consequent biomass fluctuations of small pelagic fish in this marine system.

Moreover, we detected a connection due to the advection of planktonic reproductive stages between Maltese and Sicilian waters, which are identified as different Geographical Sub-Areas (GSA) from the General Fisheries Commission for the Mediterranean (GFCM). In this framework, the adoption of common management strategies could be a successful approach in the context of sustainable fisheries and resources exploitation.

Moreover, this approach is strongly suggested in the studies addressed to the implementations of predictive models that could provide useful information in others similar systems. Our findings can indeed be easily applied to those geographical regions where fate and distribution of small pelagic larvae are potentially affected by wind effects (e.g., Gulf of Tunis, northeast Spanish coast, the northern Aegean Sea, and California Current System). We believe that our approach, paired with the use of operational oceanographic tools, can lead to very interesting and useful results for a sustainable fishery management. Moreover, it provides some insights regarding the potential of remote sensing and Lagrangian techniques, which, coupled with biological observations, allow us to clarify the dispersion dynamics involved during the planktonic stage of small pelagic species.

The expected benefits for fisheries management in strategic areas, in the Mediterranean, as well as in other ocean basins, will consist in providing a major and more detailed information about preferential

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sources and recruitment areas, in order to better estimate and possibly regulate the amount of future biomass.

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#### **CHAPTER 3: Spatial growth patterns as revealed by otolith microstructure analysis: case study of *Sardinella aurita* in the central Mediterranean sea**

##### **Abstract**

Relationship between environmental conditions and life history traits is investigated in early life stages of *Sardinella aurita* (Pisces, Clupeidae), collected in central Mediterranean Sea along a latitudinal gradient. Two consecutive surveys were carried out in southern (Strait of Sicily) and northern sites (central Tyrrhenian Sea), during summer 2013, in correspondence of the spawning peak of this species in Mediterranean Sea. *In situ* and satellite-derived environmental parameters were collected and sea surface temperature and Chlorophyll-a concentration, considered as a proxy of food availability, were analyzed and compared among areas. An estimation of the mean daily growth rate were carried out through microstructure analysis of otoliths extracted from larvae collected in both sites. Back-calculated hatching time and back-trajectories calculation using a Lagrangian approach were used in order to estimate spatio-temporal daily positions of each specimen in each day of life and multivariate models were consider with the aim to examine the relationship between width of the increments and environmental conditions. Central Tyrrhenian Sea showed higher value of temperature and food availability compared with the southern site. Length-at-age linear model showed higher growth rate in the warmer and food-enriched zones (north) compared with the south, although not significance differenced were detected. However, mean daily growth deducted by the microstructure analysis showed a significance difference in the three days of life among site, highlighting higher growth rate in larvae collected in the central Tyrrhenian Sea. Generalized Additive Mixed Model (GAMM) identified the positive non-linear contribution of temperature in affecting the larval growth performance during the first days post-hatch, becoming the most important factor when water temperature arise higher than 24.5°C. On the contrary, no significant contribution emerged considering food availability. This study highlight the importance of the temperature conditions for the larval development of this thermophilic species and shed light on the effect of the Mistral winds in the Strait of Sicily that generate colder surface water in

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the spawning area of this species. Moreover, it emphasize the presence of suitable condition for the larval growth of this species in one of the northern area of its distribution suggesting that other factors, as the water temperature occurring in the colder period of the year, could play a key role in limiting the norther distributions of this species.

#### **Introduction**

It is well-known that fish population size can exhibit large interannual fluctuations (Lloret et al., 2001 and 2004, Patti et al., 2004). These fluctuations are mainly attributable to variations in recruitment success, which in turn are mainly due to very high and extremely variable mortality (Peterson and Wroblecky, 1984; Bradford, 1992) during the critical period of the planktonic stages (Hjort, 1914, 1926; May, 1974).

In order to determining recruitment magnitude, high mortality coupled with high variability in larval traits can result in selective loss of particular individuals. However, modifications of this selective loss can be caused by environmental factors such as food availability and temperature (Sponaugle and Grorud-Colvert 2006; Sponaugle et al. 2006), but also maternal effects that could be evident at hatching or even at the egg stage (Gagliano and McCormick 2007). Advantages conferred by this early variation can influence not only survival in the pelagic larval stage (Meekan and Fortier 1996; Hare and Cowen 1997; Tanaka et al. 2006), but may carry over and affect recruitment to and survival in subsequent juvenile and adult stages (Vigliola and Meekan 2002; McCormick and Hoey 2004; Meekan et al. 2006).

In agreement with the bigger-is-better theory (Miller et al., 1988; Litvak and Leggett, 1992) the risk of death due to predation changes dramatically during the first few days after hatching. This is due to the rapid increase in length and/or changes in behavior related to development during this period (Bailey & Batty 1984, Blaxter & Batty 1985, Hubbs and Blaxter 1986, Eaton & Di Domenico 1986, Webb & Weihs 1986, Butler & Pickett 1988, Fuiman 1989). The view most commonly expressed in the literature is that larger and older larvae are less vulnerable to predation (Bailey & Batty 1984, Blaxter & Batty 1985, Blaxter 1986, Eaton & DiDomenico 1986, Webb & Weihs 1986, Butler & Pickett 1988, Miller et al.

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1988, and others). Therefore, mortality in many fish species is very high during early development due to their small size, limited swimming ability, and sensitivity to variations in their surrounding environment (Rice et al. 1987, Miller et al. 1988). During these vulnerable stages, fishes may be adversely affected by environmental factors that influence survival and subsequent year-class strength (Houde 1994, Claramunt & Wahl 2000).

Environmentally-mediated differences in somatic growth are well known in temperate marine fishes. Warmer water temperatures induce higher metabolic rates in poikilotherms and result in generally higher growth rates (Houde 1989, Blaxter 1991, Benoît et al. 2000). The relationship between growth, size, and mortality has been widely examined for larvae of economically important temperate species in the interest of understanding stock-recruit issues (e.g. Chambers & Leggett 1987, Rutherford & Houde 1995, Campana 1996, Otterlei et al. 1999). While growth must be sustained with adequate food, water temperature plays a central role in determining growth rates (Houde 1989, Pepin 1991, Rombough 1997).

However, high variability in the process that link environment to recruitment exists among species and habitat and, in general, are emphasized in species characterized by short lifespan and large fecundity (Bakun, 1996).

This is the case of small pelagic fish of the Mediterranean Sea. Previous studies attributed biomass fish variations to environmental factors that are able to change the natural mortality rate of the early life stage, affecting the recruitment of the new generation in the adult fish population (Cury & Roy, 1989; Bernal, 1991; Hunter and Alheit, 1995; Bakun, 1996; Patti et al. 2004). Features of their life history traits (i.e., short lifespan and large fecundity), makes the population size of small pelagic fish strongly dependent on the abiotic environment.

Understanding how environmental factors are able to tune the reproductive success of fish is always one of the most important challenge of the fisheries science. Scientific advances in this direction, have been addressed, especially paying particular attention to the dynamics of transport of eggs and larvae of important commercial species in the Mediterranean sea, i.e. *Engraulis encrasicolus* (e.g. Cuttitta et. al., 2003, 2006; Zarrad et.al, 2006; Sabatés et al., 2007) and *Sardina pilchardus* (e.g. Olivar et. al., 2001, 2003; Santos et. al., 2004; Alemany et. al., 2006; Tugores, 2011).

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Nevertheless *Sardinella aurita*, represent one of the most abundant coastal fish species in the summer larval fish assemblage of the Mediterranean Sea (Somarakis et al., 2002; Sabatés et al., 2006; Isari et al., 2008; Olivar et al., 2010; Zarrad et al., 2013; Cuttitta et al., 2007 and 2016). Moreover is one of the most exploited fish species over the Mediterranean fishery resource, especially in the Ionian and Levant FAO division fishing areas (FAO-GFCM, 2015).

This small pelagic fish is a thermophilic species, widely distributed throughout the tropical and subtropical seas of the world, including the entire Mediterranean and the Black Sea (Ben-Tuvia, 1960). An increasing abundance and gradual northward expansion of this species has been reported along different areas of the Mediterranean in correspondence to warming of the sea water (Sabatés et al. 2006, Tsikliras, 2008, Sinovčić et al., 2004), with expected effects on ecosystem and fisheries. The reproductive period in the Mediterranean occurs during the warmest months of the year, i.e., from July to October (Palomera and Sabatés, 1990; Tsikliras and Antonopoulou, 2006; Palomera et al., 2007), in accordance with its tropical origin (Ben Tuvia, 1960). Eggs and larvae of *Sardinella aurita* are often associated with warm coastal and chlorophyll-enriched water (Ben Tuvia, 1960; Sabatés et al., 2009).

However, a gap of knowledge exist on understanding how different conditions in term of temperature and food availability can affect growth and survival of the early life stage, in this species, as well as in others teleosts. With this goal in mind, otolith microstructure constitutes a powerful tool to determine age and growth of fish larvae and juveniles (Thorrold et al., 1997; Rooker et al., 1999; Power et al. 2000). During those stages, otoliths develop at a daily rhythm (Pannella, 1971) through deposition of a structure of annuli or increments with a translucent band (incremental zone) and an opaque band (discontinuous zone). The finding triggered the emergence of a large number of methods based on otoliths microstructure applied worldwide (Campana and Neilson, 1985; Jones, 1986; Campana and Moksness, 1991; Campana, 1992, 2001; Stevenson and Campana, 1992).

Microstructural growth patterns in otoliths of teleost fish and age validation studies of larval and juvenile otoliths have shown that microstructural characteristics are species-specific and may be influenced by nutrition and environmental variables (Campana and Neilson, 1985; Jones, 1986; Maillet and Checkley, 1989; Bradford and Geen, 1992). Recent reports on the relationship between daily somatic growth and

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incremental growth of otoliths support the use of increment widths as a measure of daily somatic growth (Secor and Dean, 1989; Hovenkamp, 1989, 1990; Moksness and Fossum, 1991). These microstructural studies of fish otoliths may provide a record of environmental and physiological condition through the larval and juvenile stages and hence important information about processes regulating recruitment in fish (Pannella, 1980; Rice et al., 1987; Moksness, 1992; Fossum and Moksness, 1993).

Using information deduced by otoliths microstructure analysis, the aim of our work was to analyze larval growth performance of early life stage of *Sardinella aurita* in two zone of the central Mediterranean Sea characterized by different conditions in terms of temperature and food availability.

#### **Materials and Methods**

##### *Ichthyoplanktonic Surveys*

Ichthyoplanktonic data were collected in two oceanographic cruises carried out on board the R/V Urania in the Strait of Sicily and central-eastern Tyrrhenian Sea in summer 2013. Surveys were conducted consecutively, from June 26<sup>th</sup> to July 16<sup>th</sup> in the Strait of Sicily and from July 17<sup>th</sup> to July 25<sup>th</sup> July in central-eastern Tyrrhenian Sea, in correspondence to the spawning period of *Sardinella aurita* in the Mediterranean Sea (Ben-Tuvia A. 1960; Whitehead et al. 1985; Palomera and Sabatés 1990).

During the two cruises, 234 and 76 station were sampled respectively following a systematic sampling on a regular grid of stations ( $1/10^\circ \times 1/10^\circ$  in both surveys along the continental shelf and  $1/5^\circ \times 1/5^\circ$  further offshore only in Strait of Sicily). Planktonic sampling was conducted by using vertical CalVET (one mouth of 25 cm inlet diameter, 150  $\mu\text{m}$  mesh) and oblique Bongo 40 net (two mouth of 40 cm inlet diameter, 200  $\mu\text{m}$  mesh, towed at 2 knots). The nets were hauled from within 5 m from the bottom to the surface, or from 100 m to the surface in deep stations. In each net mouth, calibrated flow-meters were mounted in order to calculate the volume of filtered water ( $\text{m}^3$ ). To preserve planktonic samples, borax-buffered solution of 4% formaldehyde and seawater (for CalVET and mouth 1-Bongo 40 samples) and solution of 70% ethanol (for mouth 2-Bongo 40 samples) were used. To identify eggs and larvae of *Sardinella aurita*, all samples were observed under a microscope in a land-based laboratory and fish eggs

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and larvae were sorted from the rest of the plankton following Whitehead et al. 1985 and Ditty et al. 1994.

Standard length (SL) of individual larvae was measured to the nearest 0.1 mm using a Zeiss Discovery.V20 stereomicroscope with a PlanApo S 2.3x camera, and Image Pro Plus 6.0 image analysis software (Media Cybernetics).

#### *Mean daily growth analysis*

Otoliths were used to obtain estimates of ages and larval growth. Daily deposition of increments in *Sardinella aurita* has been validated in Balza et al. (2007). Larvae were selected from two study areas in order to obtain a representative number of observation for each zone. In each zone, larvae were selected randomly from each 1 mm standard length (SL) size bin to ensure that all sizes were represented. Both *sagittae* were extracted using insect pins and fixed on a slide using a mounting medium for microscopy. Otoliths were read at 1000 X magnification (oil immersion) using a ZEISS Axio Lab A1. Daily growth increments in each *sagitta* were counted, starting from the spawning mark, as described for larval stage of *Sardinella aurita* by Balza & Marín (2000). Each otolith was read blind (i.e. with no size or collection location information available) by a single reader, following the same approach described in Sponaugle et al. (2009). When all otoliths were completed, the process was repeated. If the difference in age between the 2 readings was <5%, one was chosen randomly as the final read. If there was a discrepancy in age of  $\geq 5\%$  difference between the 2 readings, the otolith was read a third time. If after a third read a high discrepancy in the readings remained, the otolith was discarded.

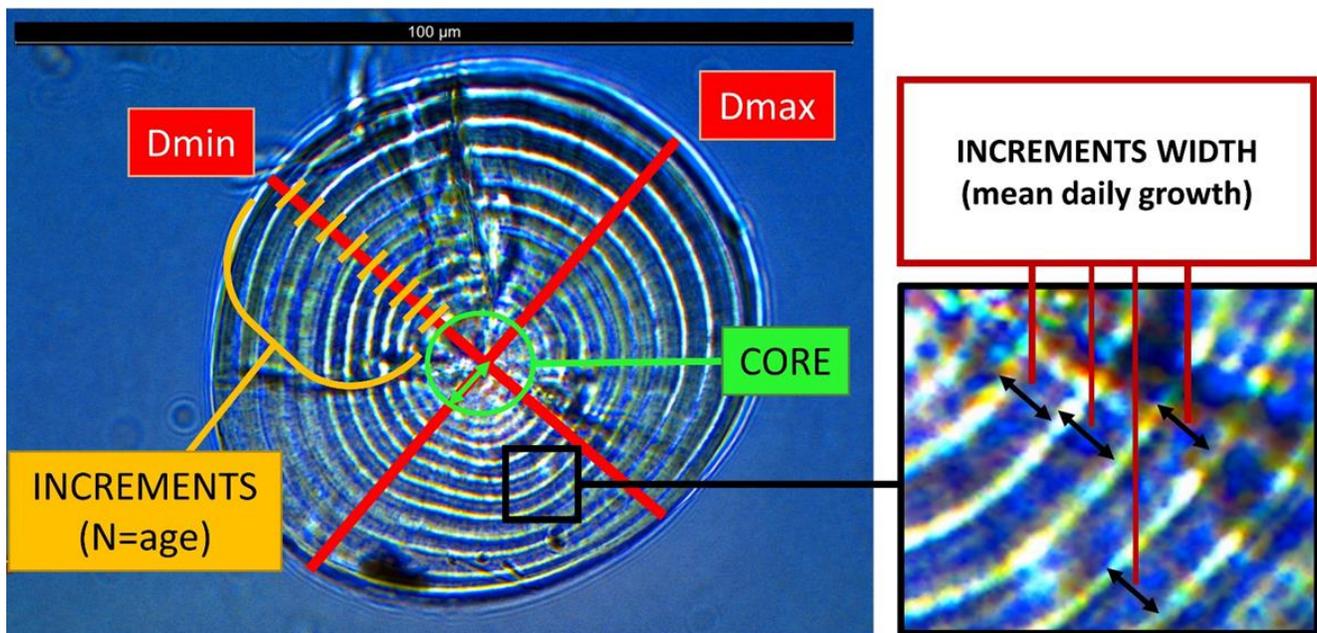
Width of the increments, minimum and maximum diameter ( $D_{\text{MIN}}$  and  $D_{\text{MAX}}$ ) in each otolith were also recorded from image analysis. For each increment, width were measured along four directions, following  $D_{\text{MIN}}$  and  $D_{\text{MAX}}$ , from the core to the edge and a mean value among them was considered (Fig. 1).

In addition, in order to assess the effect of environmental parameters on the eggs stage, we took into account the core width, measured from the primordium to the first D-zone (Balza & Marín 2000).

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$D_{\text{MIN-at-SL}}$ ,  $D_{\text{MIN-at-age}}$ ,  $D_{\text{MAX-at-SL}}$  and  $D_{\text{MAX-at-age}}$  relationships were estimated using linear regression models. Both otoliths per larva were considered. Moreover, a linear regression between SL and age were implemented in order to estimate growth rates. In all linear regression, relationships were compared among two study areas and ANCOVA were performed in order to test differences in slope and intercept estimates.

For each larva, individual hatch dates were back-calculated as sampling-date minus age and each increment were related to days between hatch and catch days.



**Figure 1** – Parameters extracted from the otoliths analysis for this study. In red, maximum and minimum diameters ( $D_{\text{max}}$  and  $D_{\text{min}}$ ) are evidenced. In yellow are showed the number of the increments used for the estimate of the age. Width of the increments, used as a proxy of the mean daily growth, are showed in the window on the left.

### *Back-Trajectories Calculation*

Backwards lagrangian simulations approach were adopted in order to estimate the trajectory of each larva and identify the daily position of larvae from catch to the back-calculated hatch date.

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Dispersal trajectories of larvae were simulated using the General NOAA Oil Modelling Environment (GNOME), a software package initially designed as an oil spill trajectory model by the NOAA Hazardous Materials Response Division (NOAA, 2002), as already carried out by (Bonanno et al. 2013, Patti et al., under review) in their paper on the distribution of anchovy early stages in the central Mediterranean Sea.

In GNOME, the movement of Lagrangian elements (particles) is simulated within a geospatially mapped environment, offering different opportunities of controlling input data (Beegle-Krause & O'Connor, 2005), from weather conditions to the surface currents, thus, allowing the authors to describe the transport of passive particles (eggs and developing larvae) released at different sites (Engie & Klinger 2007). In this study, the daily surface current fields during the survey periods, as evaluated by means of the altimeter products (Absolute Dynamic Topography) produced by Ssalto/Duacs and distributed by Aviso, with support from Cnes (<http://www.aviso.oceanobs.com/duacs/>) and wind patterns were jointly used and considered as "movers" of the anchovy eggs/larvae, without introducing any diffusion term.

The influence of wind on surface circulation patterns was evaluated using a value-added 6-hourly gridded analysis of ocean surface winds (NASA/GSFC/NOAA, 2009) estimated at the grid points with reference coordinates (14 °E; 36.5 °N) in Strait of Sicily and (14 °E; 40.25 °N) in central-eastern Tyrrhenian Sea. Specifically, wind speed and directions were calculated from zonal and meridional surface wind information included in the “Blended Sea Winds” dataset provided by the National Climatic Data Center, NOAA ([www.ncdc.noaa.gov](http://www.ncdc.noaa.gov)) (0.25° of latitude x 0.25° of longitude). Extracted wind time series were included as external movers within GNOME simulations.

In each simulation performed, wind effect on the shallow water layer was tuned taking into account the expected vertical distribution of sardinella larvae in the water column. Indeed wind is typically included in particle-tracking models assuming that the surface wind-induced current (windage effect) is about 3% of the wind speed (Pugh, 1987; Stolzenbach et al., 1977). This current decreases logarithmically to zero at approximately a depth generally assumed to be 20 m (Elliott 1986). Given that the bulk of the larval stages is likely to be found from the surface up to the depth of 20 (Sabatés et al. 2008), this reference depth layer was adopted in the simulations. The wind-induced current at depth  $x$  (in meters) can be estimated using the following equation (Pugh, 1987):

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$$u_x = u_0 - \frac{u^*}{k} \ln\left(\frac{x}{z_0}\right)$$

where  $u_0$  is the surface wind-induced current,  $k = 0.4$  is the von Karman constant,  $u^*$  is the friction velocity that can be estimated as  $0.0012 \cdot W$ , with  $W$  being the wind speed 10 m above the sea surface, and finally  $z_0$  is the sea surface roughness length, fixed at 0.001 m. Taking into account the above formulation, in GNOME simulations for the second scenario the windage effect, i.e. the movement of particles induced by the wind, was set in the range 0.93-0.23%, values corresponding respectively to the wind-induced current at the depths of 1 m and 10 m in terms of fractions of wind speed.

Backwards simulation were performed for each larva subjected to the otolith microstructure analyses and temporal and spatial position were estimated for each measured increment.

#### *Environmental Data*

During two oceanographic cruises, in all planktonic stations, continuous vertical profiles of temperature, pressure and fluorescence were obtained from the surface to the bottom by means of a CTD SBE 911 plus probe. The probe was calibrated before and after the cruise at the NURC (NATO Undersea Research Centre) in La Spezia, Italy. In order to compare temperature and food availability in both regions, shallow temperature and chlorophyll-a concentration iso-surface were produced using Ocean Data View software and “diva-gridding” as interpolation method.

Moreover, remote sensing data were considered in order to obtain a characterization of temperature and food availability conditions during the larval growth period. Therefore, mean daily spatial gap-free data (L4) of Sea Surface Temperature (SST) and Chlorophyll-a concentration (CHL) (0.0625deg. x 0.0625deg of horizontal resolution in both dataset) were downloaded from the remote sensing database available on [marine.copernicus.eu](http://marine.copernicus.eu). Days was selected in order to cover time period from the earlier hatch date deduced from larval age estimation (June 29<sup>th</sup>) to the date of the last collected larva (July 22<sup>th</sup>).

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Estimation of larval position in each day from hatch to catch allowed to attribute a values of SST and CHL for each otolith increment and a multivariate statistical approach were used in order to investigate on the relationship between increment width and environmental parameters.

#### *Multivariate Statistical Modelling*

With the aim to estimate the effect of temperature (SST) and food availability (SST) on measured increment width, we used a multivariate modelling approach.

However a positive relationship between increment width and larval age was detected ( $F=5.81$ ,  $p\text{-value}<0.01$ ). Before to apply models a transformation of data was applied following Baumann et al. (2003) and Robert et al. (2009) in order to remove age effect. A detrended growth index was computed using:

$$DIW_{ij} = (IW_{ij} - IW_j) SD_j^{-1}$$

Where DIW is the detrended increment width of individual  $i$  at age  $j$ ,  $IW_{ij}$  is the otolith growth (increment width) for individual  $i$  at age  $j$ ,  $IW_j$  is the mean of otolith growth (increment width) of all individuals at age  $j$ , and  $SD$  is the standard deviation of  $IW$  at age  $j$  (Robert et al. 2009).

We tested normality distribution of the response variable (DIW) using Shapiro-Wilk normality test. Because the response variable was normally distributed ( $p\text{-value} > 0.05$ ), a Gaussian distribution was considered in all model and an “identity” link between the mean of the response variable and the systematic part of the model.

In order to address the possible issue of multicollinearity prior to model selection (Neter et al. 1996), we calculated a Pearson product-moment correlation coefficient between covariates SST and CHL. A value of 0.26 (I.C. 95%: 0.18 - 0.34) was obtained and therefore we considered models with additive and interactive effect of WI-related covariates.

Because of different relationship between response and explanatory variables was conceivable, nominal variable of “region” (REG, 2 levels: Strait of Sicily and Tyrrhenian Sea) and “zone” (ZON, nested in

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region, 3 levels: Gulf of Naples and Gulf of Gaeta in Tyrrhenian Sea; Malta, Capo Passero and Lampedusa in Strait of Sicily) was alternatively included as interaction terms with continuous covariates and different models were compared.

When residuals showed clear pattern that identify a violation of homogeneity, the heterogeneity of the variance structure was allowed for each levels of REG or ZON nominal variables and generalized least squares (GLS) methods were also used in order to fit models.

Moreover we reflected on a possible intraclass correlation (Snijders & Bosker 2012) due to the nested nature of the dataset. Indeed, MWI observed in the same area (REG or ZON) are likely more related to each other than to the MWI measured from individuals caught in different areas. In the same way, MWI values collected from the same otolith could be more related comparing them with other MWI measured in otoliths extracted from other individuals. This could be an important source of specific within-fish variation due to specific growth of the otolith due to genetic or other factor

Therefore, Mixed Models with nominal variable “individual” (IND, identifying larva subjected to the otolith extraction: 114 levels) as a random effect (as used by Weisberg et al. 2010 for modelling fish somatic growth) were also considered.

Finally, the hypothesis of non-linear relationship between DIW and continuous covariates were tested applying natural cubic spline smoothers to SST and CHL and generalized additive (and mixed) models (GAM and GAMM) (Wood 2006) were implemented and compared. Because of the nested nature of the data, in this cases regression parameter were estimated using restricted maximum likelihood estimation method (REML) and not significant terms were dropped.

Akaike’s Information Criterion, AIC (Akaike 1973) were used to compare models and determine which model(s) served as the best approximation(s) to the data. However when examining GAMMs, is not possible to compare them to linear models or GAMs using AIC because of its reliance on maximization of full likelihoods (Wood 2006). In this case we evaluated the statistical utility of GAMM in terms of effectiveness in accounting for natural heterogeneity of data, in terms of significance of estimated parameters and in terms of non-linearity of estimated smoothers.

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For the validation model, normal Q-Q plot of the residuals, plot of residuals vs. fitted values and plot of residuals vs. variables considered in the model were used to assess normality distribution of the residuals and the assumptions of homogeneity and independence of the variables (Zuur et al. 2009).

All analysis were performed with R software version 3.3.2. (R Core Team, 2016). All statistical models were implemented using “mgcv” R package (Wood 2006).

## Results

### *Spatial distribution patterns of eggs and larvae*

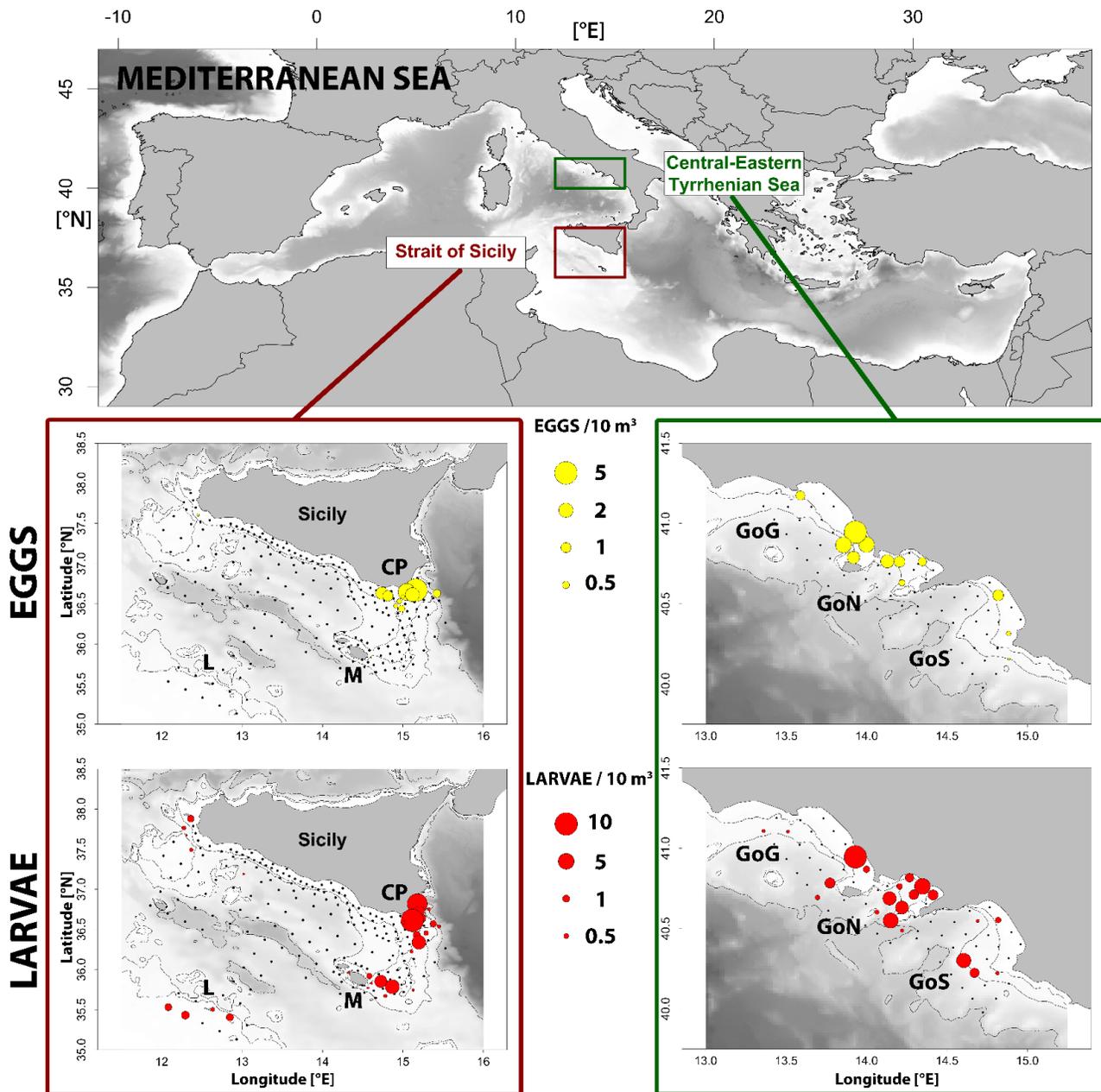
Spatially, most of eggs and larvae of *Sardinella aurita* were found between coastline and the isobath of 200 meters (Fig 2).

In the Strait of Sicily, higher abundance of ichthyoplanktonic stages were detected in the south easternmost of the study area. Eggs and larvae were found mainly in coastal waters off Capo Passero. In addition, few observations were detected close to Malta and off Mazara del Vallo, in the north-westernmost area. No eggs and low density of larvae were found around Lampedusa Island.

In central Tyrrhenian Sea, higher abundance values of eggs density were found in the Gulf of Gaeta, while larvae were more abundant in the Gulf of Naples. Lower values of larvae, were found in the Gulf of Gaeta and in the Gulf of Salerno respectively.

Summarizing, three zones per cruise were identified as the main spawning areas of *Sardinella aurita*: Capo Passero, Malta and Lampedusa in the Strait of Sicily; Gulf of Naples, Gulf of Salerno and Gulf of Gaeta in the central Tyrrhenian Sea.

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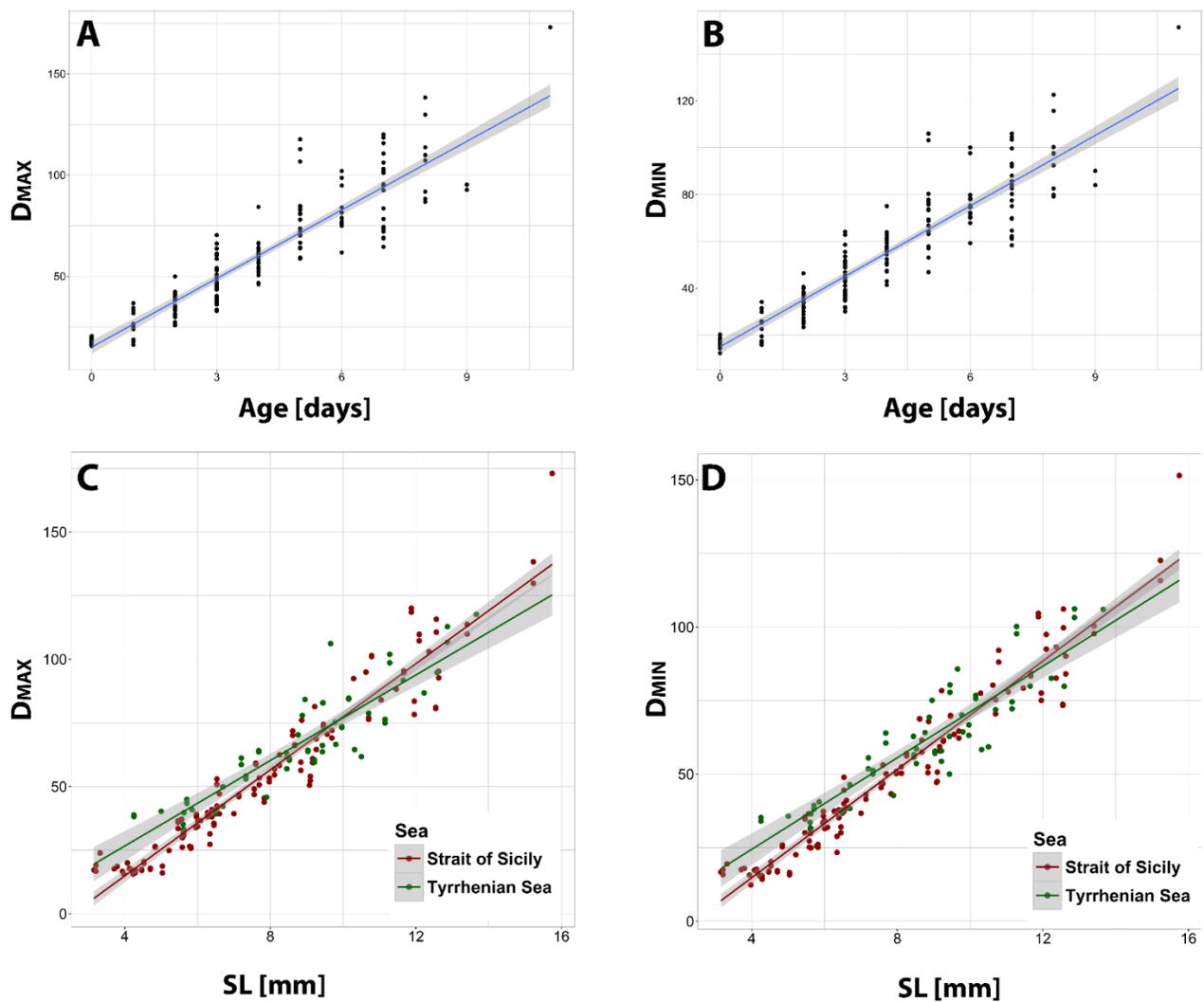


**Figure 2** – Spatial distribution of eggs and larvae of *S. aurita* in the Strati of Sicliy (left) and central-Eastern Tyrrhenian Sea (right). In Strait of Sicily: CP = Capo Passero; M = Malta; L = Lampedusa. In central-Eastern Tyrrhenian Sea: GoG = Gulf of Gaeta; GoN = Gulf of Naples; GoS = Gulf of Salerno.

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### *Otoliths microstructures and analysis of the mean daily growth*

Significant linear regression of age vs. otolith maximum diameter ( $D_{\max}$ ) and of age vs. minimum diameter ( $D_{\min}$ ) were found in the whole dataset, while not significant heterogeneity of the slope were found among regions (ANCOVA:  $p > 0.05$ ) (Tab. 1, Fig. 3 A and B). A significant relationship was found between SL and both diameters ( $D_{\max}$  and  $D_{\min}$ ) in both regions (ANCOVA:  $p < 0.01$  in both test) (Tab 1; Fig. 3 C and D).

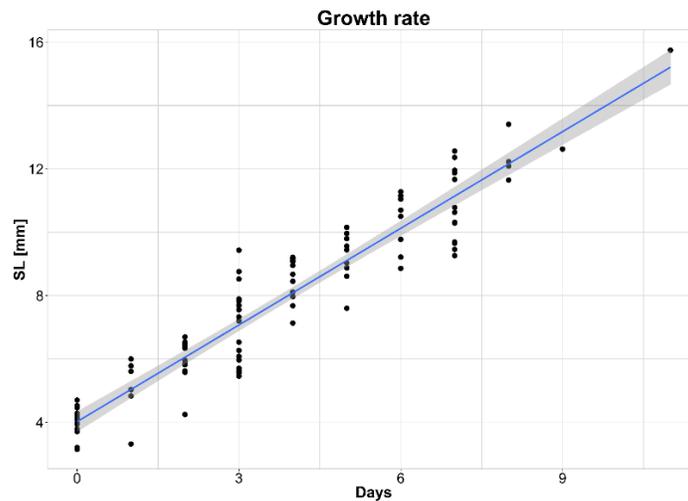


**Figure 3** – linear regression models of Diameters ( $D_{\max}$  and  $D_{\min}$ ) -at-age (A and B) and –at-SL (C and D). Where ANCOVA detected statistical difference between regions, both models per regions were showed.

## *Sardinella aurita*: larval growth performance

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A significant growth rate were obtained from linear regressions of SL-at-age (Fig. 4, Tab. 1). The obtained instantaneous growth parameters was 1.00 mm/day. Regression parameters did not vary among two regions (ANCOVA: p-values > 0.05).



**Figure 4** - liner regression model of larval growth.

Significant linear regression of SL vs. otolith maximum diameter ( $D_{\max}$ ) and of SL vs. minimum diameter ( $D_{\min}$ ), as well as significant regression of otolith  $D_{\max}$  and  $D_{\min}$  radius-at-age residuals vs. SL-at-age residuals ( $R^2= 0.53$ ,  $p<0.01$ ) confirmed that otolith increment data can be used to examine somatic growth in this species at larval stage.

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**Table 1** – linear regression estimates. N is the number of observation. In Formula, Dmax= maximum otolith diameter; Dmin=minimum otolith diameter; SL=Standard length; Age = days estiated from the number of otolith increment. In region, SC=Strait of Sicily; TS=Tyrrhenian Sea. When ANCOVA detected significant different in slope and intercept, both estimation are reported, otherwise, model were performed on the whole dataset (SC+TS).

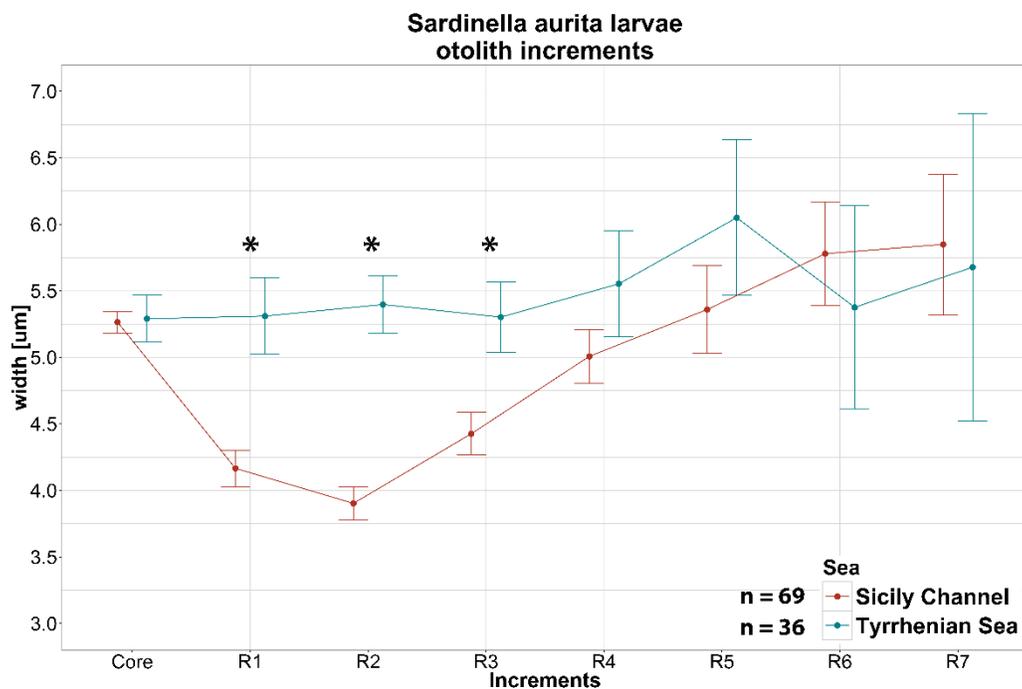
Formula	Regio n	N	SL range	Age range	Intercept	Slope ± SE	r <sup>2</sup>
Dmax ~ Age	SC + TS	183	3.15 – 15,75	0 - 11	15.01 ± 1.66	11.29 ± 0.37	0.84
Dmin ~ Age	SC + TS	183			15.03 ± 1.50	10.03 ± 0.33	0.84
Dmax ~ SL	SC	127	3.15 – 15.75	0 – 11	-26.77 ± 2.12	10.42 ± 0.26	0.93
	TS	56	4.25 – 13.66	2 – 8	-6.96 ± 5.02	8,40 ± 0.55	0.81
Dmax ~ SL	SC	127	3.15 – 15.75	0 – 11	-22.00 ± 1.78	9.20 ± 0.21	0.94
	TS	56	4.25 – 13.66	2 – 8	-6.63 ± 4.61	7.78 ± 0,51	0.81
SL ~ Age	SC + TS	99	3.15 – 15.75	0 - 11	3.95 ± 0.18	1.01 ± .03	0.91

Core width (CW) and the mean daily growth (MIW) during the first 8 days of larval life in the two investigated regions are shown in Figure 5. No statistical difference of CW were detected between two

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regions. On the contrary, CW showed the lower standard error compared with the others increment. Obtained mean values of CW in this study was  $5.27 \pm 0.82$  (mean  $\pm$  SD).

Mann–Whitney U test applied to test difference between region in terms of MIW values, showed differences for the firsts three increments (U: p-value < 0.01) while no significant differences emerged for other increments.



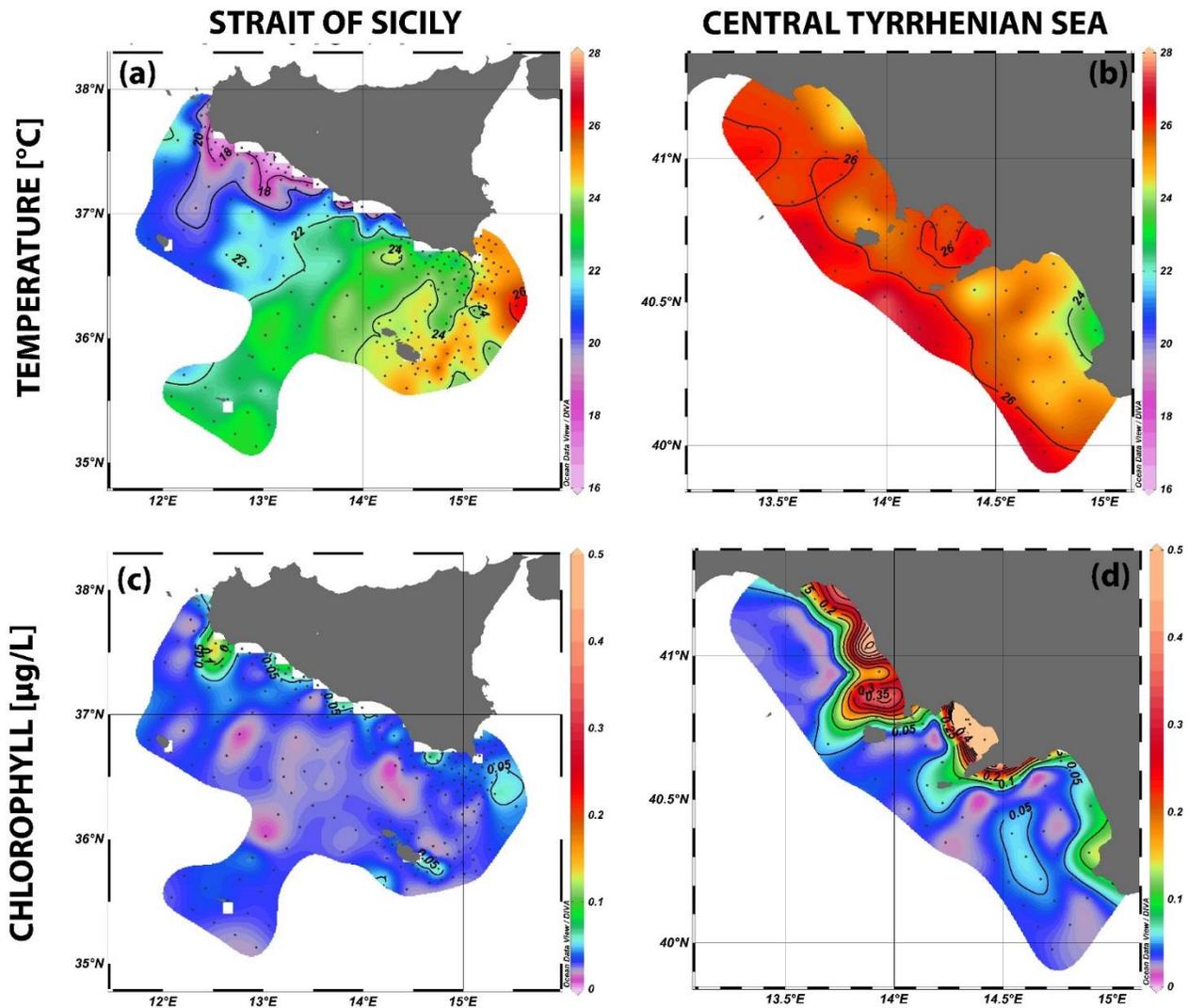
**Figure 5** – Core width and daily growth (mean  $\pm$ SE) of otolith increment width [MIW]) for *Sardinella aurita* larvae collected in Sicily Channel and Tyrrhenian Sea. Number of samples per region (n) and significance of Mann–Whitney U test (\*= p<0.01) are also showed.

### *CTD and Remote sensing data*

Spatial patterns of shallow temperature and chlorophyll-a concentration recorded by CTD in the two cruises are shown in the Figure 6. Lower mean values of temperature and chlorophyll-a concentration

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were recorded in the Strait of Sicily (Fig. 6 A and B) compared with the Tyrrhenian Sea ( $T = 22.37 \pm 2.41$ ;  $CHL = 0.037 \pm 0.019$  and  $T=25.66 \pm 0.74$ ;  $CHL=0.148 \pm 0.292$ , respectively) (Fig. 6 C and D).



**Figure 6** – Shallow iso-surfaces of temperature (upper panels) and chlorophyll-a concentration (downer panels) in Strait of Sicily (left panels) and central Tyrrhenian Sea

Specifically, physical-chemical patterns of the shallow water in Strait of Sicily are affected by the presence of a coastal upwelling in front of Mazara del Vallo, in the northwestern part of the study area

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(Agostini and Bakun, 2002; Cuttitta et al. 2016). Therefore, colder and nutrient-enriched water arise from the deep, affecting the coastal northwestern sector of the study area, where the coldest water and the highest concentration of chlorophyll-a were recorded (Fig. 6 A and C). An increasing of temperature was detected south-eastwards. Moreover, spatial distribution of temperature in front of Capo Passero suggest the presence of a cold filament (Bignami et al. 2008; Torri et al. 2015) triggered from coastal waters off Capo Passero and extend towards Malta Island. Affecting temperature and trophic conditions (Torri et al. 2015), this oceanic structures could be play a key role affecting growth performance of fish larvae in the study area.

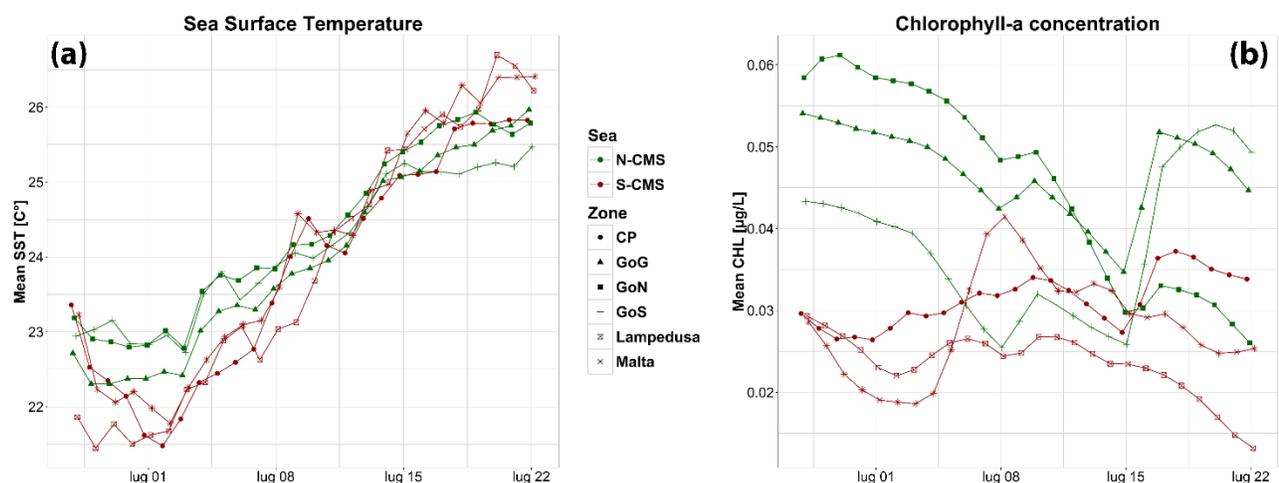
In central-eastern Tyrrhenian Sea, not evident shallow temperature patterns among three Gulfs were found (Fig. 6 B). Conversely, very high difference in chlorophyll-a concentrations were detected between inshore and offshore areas. In particular, very higher trophic condition (i.e. chlorophyll-a concentration) were detected in the coastal shallower water of the Gulf of Naples and Gulf of Gaeta (Fig. 6 D).

By remote sensing data, mean daily SST and Chlorophyll-a concentration from June 29<sup>th</sup> to July 22<sup>th</sup> in the areas characterized by the presence of planktonic stages of *Sardinella aurita* are shown in Figure 7.

These time series allowed to detect different parameters fluctuations among zones. In spite of the detection of different mean values of Temperature and chlorophyll-a concentration by CTD data, daily data allow to highlight different trends in the selected period and important fluctuations emerged in the selected period, corresponding to the time which founded larvae of *Sardinella aurita* lived.

Temperature ranged between 21 – 27 °C in the Strait of Sicily and between 22-26 °C in the Tyrrhenian Sea (Fig. 7 A) Is possible to note that difference between Tyrrhenian Sea and Strait of Sicily begin to be noticeable around June 31<sup>th</sup> until July 11<sup>th</sup>. Thereafter, no relevant mean values of temperature between two study areas are detectable where planktonic stage of *Sardinella aurita* were found.

Chlorophyll-a concentration ranged between about 0.015 – 0.04 µg/L in the Strait of Sicily's zones while higher values (0.025 - 0.06) were detected in zones within the central Tyrrhenian Sea (Fig. 7 B).

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**Figure 7** – Mean daily SST (left) and CHL (right) value in identified spawning zone for *S. aurita*. Time period from June 29<sup>th</sup> to July 22<sup>th</sup> were selected following temporal range detected from otoliths analysis.

*Multivariate statistical models*

Model selection process allowed to identify the following GAMM as the best model:

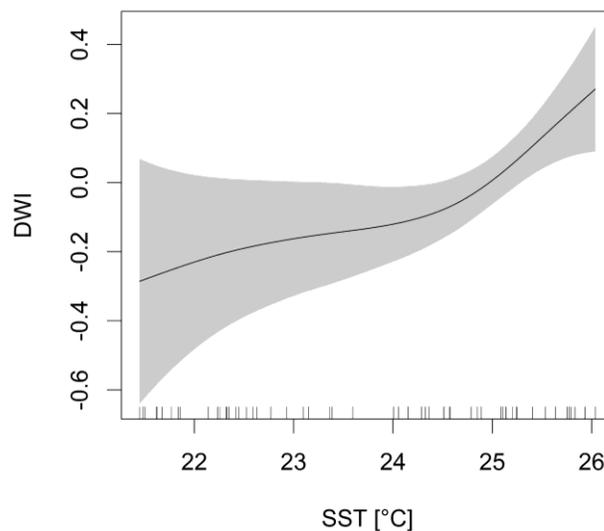
$$DIW_i = s(SST_i) + \text{random}(IND_i) + \text{residuals}_i ;$$

where DIW is the detrended increment width,  $s(SST)$  is a natural cubic spline smoother of the SST and random (IND) is the intercept random effect of the variable IND. Estimates of the regression parameter were showed in the Table 2 . Not a significant contribution emerged considering continuous variables CHL, indicating that food availability did not affect the daily mean growth of larvae collected in the study area. On the other side, temperature was identified as the most important variables. Estimated smoother highlighted a non-linear positive relationship between DWI and SST (Fig. 8). In particular, an increase in the slope were detected in relation to temperature values higher than about 24.5 °C. Spatial nominal variables REG and ZON did not added a significant contribution to selected model.

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**Table 2** – Estimates of GAAM model implemented:  $DIW_i = s(SST_i) + \text{random}(IND_i) + \text{residuals}_i$ . DIW is the detrended increment width,  $s(SST)$  is a natural cubic spline smoother of the SST and random (IND) is the intercept random effect of the variable IND.

Mixed-effects model fit by REML				
Family = Gaussian				
Link function = identity				
<b>Fixed effect:</b>				
	Estimate	Std. Error	t value	p-value (> t )
<b>Intercept</b>	- 0.06966	0.06123	- 1.138	0.256
	edf	F	p-value	
<b>S(SST)</b>	2.072	1.166	0.00219	**
<b>Random effect:</b>				
	intercept	residual		
<b>Standard</b>	0.5516995	0.6739751		
<b>Deviation:</b>				
<b>N = 549</b>				



**Figure 8** – Natural cubic spline smoother estimated by GAMM model and describing relationship between DWI and SST.

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#### **Discussion and conclusions**

Our findings concerning spatial patterns in 2013 in the Strait of Sicily confirmed results highlighted in Torri et. al. (2015) regarding the identification of the main spawning areas in the northern sector of the Strait of Sicily in the years 2010-2011. In according to these authors, waters off Capo Passero and around Maltese archipelago are identified as the main spawning area of this species, while only few observation were recorded in the North-Western part of the study area, on the adventure Bank. In addition, the present study showed the presence of planktonic stage on the continental shelf close to Lampedusa, in agreement with Zarrad et al. (2013) that obtained similar results for a nearby zone, in the Gulf of Hammamet.

Moreover, this is the first scientific effort aimed to analyze spatial patterns of eggs and larvae of *Sardinella aurita* in the central-eastern Mediterranean Sea. In 2013, higher abundance of both eggs and larvae were found in the Gulf of Naples and in the South-Eastern part of the Gulf of Gaeta. However, positive station found in the Gulf of Salerno and in the northern Gulf of Gaeta highlighted presence of this species in these areas.

Spatial correlation between planktonic stage and warmer and chl-enriched waters was detected in both regions. Specifically, in the Strait of Sicily the presence of an upwelling system in the North-Western area, leading colder and nutrient- enriched waters from the bottom to the shallower layers, could play a key role affecting adults fish distribution due their thermophilic features. Previous studies conducted in the northwestern Mediterranean indicated that the spawning of this species took place at temperatures higher than 23°C (Navarro, 1932, Andreu & Rodriguez-Roda 1951, Palomera and Sabatés 1990) because gonads maturation in this species are temperature dependent. In the zone corresponding to the upwelling system, coastal temperature values were lower, ranging between 16 and 22°C. Therefore, despite of the higher food availability characterizing this area, lower temperature values could be crucial, forcing adults to shift reproduction in warmer zone, like the South-Eastern part of the study area.

Different is the case of the central-Eastern Mediterranean Sea, where we found a very high temperature and food availability conditions compared with the Southern site. Here, higher density were spatially

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correlated with enhanced food conditions, or rather corresponding with Gulf of Naples and Southern Gulf of Gaeta. In these zone, temperature was wherever higher than 23°C and food availability could be the most important driver, affecting spatial distribution of adults, which, being zooplankton feeders, are strictly linked to areas characterized by high primary productivity (Ben Tuvia, 1960; Sabatés et al. 2009). In agreement to this scenario, lower density of eggs and larvae detected in the Gulf of Salerno were corresponded worse trophic conditions in spite of temperature higher than 23°C.

The assessment of larval growth rate allow us to understand more about environmental factor affecting larval development in these study areas. In spite of different conditions arose in terms of temperature and food availability, no statistically different growth rates were emerged by the otolith analysis between regions, although larvae collected in the central-eastern Tyrrhenian Sea showed faster growth rates. However, analysis of the mean daily growth evidenced a different growth rate limited to the first three days of the larval life, with lower values in larvae collected in the Strait of Sicily. Considering that larvae of *Sardinella aurita* start actively feeding by day 3 (Ditty et al. 1994), detected differences could be the results of differences in temperature conditions instead of food availability.

We found a good agreement with this hypothesis calculating back-trajectories of individual larvae and relating temperature and food availability daily conditions to the mean daily growth. Using a modeling approach, not a significant contribution of food availability was estimated. Conversely, model's outcome highlighted a non-linear effect of temperature, with a stronger effect when temperature are higher than 24.5 °C.

These findings provide useful insights about how modifications of temperature of water surrounding eggs and larvae of this species can affect growth and hence the natural mortality rates of the early life stages of this species.

Because difference in the mean daily growth between larvae collected in the two regions arise in the first 3 days of larval life, it is reasonably consider that different size at yolk-sac-absorption occurred between Strait of Sicily (shorter) and central-eastern Tyrrhenian Sea (longer). We found that this a direct consequence of different temperature conditions and this is in agreement with its life history traits. The rate and efficiency of yolk-sac reabsorption and the allocation of yolk for embryo development and

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metabolic energy is one of the most critical processes during early development. In our case, the effect of temperature affects larval size during yolk-sac absorption because it could play a dominant role in the efficiency of yolk conversion into body tissues, as described in Jaroszewska & Dabrowski (2011).

The increased length at yolk-sac absorption means larger mouth sizes, which might result in enhanced capture abilities, increased resistance to starvation (Shirota 1970, Hunter 1981, Fukuhara 1990). Moreover, greater larval size during this transitional period may increase the larva's ability to avoid predation (Blaxter and Hempel, 1963; Braum, 1967; Blaxter, 1969). It follows larger size at this stage could be reasonably translated in enhanced chances of survival.

This could be the case of the Strait of Sicily, where lower mean daily growth and lower temperature were found. Torri et al (2015), found high inter-annual variability of temperature in the spawning period of *Sardinella aurita* related to the presence of strong events of Mistral winds that are able to control the extension of the North-West coastal upwelling system upwelling and promote formation of cold filament in the spawning and recruitment zone of Capo Passero. This is the case of year 2010, while in 2011 no cold filaments were detected due to the absence of strong Mistral winds events. Shallow hydrodynamics conditions detected in this study reflect very similar conditions detected in 2010 by these authors. As this year, a cold filament in the zone between Capo Passero and Malta, extended north-west upwelling and lower temperature were detected.

Considering what has been highlighted in this study, formation of cold filaments in correspondence of the spawning period of this species in the Strait of Sicily could play a key role for the larval survival, affecting the natural mortality rates and recruitment of the new generation in the adult fish population (Cury & Roy, 1989; Bernal, 1991; Hunter and Alheit, 1995; Bakun, 1996; Patti et al. 2004). Therefore this study highlights the role of physical forcings and temperature-induced and suggests the crucial role of the surface hydrodynamic conditions in affecting the larval growth rate of this thermophilic species. These evidences were supported also by the higher mean daily growth rate detected in central Tyrrhenian Sea in the following two days, when larvae start to feed actively. The weak contributions of the food availability compared with temperature conditions suggest an important role of the metabolic rate of the individuals during these stages. Larval daily ration needs increase with increasing temperature because

### *Sardinella aurita: larval growth performance*

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of the positive relationship between temperature and metabolism but also because larvae need to spend more energy swimming to be able to capture more prey (Clarke and Jonhston 1999). Therefore, in lower temperature conditions, larval development could be affected by a lower metabolic rate joined with lower needs to feed. In our case, enhanced trophic conditions occurring with lower temperature (i.e. upwelling conditions), could be play a secondary role compared with the reduced metabolic rate and feeding needs.

Finally, this study pointed out on the variability of the otolith core width in larvae collected from the two regions. In spite of the exposure of this larvae under different temperature conditions, a very low variability of this parameter were detected in all larvae examined for this study and no statistical difference emerged between areas. No information about the core size in relation to environmental parameters is present in literature. In other species, this parameter is not always associated with the larval size at hatching (e.g. Neilson et al. 1985). Therefore, similar otoliths core size could not be translated as similar size at hatch occurring in the two site and specific studies needs to be carry out aimed to evaluate other factors, such as maternal influence on the egg size and quality of the yolk (Hinckley, 1990; Buckley et al., 1991; Trippel et al., 1997).

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## CONCLUSIONS

This work allowed to clarify the role of physical forcings in affecting the spatial distribution of planktonic stage of marine fish species that spawn during summer in the central Mediterranean Sea.

The Strait of Sicily is an hotspot of biodiversity (Coll et al., 2010), where numerous species find suitable conditions for reproduction and feeding. Most of species produce planktonic stages that can be not only transported far to the spawning area but also concentrated in zone where thermohaline fronts and gyre act originating retention zones (Agostini and Bakun, 2002). In this study, variables and intense surface hydrodynamic conditions of the upper layers were detected among years of sampling. Atlantic Ionian Stream (AIS) is a semi-permanent oceanographic structure that govern the surface dynamics at mesoscale level and showed different pathways among investigated years. The strength of the upwelling occurring in the coastal-eastern part of the study area was highly variable in relation to the blowing of the Mistral winds. Several oceanographic structure were affected by the occurring of intense upwelling events in this zone: cyclonic and anti-cyclonic gyres, along-shore southeastward currents and temporary cold filaments occurring in the coastal-western part of the study areas. In this hydrodynamic framework, planktonic stages of marine fish were advected following different pathways linked to physical forcings that involved firsts layers of the water column, where most of eggs and larvae were located. Direct consequence of this advection is the larval advection in zones more or less suitable for their development as well as the co-occurrence of different larval species in spite of the adults spawning behavior in different environments (e.g. in different bottom depth layers). Therefore, meso and bathypelagic larve resulted abundant on the continental shelf (e.g. Adventure and Maltese Bank) in relation to the AIS flowing, while small pelagic fish resulted be common in the offshore zone due to the Ekman transport linked to the Mistral winds and the upwelling system. Moreover, the occurring of different hydrodynamic events affect environment conditions of the larval surrounding, especially in terms of temperature and food availability. It has been highlighted that persistent Mistral winds occurring in the summer period are linked with colder and nutrient-enriched water arising from deeper layers in the spawning zone. As consequence, these conditions were able to tune the spawning period of summer temperature-linked spawner fish. Moreover, the higher concentration of nutrients in the shallow waters stimulate primary

production, triggering the trophic net and making plankton available for fish larvae as well as for zooplanktonic feeders species (e.g. small pelagic fish). It follows that physical forcing occurring in this area can play a key role for the spawning and larval development, affecting not only spatio-temporal dynamics but also environmental conditions occurring in one of the most critical stage of the life history traits of marine fish.

Differently, southern and central Tyrrhenian Sea showed lower hydrodynamic features compared with the Strait of Sicily. Only a permanent anticyclonic gyre able to advect mesopelagic larvae toward coastal zone were detected in the Southern Tyrrhenian Sea, while no mesoscale oceanographic structure emerged in the central Tyrrhenian Sea. In agreement with this scenario, it has been showed that local environmental conditions not linked to hydrodynamic conditions play the major role affecting spatio-temporal distributions and survivals of the early life stages in these zones.

In areas where permanent or semi-permanents oceanographic mesoscale structures govern the environmental conditions and affect essential parameters for the development of early life stage, the evaluation of the role of physical forcings occurring in the surface waters can be hence crucial in order to clarify mechanisms underlying the recruitment process and fish biomass fluctuations as result of stochastic processes (Hinrichsen et al., 2001). Biological and physical processes act to create windows of episodic survival (Houde, 1989), which may be determined by better growth (Campana, 1996; Meehan and Fortier, 1996), reduced predation (Houde, 1989; Paradis et al., 1996), and enhanced transport (Campana et al., 1989). Oceanographic studies addressed to the determine physical and environmental process involved during early life stage represent the first step and further effort needs for the evaluation of the effect in terms of growth, mortality rate, recruitment and adult fish biomass.

In this framework, an interesting case study considered in this work was the evaluations of the physical forcings on spatial distribution of the early life stage of *Sardinella aurita* and of the growth performance related to environmental induced modifications. It has been showed that Mistral winds blowing intensely during the spawning period of this species can generate cold filaments able to advect larvae and connect spawning area as well as move them far away from the spawning zone. Such filaments lead colder and nutrient enriched water in the upper layers, modifying larval environmental surrounding. Therefore, a

further study was carried out in order to assess larval growth performance of *Sardinella aurita* in different environmental conditions, considering the distribution of this species along a latitudinal gradient. Faster growers were detected in the northernmost site in correspondence to warmer and food-enriched site compared with other sites. Moreover, multivariate models identified the key role of temperature in affecting the larval growth rate compared with the food availability. This work opens doors to new research questions concerning the role of cold filaments detected in the Strait of Sicily and underline the necessity to take into account hydrodynamics conditions occurring during the larval stages into studies addressed to recruitment and stock assessment, especially in areas where physical forcings play a crucial role in the environment of the upper water layer. In addition, it has been highlighted the occurrence of suitable conditions spawning and larval development in one of the northern limit of the distribution of this species in Mediterranean Sea, suggesting that other factors can play a key role to limiting its spatial range, such as the water temperature in the colder periods of the year (Furnestin, 1952; Sabatés et al., 2006).

*Sardinella aurita* represent an interesting case on how physical forcings can affect early life history traits, with expected, but not explored, effects on mortality rates and recruitment of the new generations into the adult population. However, the same approach could be used in other study addressed to the understanding of the causes underlying the biomass fluctuations of important commercial fish, especially if concern short life span species like small pelagic fish. Evaluation of the geostrophic currents and the wind effect as well as Ekman transport calculations and Lagrangian transport techniques represent a set of useful tools able to give an answer to issues concerning larval growth and mortality rates and can be applied to a wide range of case study over the world taking advantage of availability of satellite-derived oceanographic data covering a wide spatio-temporal ranges.

Operational oceanographic tools coupled with long spatio-temporal data series collected by ichthyoplanktonic monitoring system potentially represent the ground whereon stock assessment and fisheries science studies can lie in order to understand mechanisms underlie the relationships between environmental factors and reproductive success of fish. Finally yet importantly, this multidisciplinary

approach provide fundamental insights in view to predictive models aimed to the estimation of biomass fish fluctuations and hence to the correct and sustainable use of the fishery resources.

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## Different key roles of mesoscale oceanographic structures and ocean bathymetry in shaping larval fish distribution pattern: A case study in Sicilian waters in summer 2009



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### ABSTRACT

Fish larvae data collected in year 2009 were used to examine the effects of particular environmental conditions on the structure of larval assemblages in two oligotrophic Mediterranean areas (the Southern Tyrrhenian Sea and the Strait of Sicily). For this purpose, relationships with environmental variables (temperature, salinity and fluorescence), zooplankton biomass, water circulation and bathymetry are discussed. Hydrodynamic conditions resulted very differently between two study areas. The Southern Tyrrhenian Sea was characterized by moderate shallow circulation compared to the Strait of Sicily. In this framework, distribution pattern of larval density in the Tyrrhenian Sea was mainly driven by bathymetry, due to spawning behavior of adult fish. There, results defined four assemblages: two coastal assemblages dominated by pelagic and demersal families and two oceanic assemblages dominated by mesopelagic species more abundant in western offshore and less abundant in eastern offshore. The assemblage variations in the western side was related to the presence of an anti-cyclonic gyre in the northern side of the Gulf of Palermo, while in the eastern side the effect of circulation was not very strong and the environmental conditions rather than the dispersal of species determined the larval fish communities structure. Otherwise in the Strait of Sicily the currents were the main factor governing the concentration and the assemblage structure. In fact, the distribution of larvae was largely consistent with the branch of the Atlantic Ionian Stream (AIS). Moreover, very complex oceanographic structures (two cyclonic circulations in the western part of the study area and one anti-cyclonic circulation in the eastern part) caused the formation of uncommon spatial distribution of larval fish assemblages, only partially linked to bathymetry of the study area. Typically coastal larvae (pelagic families: Engraulidae and Clupeidae) were mostly concentrated in the offshore areas and off Capo Passero, where the presence of a thermo-haline front maintained their position in an area with favourable conditions for larval fish feeding and growth.

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### 1. Introduction

The studies on ichthyoplankton distribution, mainly of commercially important species, play an important role in ecology and evolution of fish populations (Moser and Smith, 1993; Neilson and Perry, 1990), because the spatial distribution of early life stages can be the major determinant of recruitment success and consequently of the adult population sizes (Boehlert and Mundy, 1993; Govoni, 2005; Moser and Watson, 2006; Sinclair, 1988). The early life history of the fish populations

depends on several factors that affect mainly the spawning biomass of fish adults (Basilone et al., 2013; Giannoulaki et al., 2013; Somarakis et al., 2004) and the larval fish conditions (Riveiro et al., 2011), mainly due to feeding success (Pepin et al., 2014), to optimal habitat (Sabatés et al., 2006, 2007; Valavanis et al., 2008) and to predation (Litvak and Leggett, 1992; Steele and Forrester, 2002; Yin and Blaxter, 1987). Moreover, mesoscale oceanographic structures including geostrophic and wind influenced current regimes (wind-induced mixing of the surface layer, upwelling) play an important role in determining the patterns of abundance and distribution of larval fish populations. This is because they act as mechanisms of retention and concentration of fish larvae in recruiting areas creating oceanic conditions favorable for the growth and survival (Alemany et al., 2006; Falcini et al., 2015; Paris et al., 2007).

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In the past, the influence of these factors on larval distribution was widely analyzed in the Mediterranean Sea, a semi-enclosed basin of which environmental characteristics are sensitive to both basin scale and local effects (e.g. winds, topography) and fluctuations of fish populations are not exclusively linked to fishing activity, but rather significantly affected by ocean conditions (Falcini et al., 2015; Lloret et al., 2000). Extensive work has mainly covered the coastal waters of the north-western part of the Mediterranean Sea (Alemany et al., 2006; Alvarez et al., 2012; García and Palomera, 1996; Olivar et al., 2012; Olivar and Sabatés, 1997; Palomera and Olivar, 1996; Palomera and Sabatés, 1990; Sabatés, 1990, 2004; Sabatés and Olivar, 1996; Sabatés et al., 2007) and to a lesser extent the central (Cuttitta et al., 2003, 2004) and the eastern parts (Aegean Sea: Isari et al., 2008; Somarakis et al., 2011; Tsikliras and Koutrakis, 2011; Tsikliras et al., 2014; eastern Ionian waters: Granata et al., 2011; Tunisian waters: Koched et al., 2013; Zarrad et al., 2013). These studies carried out in the Mediterranean Sea showed that there was a clear bathymetric separation of larval fish assemblages, due to different spawning behavior of adults: inshore assemblages have a different composition from offshore ones (Alemany et al., 2006; Giordano et al., 2014; Granata et al., 2011; Sabatés and Olivar, 1996). Generally, bathy and mesopelagic families were characteristic members of the oceanic group, while the inshore species consist of pelagic and demersal (Beldade et al., 2006; Sabatés et al., 2003; Tsikliras and Koutrakis, 2011). However, this trend can be disrupted by peculiar mesoscale oceanographic structures, because currents, fronts and gyre can reshape the distribution of fish larvae with advection and concentration (Agostini and Bakun, 2002; Falcini et al., 2015; Sabatés et al., 2013). On the other hand, the environmental parameters that can affect the distribution of fish larvae, such as temperature and food availability, are strictly dependent on local conditions and vary considerably at reduced spatial scale (García Lafuente et al., 2002).

This is the case of spatial distribution of larval fish assemblages in two oligotrophic areas in the Mediterranean Sea with different and peculiar environmental conditions: southern Tyrrhenian Sea and Strait of Sicily. The southern Tyrrhenian Sea has high relevance as a probable nursery area for many commercially important pelagic and coastal fishes such as *Seriola dumerili*, *Xiphias gladius* and *Thunnus thynnus* and it is a habitat for mesopelagic fishes and squids, and plays a key role in deeper ecosystem energy flux (Bruno et al., 2001; Giordano et al., 2014; Granata et al., 2011). The Strait of Sicily is one of the main fishing sites in the Mediterranean (García Lafuente et al., 2002). It is a region characterized by a dominant hydrographic feature (the Atlantic Ionian Stream (AIS)) that has clear influence on the spawning strategy and the recruitment success of many fish species (García Lafuente et al., 2002, 2005; Mazzola et al., 2000, 2002).

In this paper, the different roles of hydrographic, physical, chemical and biological conditions in these two study areas during summer 2009 were showed, with the aim of determining what are the conditions in which the dynamics of transport and the chemical–physical and biological properties can be decisive in affecting the spatial composition of fish larvae in Mediterranean Sea.

## 2. Material and methods

### 2.1. Ichthyoplankton and oceanographic sampling

The two study areas are located in the southern side of the Tyrrhenian Sea and in the Strait of Sicily (Fig. 1).

Oceanographic data and ichthyoplanktonic samples were collected during two oceanographic surveys on board of the O/V Urania: Bansic 2009 carried out in the period 04–21 July 2009 in the Strait of Sicily and MedSudMed 2009 carried out from 26 July to 3 August 2009 in the Southern Tyrrhenian Sea. The sampling was made on a station

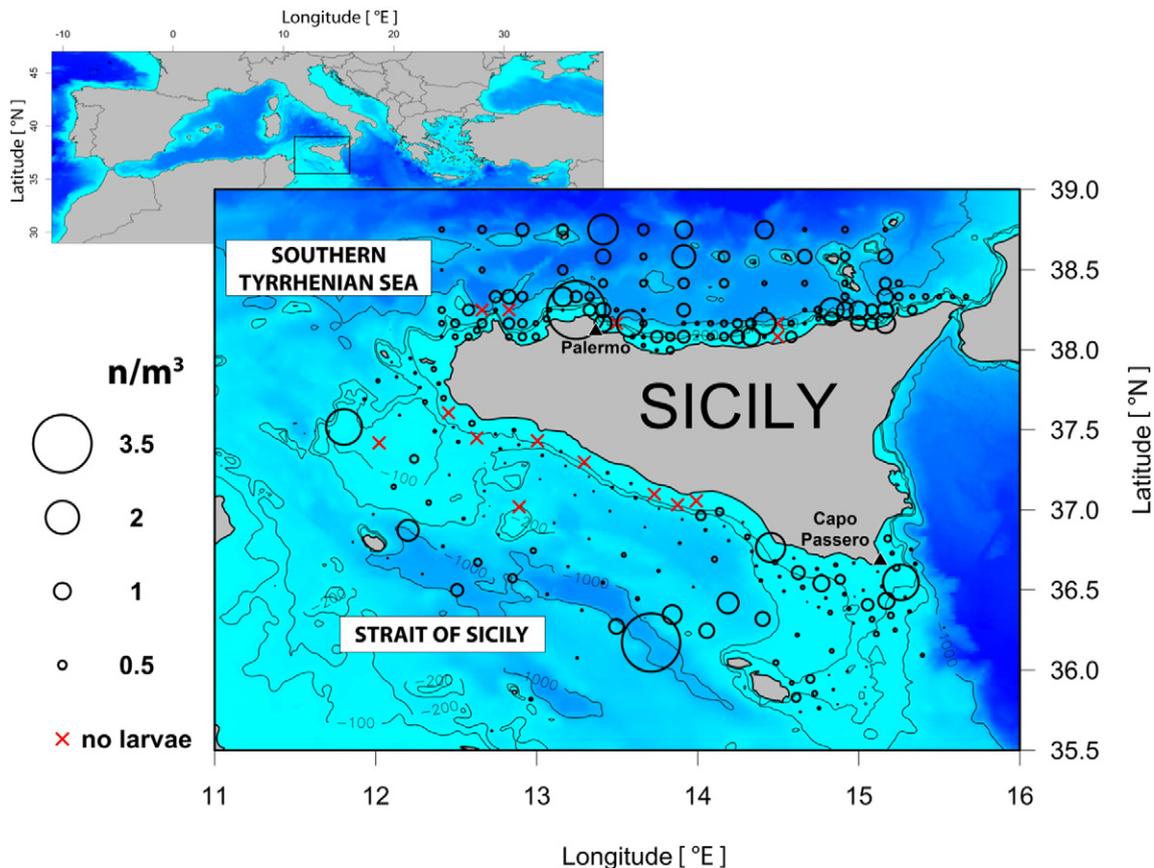


Fig. 1. Larval fish distribution in the study areas. The circle dimension is proportional to the total larval fish abundance.

**Table 1**

Analysis of deviance for GAM covariates of the final model fitted on total larval fish abundance.

Selected variables	Degree of freedom	F	p-value
<i>Tyrrhenian Sea</i>			
Zooplankton	1	23.71	4.15e−06
Temperature	1	6.50	0.0124
Fluorescence	3.19	3.03	0.0251
<i>Strait of Sicily</i>			
Salinity	1.28	6.52	0.0046
Zooplankton	1	3.07	0.0001
Temperature	3.54	3.66	0.0078

grid of 4 × 4 nautical miles within the 200 m bathymetry and a grid of 12 × 12 nautical miles for the off-shore areas over the 200 m bathymetry. In each station ichthyoplankton samples were collected by means of a Bongo40 net, which is composed by two coupled nets with the inlet mouth diameter of 40 cm and mesh size 200 μm. The plankton oblique tows were carried with a constant speed of 2 knots lasting from 12 to 15 min out to a depth of 100 m, wherever possible, because this layer characterizes the majority of the fish larvae in the study areas (Olivar et al., 2001, 2014; Sabatés et al., 2008). The larval fish collecting procedure was conducted during day and night, because, during the summer, fish pelagic larvae are more concentrated in the surface layer during the night and more dispersed along the column water during the day (Olivar et al., 2001; Sabatés et al., 2008), whereas the opposite occurs at least for most abundant mesopelagic species larvae (Olivar et al., 2014).

The filtered water volume of each mouth was measured by a calibrated flow-meters (type G.O. 2030). The samples were stored in formaldehyde at 4% buffered with borax. Fish larvae were sorted from the

rest of the plankton and identified to the family taxonomic level. Taxonomic identification was based on Bertolini et al. (1956); Costa (1999); Moser and Ahlstrom (1996) and Tortonese (1970). The number of fish larvae from each sample was standardized to n/m<sup>3</sup> according to Perez Ruzafa et al. (2004).

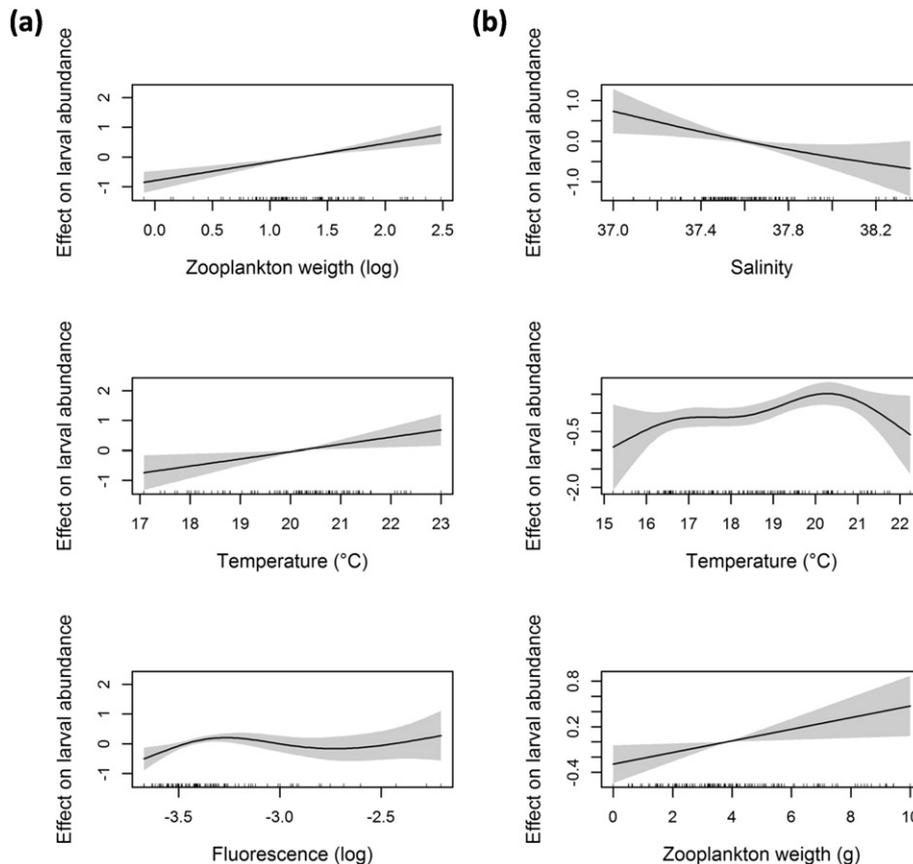
In all the stations, continuous vertical profiles of temperature, salinity and fluorescence were obtained from the surface to the bottom by means of a multiparameter probe SEABIRD mod. 9/11 plus mounted on a General Oceanics rosette equipped with 24 Niskin Bottles. The probes were calibrated before and after the cruise at the NURC (NATO Undersea Research Centre) in La Spezia, Italy. Each parameter has been calculated as the average of the values for each meter of the layer from surface to 50 m depth, that corresponds about to the average thermocline calculated in the study areas.

The circulation features were evaluated by means of the altimeter products (Absolute Dynamic Topography) and geostrophic velocity field, produced by Ssalto/Duacs and distributed by Aviso, with support from Cnes (<http://www.aviso.oceanobs.com/duacs/>). The period selected to evaluate the circulation pattern was 15 July–14 August 2009 for the Tyrrhenian Sea and 01–31 July 2009 for the stations in Strait of Sicily.

Since results of this study about the larval fish distribution in Strait of Sicily suggested a relationship with offshore wind-induced currents, the influence of wind on surface circulation was evaluated by means of satellite wind stress ( $\vec{\tau}$ ) and Ekman transport ( $\vec{m}$ ) from ocean surface 6-hourly wind data ( $\vec{U}_{wind}$ ), provided by the Cross-Calibrated Multi-Platform project (25 × 25 km, <http://podaac.jpl.nasa.gov>).

Wind stress was obtained as:

$$\vec{\tau} = \rho_{air} C_d |\vec{U}_{wind}| \vec{U}_{wind},$$



**Fig. 2.** (a) Plots of the smoothing response of the generalized additive model for larvae abundance in the Tyrrhenian Sea. (b) Plots of the smoothing response of the generalized additive model for larvae abundance in the Strait of Sicily. Black thick line indicates the value of the GAM smoothing response and grey area represents the 95% confidence intervals.

where  $\rho_{air}$  is the air density and the dimensionless friction coefficient  $C_d = 0.0012$  for  $0 < |\vec{U}_{wind}| < 11$  m/s and  $C_d = 0.00049$  for  $|\vec{U}_{wind}| \geq 11$  (Large and Pond, 1981; McClain and Firestone, 1993).

Ekman transport was calculated as:

$$\vec{M} = (\rho_{water} f)^{-1} \vec{\tau} \times \hat{k}$$

where  $\rho_{water}$  is the water density,  $f$  the Coriolis parameter, and  $\hat{k}$  is the vertical unit vector (Pickett and Paduan, 2003).

Finally, the Ekman transport  $\vec{M}$  was spatially represented in daily maps corresponding to survey period (from 4 to 21 July 2009).

### 2.2. Data analysis

A generalized additive model (GAM; Hastie and Tibshirani, 1986) for each study area was applied on total larval fish abundance and environmental parameters to define the factors that influenced larval fish concentration. Fluorescence and zooplankton weight of the Tyrrhenian Sea were transformed into natural logarithm in order to achieve uniform distribution for GAM application. The quasi-Poisson error distribution with the log link function was used and the natural cubic spline smoother was applied for smoothing the GAM fitting. The selection of the final model was performed by minimizing the Generalized Cross-Validation (GCV) and maximizing the level of explained deviance (0–100). All first-order interactions of the main effects were tested. Validation graphs (e.g. residuals versus fitted values, QQ-plots and residuals versus the original explanatory variables) were then observed to detect the existence of any pattern and possible model misspecification.

Afterwards, the stations were classified according to their species composition similarity by hierarchical clustering using the Bray-Curtis distance measure (Bray and Curtis, 1957) and Ward's linkage as grouping method (Ward, 1963). Only dominant families, defined as those with total density > 0.5 individuals per m<sup>3</sup>, and the stations with presence of fish larvae were used in order to avoid problems, e.g. biasing or dominating the ordination. No scaling of the data was preventively carried out for the taxonomic dataset of the Tyrrhenian Sea, while the density values of the Strait of Sicily were transformed into square root, because some larval fish taxa exhibited a large variance and they could be decisive in determining the main sources of information. Analysis of similarities (ANOSIM) was performed to test the significance of differences in groupings separated in the cluster analysis (Clarke, 1993). Levels of occurrence, abundance of species and geographical distinctness among species and station groups were then used as subjective criteria to fine-tune the identification of clusters.

A Principal Coordinate Analysis (PCoA; Gower, 1966) was performed on Bray-Curtis dissimilarity of the larval fish assemblages to describe relationships among clusters and to show their general spatial pattern. The equilibrium circle (Legendre and Legendre, 1998) was used to select the families, that significantly contributed to the axes shown in the ordination graph. Specifically, they must have vectors outside of the equilibrium circle. The first and second dimensions of the PCoA were mapped to reveal gradients and patchiness.

A distance-based redundancy analysis (dbRDA), based on the Bray-Curtis dissimilarities and limited to 2 axes, was performed to explore which group of habitat variables best explained the spatial variation observed in larval fish assemblage structure (McArdle and Anderson, 2001). Environmental variables were standardized to obtain comparable scales (Clarke and Warwick, 1994). Distance based redundancy analysis (dbRDA) biplots were generated to visually display the direction and magnitude of the relationships between habitat factors and larval fish families (Legendre and Anderson, 1999). All the statistical analyses were carried out using statistical software R (R 3.0.1; R Core Team, 2013).

### 3. Results

#### 3.1. Larval fish abundance

In the 109 stations in the Tyrrhenian Sea, total fish larvae density ranged between ~0.027 n/m<sup>3</sup> and ~1.941 n/m<sup>3</sup> (mean 0.296 n/m<sup>3</sup>; standard deviation 0.243). The station with the most concentrated density was in the western coastal side of the study area in the gulf of Palermo. In general, ichthyoplankton showed a patchwork distribution with no difference between offshore and coastal area (KW = 0.791; p-value > 0.05) and with higher concentrations of larvae in the eastern coastal side compared to the western coastal side (KW = 5.237; p-value < 0.05) (Fig. 1).

In the 150 stations in the Strait of Sicily total fish larvae density ranged between ~0.011 n/m<sup>3</sup> and ~3.572 n/m<sup>3</sup> (mean 0.433 n/m<sup>3</sup>; standard deviation 0.561). Largest abundance values were found in the eastern part of the study area (KW = 5.868; p-value < 0.05), over the continental shelf area separating Sicily coasts from Maltese Islands (Fig. 1).

The generalized additive model for the Tyrrhenian Sea indicated the zooplankton weight, the temperature and the natural logarithm of fluorescence as the most important factors to explain the variability of larval fish abundance. The explained deviance by the model was 34.2% of the

**Table 2**

Larval fish families recorded in Tyrrhenian Sea and the Strait of Sicily. Ecological group (B: bathypelagic fish, P: pelagic fish, M: mesopelagic fish, D: demersal fish), abundance (number of fish larvae for each family) and percentage of total abundance (number of fish larvae for each family/total number of fish larvae) are reported.

Family	Ecological group	Abundance Tyrrhenian Sea	Percentage Tyrrhenian Sea	Abundance Strait of Sicily	Percentage Strait of Sicily
Ammodytidae	D	14	0.617	2	0.077
Apogonidae	D	3	0.132	1	0.039
Blennidae	D	1	0.044	7	0.270
Bothidae	D	24	1.057	35	1.352
Bramidae	P	2	0.088	1	0.039
Callionymidae	D	5	0.220	20	0.773
Carangidae	P	10	0.441	31	1.198
Centracanthidae	P	7	0.308	17	0.657
Centriscidae	D	1	0.044	1	0.039
Cepolidae	D	6	0.264	1	0.039
Clupeidae	P	238	10.485	557	21.522
Congridae	D	2	0.088	20	0.773
Coproidae	D	1	0.044	0	0.000
Cynoglossidae	D	1	0.044	0	0.000
Engraulidae	P	132	5.815	1127	43.547
Evermannellidae	B	0	0.000	5	0.193
Gadidae	D	4	0.176	37	1.430
Gobiidae	D	110	4.846	153	5.912
Gonostomatidae	B	848	37.357	195	7.535
Labridae	D	25	1.101	60	2.318
Myctophidae	M	387	17.048	124	4.791
Ophichthidae	D	1	0.044	2	0.077
Ophidiidae	D	1	0.044	0	0.000
Paralepididae	M	113	4.978	14	0.541
Phosichthyidae	B	207	9.119	23	0.889
Pomacentridae	P	32	1.410	14	0.541
Scombridae	P	18	0.793	22	0.850
Scophthalmidae	D	0	0.000	2	0.077
Scorpaenidae	D	3	0.132	12	0.464
Serranidae	D	25	1.101	27	1.043
Soleidae	D	0	0.000	3	0.116
Sparidae	D	34	1.498	57	2.202
Sternoptychidae	B	1	0.044	1	0.039
Stomiidae	P	1	0.044	0	0.000
Stomiidae	B	1	0.044	0	0.000
Synodontidae	D	4	0.176	0	0.000
Trachinidae	D	1	0.044	9	0.348
Trichiuridae	M	1	0.044	1	0.039
Triglidae	D	0	0.000	5	0.193
Tunnidae	P	6	0.264	2	0.077
Total		2270		2588	

total deviance (Table 1). The relationship between larval fish abundance and zooplankton weight and temperature was linearly increasing, indicating a positive effect on abundance for zooplankton values higher than about 4.40 g and temperature values higher than 20.5 °C. The effect of fluorescence values was increasing until 0.04 mg L<sup>-1</sup> and almost absent for higher values (Fig. 2a).

The selected parameters for the best model to explain the larval fish abundance in the Strait of Sicily were salinity, zooplankton weight and temperature. The explained deviance by the model was 42.5% of the total deviance (Table 1). The higher larval fish abundance was associated with the lowest salinity values (<37.6) and with optimal temperature in the range 19–21 °C. The effect of the zooplankton on larval fish abundance was linearly increasing and positive for zooplankton values higher than 4 g (Fig. 2b).

### 3.2. Larval fish composition

A total of 2270 fish larvae from 36 families in the southern Tyrrhenian Sea and 2588 fish larvae from 34 families in the Strait of Sicily were identified (Table 2). The three most abundant families accounted for 64.89% of the total catch in the Tyrrhenian Sea. They were Gonostomatidae (37.36%), Myctophidae (17.05%) and Clupeidae (10.48%). Except Phosichthyidae (9.12%), Engraulidae (5.81%) and Gobidae (4.85%), all other 30 families comprised <2% of the total number of larvae collected. In the Strait of Sicily, the most abundance families were Engraulidae (43.55%) and Clupeidae (21.52%), and except Gonostomatidae (7.53%), Gobidae (5.91%), Myctophidae (4.79%), Labridae (2.32%) and Sparidae (2.20%), all other families comprised <2% of the total larvae.

Four larval fish assemblages were defined by the Bray–Curtis dissimilarity index for sample from Tyrrhenian Sea and five assemblages from Strait of Sicily, as suggested by their respective dendrograms (Fig. 3 a and b). The clusters differed substantially in family composition and

abundance, presenting distinct assemblage structures in both sampling areas. The ANOSIM analysis showed significant dissimilarity between families assemblages obtained from cluster analysis in Tyrrhenian Sea (Global R = 0.6625; p < 0.001) and in the Strait of Sicily (Global R = 0.5523; p < 0.001).

The families that significantly contributed to the axes shown in the ordination graph of PCoA applied on data from Tyrrhenian Sea were Gonostomatidae, Myctophidae, Gobidae, Engraulidae and Clupeidae. The first dimension of the PCoA well discriminated two groups: the lower values characterized clusters 1 and 2 and families Gonostomatidae and Myctophidae and the higher values characterized clusters 3 and 4 and families Engraulidae, Gobidae and Clupeidae (Fig. 4a). This dimension showed a strong coastal–offshore gradient, indicating that the first assemblage (clusters 1 and 2 with mesopelagic families) was mainly present in the offshore area unlike families belonging to the second assemblage (clusters 3 and 4 with neritic families) located in coastal areas (Fig. 4b). The second dimension of the PCoA discriminated larvae of clusters 1 and 3 with small pelagic families from larvae of cluster 2 with mesopelagic families (Fig. 4a). This dimension showed different values in specific areas. In particular, higher values (clusters 1 and 3 with neritic families) were found in the Gulf of Palermo and in the easternmost part of the study area (Fig. 4c). Otherwise, negative values (cluster 2, mesopelagic fish larvae), were present in the offshore zone and in central-east coastal zone with exception of areas from 14.75° and 15° of longitude (Fig. 4c).

The families that significantly contributed to the PCoA axes defined by data from Strait of Sicily were Clupeidae, Engraulidae, Gobiidae, Labridae, Bothidae, Myctophidae. The first dimension of the PCoA discriminated two groups: the lower values characterized clusters 1 and 4 and families Clupeidae and Engraulidae and the higher values clusters 2 and 5 and families Gonostomatidae and Myctophidae (Fig. 4d). This dimension showed the presence of the second assemblage (clusters 2 and 5 with mesopelagic families) in the western

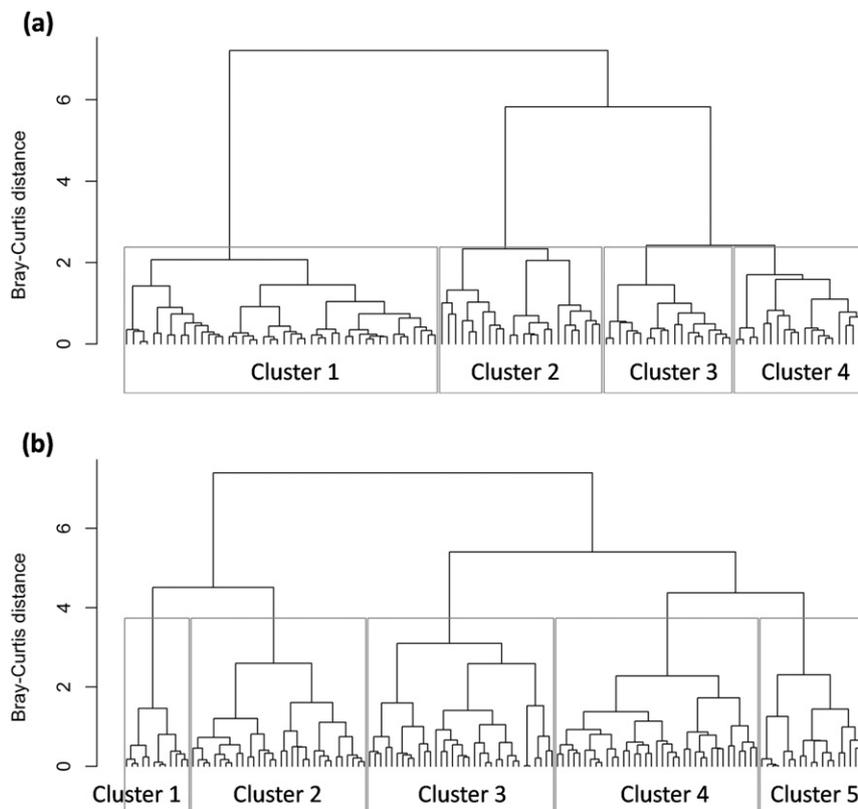
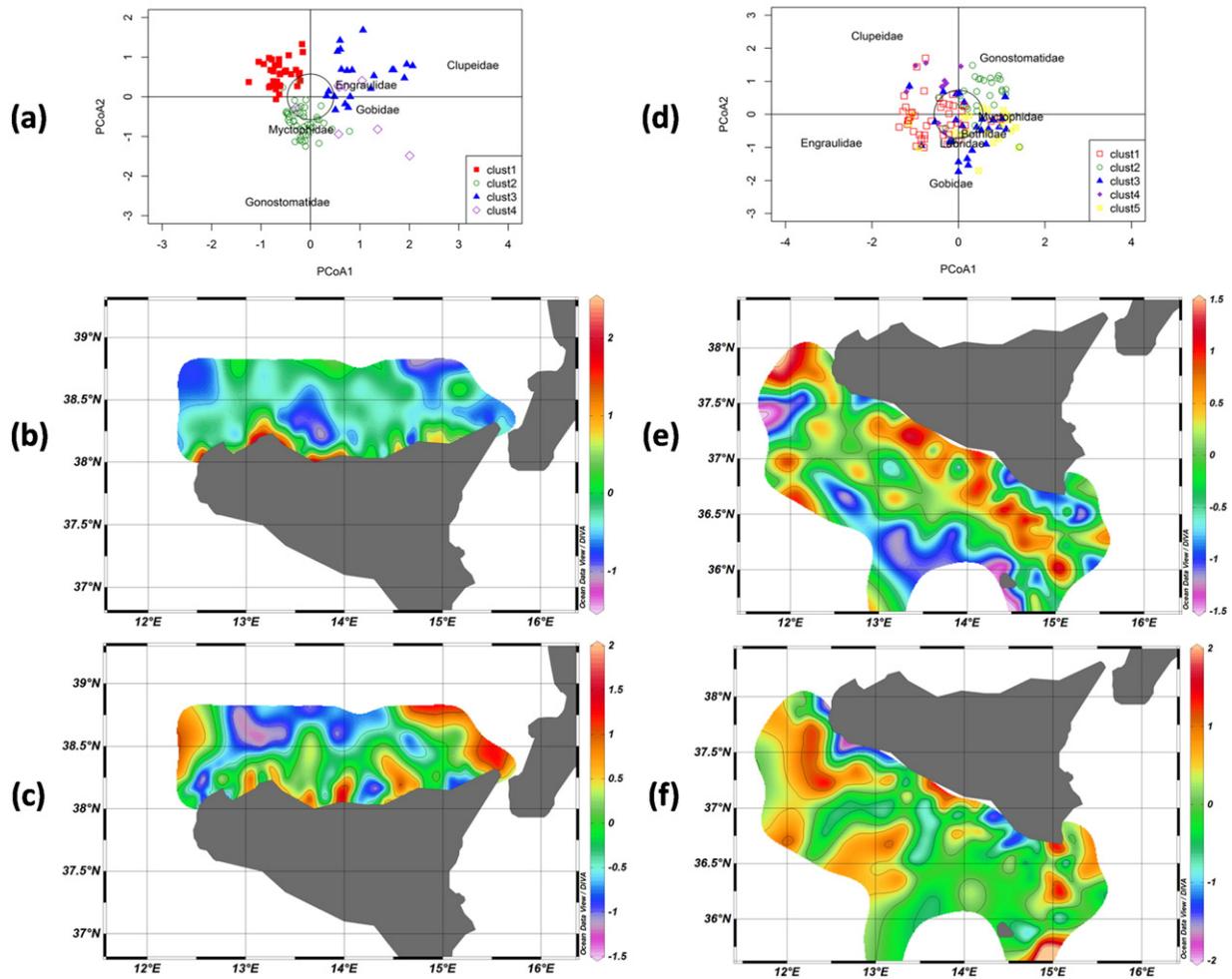


Fig. 3. Dendrograms generated by hierarchical cluster analyses with Bray–Curtis distance and Ward's grouping method applied on larval families composition in the southern Tyrrhenian Sea (a) and Strait of Sicily (b).



**Fig. 4.** (a) Bivariate plot of first and second principal coordinates on larval fish composition in the Tyrrhenian Sea. The equilibrium circle was added to a PCoA ordination diagram; (b) geographical distribution of the first principal coordinates on larval fish composition in the Tyrrhenian Sea; (c) geographical distribution of the second principal coordinates on larval fish composition in the Tyrrhenian Sea; (d) bivariate plot of first and second principal coordinates on larval fish composition in the Strait of Sicily. The equilibrium circle was added to a PCoA ordination diagram; (e) geographical distribution of the first principal coordinates on larval fish composition in the Strait of Sicily; (f) geographical distribution of the second principal coordinates on larval fish composition in the Strait of Sicily.

coastal area up to 14.5° of longitude, while the first assemblage (clusters 1 and 4 with small pelagic families) was dominant in the eastern coastal area and offshore (Fig. 4e). The second dimension of the PCoA discriminated larvae from cluster 5 with families Bothidae, Labridae and Gobiidae (lower values) from larvae from clusters 2 and 4 with families Clupeidae and Gonostomatidae (higher values) (Fig. 4d). This dimension showed lower values in the central area (from 13° to 15° of longitude) and higher values in the remaining areas (Fig. 4f).

### 3.3. Environmental parameters

#### 3.3.1. Tyrrhenian Sea

The surface waters dynamics evidenced the presence of an anti-cyclonic eddy in the Tyrrhenian Sea located in the area from longitude 13°E to 14.5°E (Fig. 5a). This structure involved a sub-surface section of the water column until about 300 m of depth (Fig. 5b) and it was particularly evidenced by the isopycnal line  $29 \text{ kg m}^{-3}$  that in the middle sank from 200 m to 300 m.

The areal distribution of the parameters (temperature, salinity, fluorescence and zooplankton weight) showed the variability of the sub-surface layer. The isosurface of temperature (Fig. 6a) showed the presence of colder water in the western area of the study area and simultaneously an increase of temperature in the central area due essentially to the presence of anti-cyclonic eddy that generated a flexion of isotherms (not shown), such as of isopycnal lines (Fig. 5b). The salinity

(Fig. 6b), fluorescence (Fig. 6c) and zooplankton (Fig. 6d) minimum values were in correspondence with this eddy. The zooplankton isosurface (Fig. 6d) showed higher values in the entire area further to the west and in the coastal eastern side.

#### 3.3.2. Strait of Sicily

Geostrophic velocity field showed that the surface circulation was mainly affected by the eastward flow of AIS, which was characterized by its typical meandering path that borders the eastern coast of Sicily and reached maximum values of  $\sim 44 \text{ m s}^{-1}$  in the core (Fig. 5a). Moreover satellite images (Fig. 5a) and density interfaces (Fig. 5c) showed the presence of two cyclonic structures in western side of the Strait of Sicily and a modest and superficial anti-cyclonic structure in eastern part. Specifically, the isopycnal 28.5 was about 50 m depth in the western part of Sicily and sank to about 120 m depth in the eastern part (Fig. 5c). Analysis of temperature and salinity data in the layer 0–50 m evidenced colder (16–18 °C) and saltier (37.75–38 PSU) water in coastal zone, especially in the Adventure Bank (western part of the study area) (Fig. 6a and b). The Bank was also characterized by higher fluorescence (Fig. 6c) and zooplankton (Fig. 6d) values. This may indicate the presence of wide coastal upwelling phenomena, generated by wind. Daily Ekman transport maps ( $\vec{m}$ ) showed a clear transport of water from coastal to offshore zone for most of the days during the survey (Fig. 7). Indeed moderate ( $4 < \vec{m} < 8 \text{ m}^2 \text{ s}^{-1}$ ) and intense ( $\vec{m} > 8 \text{ m}^2 \text{ s}^{-1}$ ) values of cross-shore

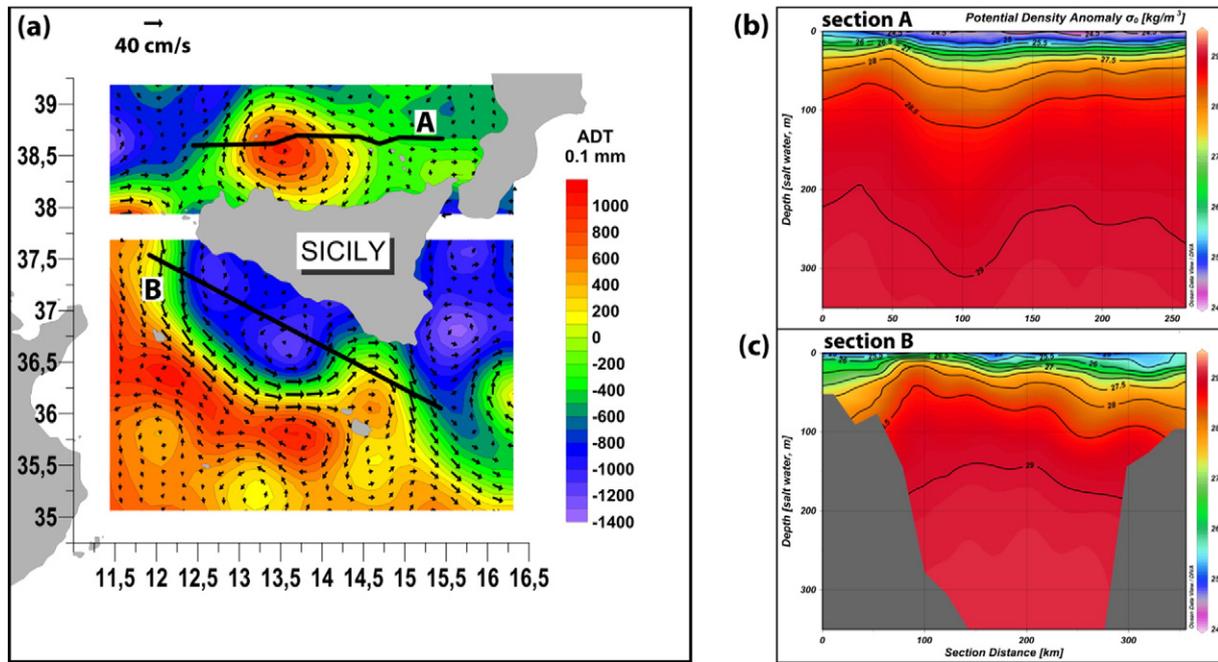


Fig. 5. (a) Absolute Dynamic Topography (ADT) and mean geostrophic velocity field by Aviso; (b) vertical section of density profiles across the transect A (Fig. 5a) in the upper 350 m; (c) vertical section of density profiles across the transect B (Fig. 5a) in the upper 350 m.

transport were evident in 11 over 18 analyzed days while in the other days the transport is weak or absent, especially in the coastal zone.

Moreover, a marked thermo-haline front was present in the easternmost part, beyond 15° of longitude and high zooplankton values were found in the western area adjacent to this front (from 13.5 to 14.5° of longitude; Fig. 6d).

### 3.4. Correlation between larval fish assemblages and environmental variables

The dbrDA provided an ordination that was constrained by some environmental variables. Results of the routine for the Tyrrhenian Sea showed that only the first two axes were significant which explained 77.20% of the overall variability. All environmental parameters, except the salinity, were significant. The biplot of the first two dbrDA axes (Fig. 8a) revealed a separation among clusters based on the environmental parameters. The first RDA axis revealed a separation between cluster 1, negatively correlated to temperature, zooplankton weight and fluorescence from cluster 2 positively correlated to the same parameters. The second axis identified neritic families (cluster 4) positively correlated with fluorescence and zooplankton weight and negatively correlated with temperature.

Results of the dbrDA for the Strait of Sicily showed that the first two axes were significant explaining the 63.41% of the overall variability. All parameters were significant. The first RDA axis revealed a separation between the first assemblage with small pelagic larvae (clusters 1 and 4) and the second assemblages (clusters 2 and 5). The second RDA axis separated clusters 3 and 5, positively correlated with zooplankton weight and salinity, from clusters 2 and 4, positively correlated with fluorescence and temperature (Fig. 8b).

## 4. Discussion

This study aims to examine the responses of larval fish assemblages to different hydrodynamic conditions, in order to clarify the role that mesoscale oceanographic structures, such as gyres, fronts and currents, can play compared to different chemical, physical and biological conditions. These factors can contribute to the survival and distribution of

fishes at early development stages and consequently to the recruitment of marine fish populations.

The southern Tyrrhenian Sea is a typically oligotrophic area but little is known about the effects of physical forcings on the biology and ecology of fish populations (Azzaro et al., 2003; Carrada et al., 1992; De Domenico, 1979; Giordano et al., 2014). Open southern Tyrrhenian Sea is considered as an important spawning area for the swordfish (*Xiphias gladius*; Romeo et al., 2009) and the Atlantic blue fin tuna (Sinopoli et al., 2004). Moreover, a previous study showed that it is a relevant nursery area for the short-finned squid *Illex coindetii* (Perdichizzi et al., 2011), which represents an important prey for several adults fish species.

Our results in Tyrrhenian Sea generally permitted the identification of two main larval fish assemblages, separating a group of shallower stations where most larvae belonged to neritic families and another group of deeper stations where mesopelagic families were dominant. This bathymetric separation confirmed a study on ichthyoplankton composition carried out in June 2006 that covered only the eastern coastal side of southern Tyrrhenian Sea, from Cape Cefalù to Cape Rasocolmo. In fact, this study showed that in this region three main assemblages can be distinguished: shore fish larvae (Labridae, Serranidae) which dominate in coastal areas, larvae found over the continental shelf (Sparidae, Scorpaenidae, Bothidae, Mullidae, Engraulidae, Gobiidae, Trachinidae, Blennidae) and larvae of meso- and bathypelagic species, which mainly over slope and open waters (Gonostomatidae, Myctophidae, Sternoptychidae). This inshore-offshore gradient was explained with respect to the hydrographic features and structures determined by the surface circulation path (Giordano et al., 2014). Moreover, these results were very similar in terms of species composition and distribution to what found in nearby areas in the Mediterranean Sea (Alemany et al., 2006; Sabatés and Olivar, 1996). However, mesopelagic larvae (e.g. Myctophidae), which presented an irregular distribution, were abundant not only offshore but also in coastal stations. This situation could be linked to larval fish transport by oceanographic structures characterizing the southern Tyrrhenian Sea and affecting strictly the habitat (García Lafuente et al., 2002). There, the main feature observed was the presence of an anti-cyclonic gyre in the northern side of the Gulf of Palermo, which was characterized by warmer and less salty water, with low values of linked-productivity

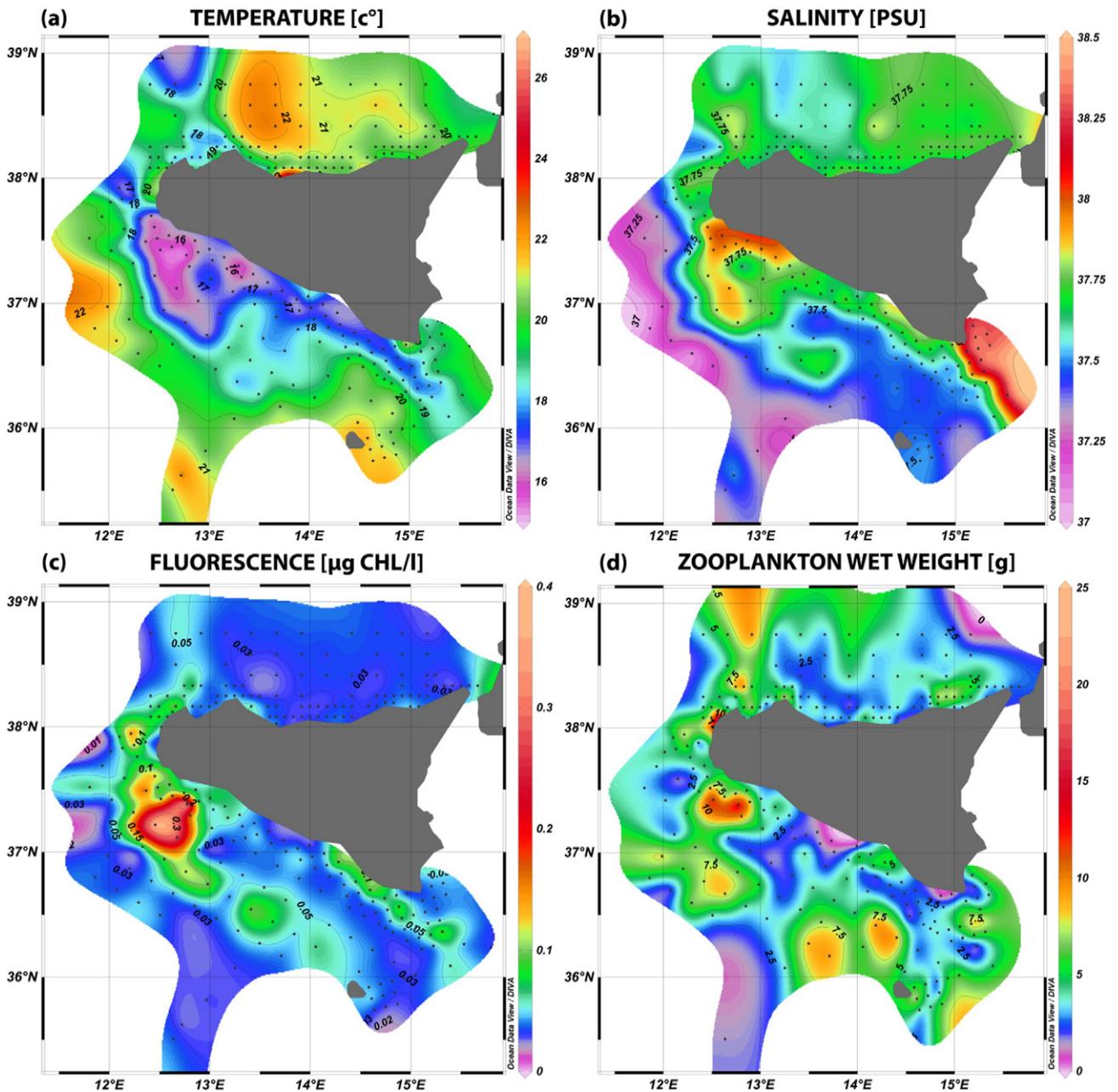


Fig. 6. Horizontal distribution pattern of mean values of temperature (a), salinity (b) and fluorescence (c) salinity in the layer 0–50 m and of total zooplankton weight (d).

parameters (fluorescence and zooplankton weight) in the core. This structure generated a near-coast accumulation area, exhibiting the maximum larval fish concentration. Specifically, the anti-cyclonic eddy could have acted as mechanisms of retention and concentration of different larval fish taxa, especially belonging to Clupeidae, Engraulidae and Gobiidae families and facilitated the transport inshore of mesopelagic larvae. In fact, it was shown that the dynamics of anticyclonic flow patterns imply convergence and downwelling (Bakun, 1996), with associated opportunities for concentrating small organisms in convergent frontal formations (Agostini and Bakun, 2002). The occurrence of mesopelagic species in neritic zones, where the spawning is not present, was observed also in other areas of the Mediterranean Sea, particularly in the Balearic Sea (Alemany, 1997), in the northern Aegean Sea (Koutrakis et al., 2004; Tsikliras et al., 2009) and in Costa Brava (Palomera and Olivar, 1996). Conversely, the eastern side of Tyrrhenian Sea was an area with apparently more stable hydrodynamic conditions. In this area, the larval fish assemblages were

more regular, corresponding to the absence of relevant mesoscale oceanographic forcings. The environmental conditions rather than the dispersal of species could have partially determined the structure and dynamics of the eastern communities. In fact, larvae belonged to demersal and pelagic families were mainly concentrated in the coastal part, characterized by higher surface temperature values and mesopelagic larvae could be found mainly offshore as well as total zooplankton, with lower density values compared to the western offshore areas.

A different situation was found in the Strait of Sicily, which was also classified as an oligotrophic area (Malanotte Rizzoli et al., 1997; Van Wambeke et al., 2002). It was characterized by a very complex sea circulation that can have strongly influenced the spawning strategy and the recruitment success of many fish species (Cuttitta et al., 2003, 2015; Fortibuoni et al., 2010; Garofalo et al., 2010). In particular, many studies (Basilone et al., 2013; Cuttitta et al., 2015; Falcini et al., 2015; García Lafuente et al., 2002) have focused on the influence of oceanographic factors on the ecology and reproductive biology of

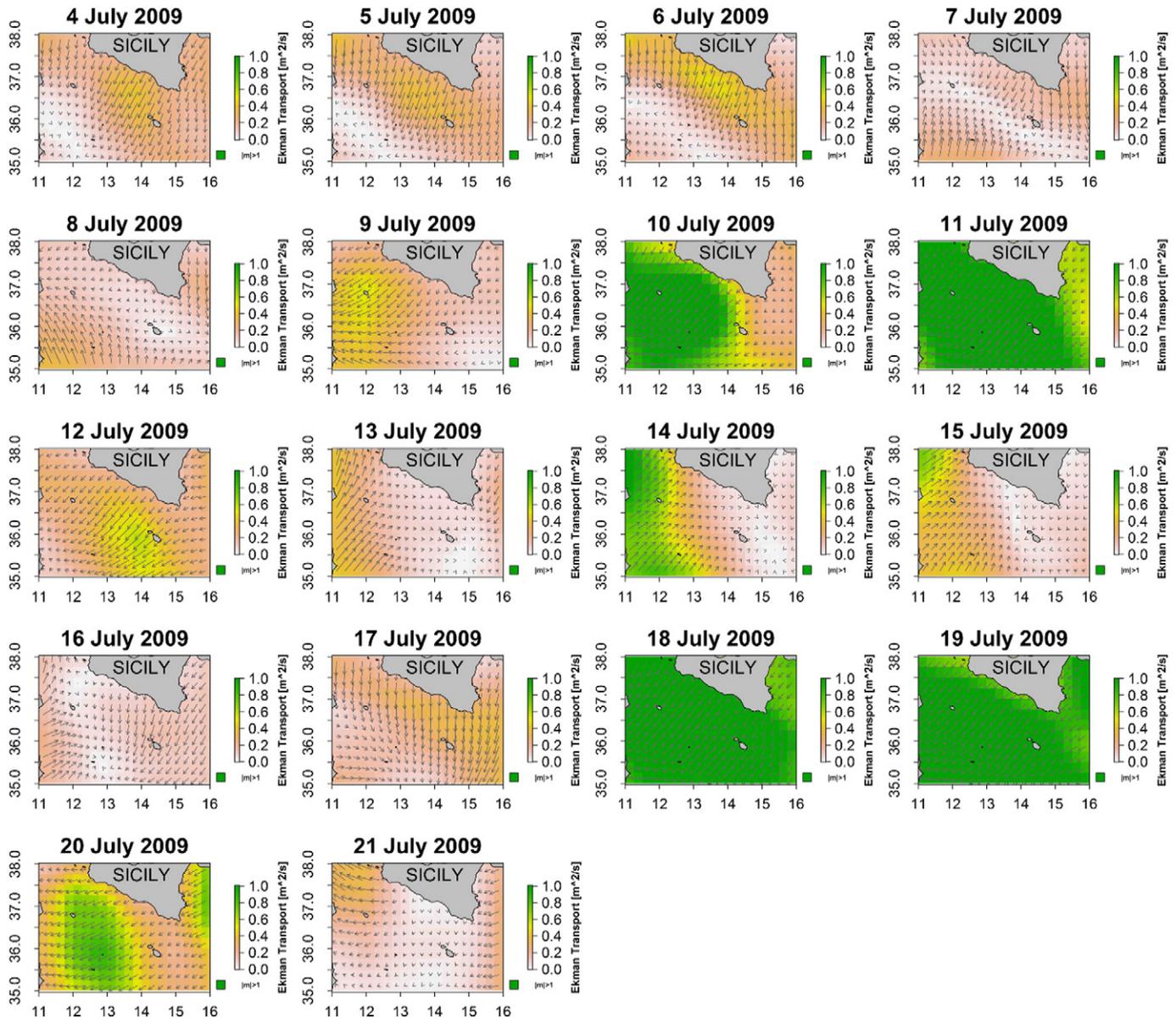
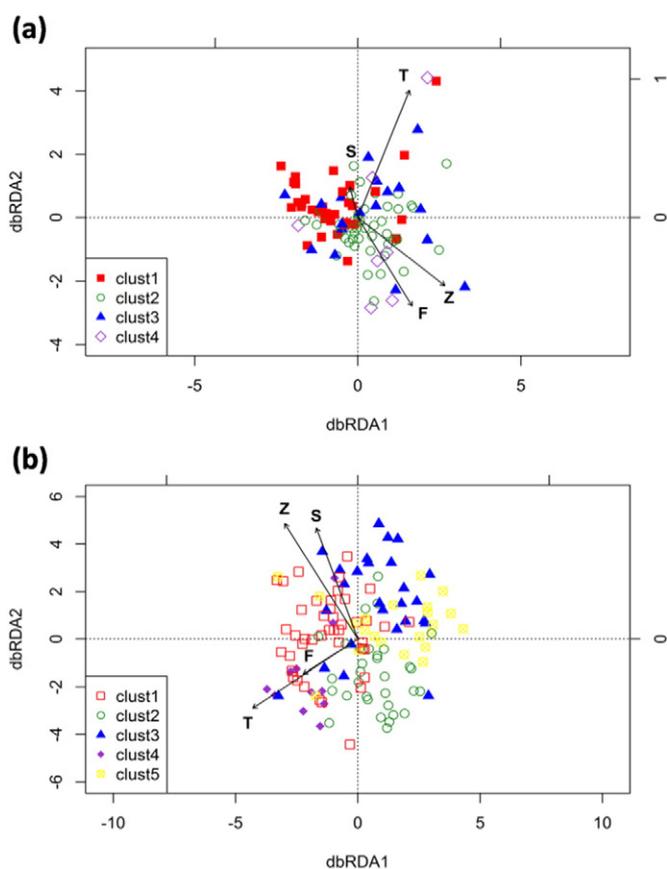


Fig. 7. Daily Ekman transport ( $\text{m}^2 \text{s}^{-1}$ ) during the cruise (Bansic 09) in Strait of Sicily. Arrows and colors indicate the direction and the intensity of the transport respectively.

the European anchovy (*Engraulis encrasicolus*), which is the target species of fisheries along the southern coast of Sicily, representing one of major fish resource in terms of economic importance (Patti et al., 2005, 2012). In this study, the influence of the circulation pattern in the Strait of Sicily on the distribution of fish larvae was very clear and it can be considered the main factor governing the larval fish retention and shaping the observed assemblages. In fact, the distribution of total larvae was largely consistent with the branch of the AIS, running roughly parallel to the southern Sicilian coast. AIS was able to advect the larvae towards a retention area at the south-easternmost corner of Sicily (off Capo Passero). Here, the presence of a thermo-haline front, as evidenced by temperature and salinity fields, allowed the larvae to maintain their relative position in an area with enhanced trophic condition, so probably providing more favourable conditions for larval fish feeding and growth (García Lafuente et al., 2002, 2005; Mazzola et al., 2002). As support of this finding, our results showed that this area was characterized by the mixing of all clusters. This happened because AIS transport and the thermo-haline front conveyed there the larvae belonged to families different from ecological point of view (demersal and small pelagic

together with meso and bathypelagic fish larvae). In addition, the spatial extension of AIS path may have had important consequences on the predominant hydrological phenomena occurring in the region, such as the offshore extension of coastal upwelling (García Lafuente et al., 2005). In summer 2009, the flow of the stream moved further offshore (Bonanno et al., 2014) and so the study area showed a broader upwelling extension centred in the western zone on Adventure Bank and expanded eastward, modifying surface temperature, salinity and linked-productivity parameters (fluorescence and zooplankton weight). This feature was confirmed by the analysis of Ekman transport, which showed that strong and persistent transport of water induced by Mistral (NW) and Westerly winds and directed offshore was detected during the survey. These winds could have played a key role in shaping larval fish distribution pattern, because they could have modulated the capacity of advection of fish larvae offshore, similarly to what observed by Falcini et al. (2015) for the anchovy larvae in year 2004. In fact, in correspondence with these processes, typically neritic larvae (pelagic families: Engraulidae and Clupeidae) were found in the offshore stations.



**Fig. 8.** Db-RDA ordination graph for the first two axes for the larval fish composition using Bray-Curtis distance and the CTD parameters (T: temperature; S: salinity; F: fluorescence; Z: zooplankton weight) as a constraining variable for the southern Tyrrhenian Sea (a) and for the Strait of Sicily (b).

Therefore, in contrast to the findings for the southern Tyrrhenian Sea, complex mesoscale oceanographic structures (e.g. AIS pathway, coastal upwelling, gyres and fronts), that interacted synergistically, were able to drive the mainly patterns of distribution of fish larvae in the Strait of Sicily. This evidence was supported also by the analysis of significance of environmental parameters affecting larval fish distribution. Indeed, only salinity and zooplankton weight were found to determine the taxonomic composition, discriminating small pelagic fish larvae species from larvae belonging to Gonostomatidae family. The association between salinity and zooplankton is mostly linked to the features of AIS surface currents, characterized by relatively low salinity values and able to transport zooplankton along its path off southern Sicilian coasts (Patti et al., 2005).

## 5. Conclusions

This case study evidenced the need to assess specific environmental conditions driving the dynamics of development of fish larvae and consequently the recruitment success of marine populations in a framework of efficient monitoring plans. The results underlined the different roles of mesoscale oceanographic structures compared with chemical, physical and biological parameters in shaping the spatial pattern of larval fish distribution in the central Mediterranean Sea in a case study of Summer 2009. In the Southern Tyrrhenian Sea more stable hydrodynamic conditions were observed. In this framework, although the anticyclonic eddy in the central part of the study area was able to transport part of mesopelagic larvae (e.g. mainly Myctophidae) in a restricted portion of the northern Sicilian coast, bathymetry and environmental conditions played a key role in determining larval fish distribution. Conversely, in the Strait of Sicily the distribution of early life stages of

fish marine species was highly dependent on marked hydrodynamic features (current speed up to ~44 cm/s). In this context, larval fish distribution showed complex spatial patterns, not associated with bottom depth as found in other similar studies in Mediterranean (Alemany et al., 2006; Giordano et al., 2014; Granata et al., 2011; Sabatés and Olivar, 1996) but with mesoscale oceanographic structures (e.g. surface currents, gyres and fronts), as reported in different cases by Bakun (2006); Basilone et al. (2013); Palomera (1992) and Sabatés et al. (2001). These structures conveyed the larvae in retention areas with conditions favorable for their survival and growth (Cushing, 1990). Additionally, it was important to underline the effect of Mistral (NW) and Westerly winds in modulating the process linked to upwelling and the transport of neritic fish larvae offshore, as described by Falcini et al. (2015) about dispersion of anchovy larvae in year 2004.

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**Wind forcing and fate of *Sardinella aurita* eggs and larvae**

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# Wind forcing and fate of *Sardinella aurita* eggs and larvae in the Sicily Channel (Mediterranean Sea)

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## Abstract

Multidisciplinary studies are recently seeking to define diagnostic tools for fishery sustainability by coupling ichthyoplanktonic datasets, physical and bio-geochemical oceanographic measurements, and ocean modelling. The main goal of these efforts is the understanding of those processes that control fate and dispersion of fish larvae and eggs and thus tune the inter-annual variability of biomass of fish species. We here analyzed eggs and larvae distribution and biological features of *Sardinella aurita* in the northeast sector of the Sicily Channel (Mediterranean Sea) collected during the 2010 and 2011 summer cruises. We make use of satellite sea surface temperature, wind, and chlorophyll data to recognize the main oceanographic patterns that mark eggs and larvae transport processes and we pair these data with Lagrangian runs. To provide a physical explanation of the transport processes that we observe, we hire a potential vorticity (PV) model that takes into account the role of wind stress in generating those cold filaments responsible for the offshore delivery of eggs and larvae. Our results show that the strong offshore transport towards Malta occurring in 2010 is related to a persistent wind forcing along the southern Sicilian coast that generated an observable cold filament. Such a pattern is not found in the 2011 analysis, which indeed shows a more favorable condition for sardinella larvae recruiting with a weak offshore transport. Our results want to add some insights regarding operational oceanography for sustainable fishery.

## 1 Introduction

Small pelagic fishes are essential elements of marine ecosystems due to their significant biomass at intermediate levels of the food web, playing a considerable role in connecting the lower and upper trophic levels (Rice, 1995; Bakun, 1996; Cury et al., 2000).

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between spawning and nursery areas and, in particular, the role of wind forcing in delivering *Sardinella aurita* larvae offshore. We finally set and confirm some hypothesis regarding the causes behind the observed and simulated patterns by giving a physical interpretation of those Lagrangian dynamics (Falcini et al., 2015).

## 2 Data and methods

### 2.1 The biological dataset

Ichthyoplanktonic data were collected during two cruises carried out from 25 June to 14 July 2010 (“Bansic 2010”) and from 8 to 27 July 2011 (“Bansic 2011”) on board the R/V *Urania*, in correspondence with the main reproductive activity of this species (Whitehead, 1985). 190 and 131 stations were sampled in Bansic 2010 and Bansic 2011, respectively (Fig. 1). Systematic sampling is constituted by a regular grid of stations ( $1/10^\circ \times 1/10^\circ$  along the continental shelf, and  $1/5^\circ \times 1/5^\circ$  offshore) placed along transects perpendicular to the coast. Planktonic sampling was conducted using vertical CalVET (one mouth of 25 cm inlet diameter, 150  $\mu\text{m}$  mesh) and oblique Bongo 40 net (two mouth of 40 cm inlet diameter, 200  $\mu\text{m}$  mesh, towed at 2 knots). The nets were hauled within 5 m from the bottom to the surface, or from 100 m to the surface ad deep station. In each mouth, calibrated flow-meters were mounted on in order to calculate the volume of filtered water ( $\text{m}^3$ ). To preserve planktonic samples, borax-buffered solution of 4 % formaldehyde and seawater (for CalVET and mouth 1-Bongo 40 samples) and solution of 70 % ethanol (for mouth 2-Bongo 40 samples) were used. In land based laboratory, all samples were observed at binocular and fish eggs and larvae were sorted from the rest of the plankton. Eggs and larvae of *Sardinella aurita* were identified (Whitehead, 1988).

The number of fish eggs and larvae collected at each station was standardized to the number beneath a unit of sea surface ( $10 \text{ m}^2$ ) using the equation of Nonaka et al. (2000):  $Y_i = \frac{(10 \times d_j \times x_i)}{v_i}$ , where  $Y_i$  is the number of larvae/eggs of each species under

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10 m<sup>2</sup> of sea at station  $i$ ,  $x_i$  is the number of larvae/eggs taken at station  $i$ ,  $v_i$  is the volume of water filtered in m<sup>3</sup> and  $d_i$  is the maximum depth reached by net.

Eggs and larvae preserved in formaldehyde were used for the determination of the stage of development. In particular, staging of eggs were performed assigning number from 1 (stage after fecundation, with a single cell) to 11 (stage pre-hatching) follow (Gamulin and Hure, 1955; Whitehead, 1988). We considered stage from 1 to 4 “early stage”, from 5 to 8 “middle stage” and from 9 to 11 “late stage”.

Larvae were photographed through binocular stereo microscope with integrated camera and total length (TL, mm), from the analysis of image performed with suitably calibrated software (Image Pro Plus 6.0, Image Cybernetics, RoperIndistries, Silver-Spring, MD, USA), were obtained. Then, classes length of 1 mm of magnitude were considered.

## 2.2 The remote sensing dataset

We pair the biological dataset with remote sensing data for (Table 1): sea surface temperature (SST), chlorophyll-a concentration (Chl). From these daily satellite data we evaluated cruise-averaged spatial maps (for each environmental parameter) that were superimposed to the entire ichthyoplanktonic data set, for both 2010 and 2011 datasets. This allowed to first recognize the main hydrographic features that occurred at Sicily Channel sea surface and the relations between *Sardinella* eggs and larvae distributions and environmental datasets.

We also analyze wind stress ( $\tau$ ) and Ekman transport ( $M$ ) from remote sensing. These quantities are derived from ocean surface 6-hourly wind data ( $U_{wind}$ ), provided by the Cross-Calibrated Multi-Platform project (Table 1). Wind stress is obtained as

$$\tau = \rho_{air} C_d |U_{wind}| U_{wind}, \quad (1)$$

where  $\rho_{air}$  is the air density and the dimensionless friction coefficient  $C_d = 0.0012$  for  $0 < |U_{wind}| < 11 \text{ m s}^{-1}$  and  $C_d = 0.00049$  for  $|U_{wind}| \geq 11$  (Large and Pond, 1981; Mc-

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Clain and Firestone, 1993). Ekman transport is then calculated as (Pickett and Paduan, 2003)

$$\mathbf{M} = (\rho_{\text{water}} f)^{-1} \boldsymbol{\tau} \times \hat{\mathbf{k}}, \quad (2)$$

where  $\rho_{\text{water}}$  is the water density,  $f$  the Coriolis parameter, and  $\hat{\mathbf{k}}$  is the vertical unit vector.

### 2.3 The Lagrangian simulations

Modern Lagrangian modelling techniques have been developed to simulate tracer trajectories from available velocity fields, such as those provided by the Mediterranean Forecasting System (MFS) (Tonani et al., 2008). Broadly speaking, there are two main issues related to the simulation of transport and mixing of particles from an ocean circulation model: (i) the lack of resolution of meso- and submeso-scale horizontal motions and (ii) the underestimation of the vertical mixing in the upper layer. In our Lagrangian approach both instances are treated by adopting a kinematic Lagrangian modelling strategy. Deterministic chaotic flows work very efficiently at this scope, since they can generate trajectories that accurately simulate the typical small-scale turbulent motions, which in turn affect the dispersion of a given tracer distribution at early stage (Palatella et al., 2014). In order to capture such a dispersion the kinematic velocity field is composed by 2-D or 3-D time oscillating convective cells of various length sizes and with a given spatio-temporal scaling relationship (e.g. Kolmogorov's scaling). Anomalous behaviors due to the "sweeping effect", i.e., a known drawback affecting kinematic simulations of turbulence, are ruled out by adopting the quasi-Lagrangian coordinates technique (Lacorata et al., 2008).

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### 3 Results

#### 3.1 Spatial distribution pattern of eggs and larvae

Ichthyoplanktonic data have revealed the main principal spawning and retention area of *Sardinella aurita* in the Italian side of the Sicily Channel (Fig. 1). The south-eastern part of the Sicilian coastal zone, off Capo Passero, has been identified as the main spawning area for this species (Fig. 1). Indeed this area exhibited the highest value of density of eggs for both years. However, we also found different spatio-temporal patterns of abundance and distribution of eggs and larvae. In 2010 the overall density of eggs and larvae was higher with respect to the 2011 (mean of 36.65 against 14.13 eggs m<sup>-2</sup> in survey 2010 and 2011; mean of 22.83 against 9.38 larvae m<sup>-2</sup> in survey 2010 and 2011). The main abundance differences between 2010 and 2011 in Malta zone (Fig. 1). Eggs and larvae were found also in the northwestern part of the study area, the Adventure bank, although in very low abundance in both years (Fig. 1).

Analysis of staging of eggs revealed a different spatial distribution of stage in the south-eastern part of the Sicily Channel. In 2010, the areas off Capo Passero was characterized by presence of all different stage in similar amounts (early stage: 36 %; middle stage: 38 %; late stage: 26 %). Otherwise, in the region of Malta we observed a predominance of middle and late stage (early stage: 20 %; middle stage: 53 %; late stage: 27 %). Differently, in 2011 we observed a dominance of late stage off Capo Passero (early stage: 7 %; middle stage: 31 %; late stage: 62 %). For this year, we also recognize that *Sardinella aurita* eggs are exclusively found only off Capo Passero, with exception of one eggs of stage 6 (middle stage) on Adventure Bank, and a dominance of late stages was emerged.

Spatial distribution of the length classes of larvae (total length ranging from 2 to 12 mm) are shown in Fig. 2. In 2010 the zone off Capo Passero was characterized by a dimensional range of 3–9 mm while in the zone off Malta we observed larvae longer than 8 mm (range 2–12 mm). This indicates that *Sardinella aurita* larvae did not find the ideal dynamic conditions for a local recruiting and were likely delivered offshore. The

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2011 showed a more different pattern: off Capo Passero we observe larvae that belong to a wide dimensional range (from 2 to 11 mm) while, around Malta, we did not record any larvae.

These evidences mark a joint action of in situ spawning (early stage) and eggs/larvae advection due to mesoscale oceanographic structures. In 2010, the presence of several stages of *Sardinella aurita* eggs and a less dimensional range of larvae in the southeast coast of Sicily could be the mutual effect of:

1. coastal, upwelling induces current from northwest to southeast (Agostini and Bakun, 2002; Cuttitta et al., 2006; Falcini et al., 2015) that delivers those eggs released in the 24–48 h days before along the Sicilian coasta and mixes them with in situ spawning eggs;
2. offshore advection of larvae of dimension higher than 8 mm due to the resulting cold filament that generates off Capo Passero due to the wind forcing.

Conversely, results about distribution of stages of development of eggs and larvae can be explained by the lack of durable oceanographic structure able to remove early life stages of *Sardinella aurita* from the spawning area (i.e., off Capo Passero).

### 3.2 Results from Lagrangian simulations

The hypothesis of a more intense offshore transport of *Sardinella aurita* occurred from the recruiting area of Capo Passero during the summer 2010 with respect to the 2011 is here explored by means of Lagrangian runs (see Fig. 3 and in the Supplement). These simulations confirm the presence of a narrow filament that dynamically connect the spawning/recruiting area off Capo Passero with Malta. During the middle of June 2010 we notice a strong southward advection of particles that are thus delivered to Malta in a few days (i.e.,  $\sim 50$  km in 5 days that corresponds to a surface current of  $\sim 10$  cm s<sup>-1</sup>). In particular, we observe two events with this intensity: one around the 10 June and the second at between the 30 June and the 8 July. The 2011 shows a similar pattern,

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although the southward advection is much weaker with respect to the 2010 case. The comparison between these two scenarios is further stressed by the Eulerian velocity fields, averaged through the two oceanographic surveys (Fig. 4). The 2011 shows a much weaker velocity field – and the absence of the cold filament – that did not deliver larvae offshore.

### 3.3 Results from the satellite datasets

SST and Chl concentration satellite patterns confirm the hypothesis of two different oceanographic conditions in the two study years (Fig. 5). In the 2010 the Sicily Channel was characterized by a colder surface water and a higher Chl concentration (mean SST = 23.59 °C; mean Chl = 0.044  $\mu\text{g L}^{-1}$ ) with compared to the 2011 (mean SST = 25.08; mean Chl = 0.042  $\mu\text{g L}^{-1}$ ). In particular, in 2010 maps evidenced a cold and Chl-rich structure that protrudes offshore from the Capo Passero (Fig. 6a, b). Such a structure is characterized by a SST  $\approx$  23.20 °C and a Chl  $\approx$  0.07  $\mu\text{g L}^{-1}$ , and traces a curved path (i.e., a filament). The 2011 does not show a similar pattern.

In seeking to understand the role of upwelling in the formation of such a cold, Chl-rich filament we find a comforting agreement from wind stress and Ekman transport maps (Supplement). Between the 30 June and 8 July 2010 a significant Ekman transport likely induced the formation of an upwelling induced coastal current (Fig. 6). Although the 2011 is also characterized by strong wind events, it does not record the same persistency that we observe in the 2010, where the Mistral wind steadily blown for 8 days (see in the Supplement).

### 4 The surface cold filament model

Based on our results we reasonably hypothesize that the particularly strong Mistral wind pattern, and thus the offshore Ekman transport, occurred during the summer spawning in 2010, triggered the southward transport of *Sardinella aurita* larvae and

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eggs along the Capo Passero and chlorophyll reach filament. To diagnose this pattern and to provide a mechanistic explanation regarding the link between the wind field and the onset of the cross-shore transport we hire the surface cold filament model (Bignami et al., 2008). Wind forcing can directly produces shelf-blocked jets that are subsequently driven offshore by the general circulation (Crépon and Richez, 1982; McCreary et al., 1989; Salusti, 1998). The model describes the origin to these cold filaments and jets generated by strong input of potential vorticity (PV) into the sea due to upwelling and/or the funneling of strong, cold and short-term wind bursts that blow over a restricted, shallow area of the sea surface near the coast (Holland, 1967). This PV ( $\Pi$ ) increase due to the wind stress ( $\tau$ ) is described by

$$\frac{d\Pi}{dt} = \frac{1}{\rho h} (\nabla \times \tau)_z, \quad (3)$$

where  $\rho$  is the water density,  $h$  is the cold water thickness, and the subscript  $z$  indicates the third component (i.e., the vertical one) of the curl.

Equation (1) can be integrated in order to estimate, and to compare, the amount of PV accumulated on the shelf area during the two summer spawning periods in 2010 and 2011.

$$\Pi = \frac{1}{\rho h} \int_0^t \left( \frac{\partial \tau_y}{\partial x} - \frac{\partial \tau_x}{\partial y} \right) dt \quad (4)$$

Figure 7 shows the temporal integral of the curl of wind stress in Eq. (4) and fully confirms our hypothesis. Based on the surface cold filament model the higher PV (i.e., higher  $(\nabla \times \tau)_z$ ) we observe in the 2010 – with respect to the 2011 – mark the strong role of the wind stress in “loading” the coastal water PV. Once the high PV is set such a strong and localized input does not remain confined to the coastal zone, but propagates offshore as filaments or jets (Bignami et al., 2008).

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## 5 Discussions and conclusions

The dynamics of marine surface layer plays a fundamental, and for many aspects unpredictable, role as far as the life and the evolution of pelagic species are concerned. In the early stage, fish larvae move as passively advected by the currents, and their fate is strictly related to their Lagrangian pathways across the sea and to the selection rules that may strongly affect their population. A systematic study of the dynamical evolution of marine species can only be assessed by means of accurate modeling of velocity fields and Lagrangian transport, as well as by a deep understanding of the physical processes that rule larvae fate and dispersion.

Our work provide some insights regarding the potential of remote sensing and Lagrangian techniques to monitor and predict the abundance of small pelagic larvae in recruiting areas. Cross-shore transport phenomena remove small pelagic eggs and larvae from the main, coastal conveyor belt that would deliver them to the recruiting areas (Garcia-Lafuente et al., 2002; Falcini et al., 2015). Estimating the rate of this removal is at the base of the prediction of the subsequent biomass, especially for short living species.

Our multidisciplinary analysis, by comparing two summer spawning season in 2010 and 2011, shows that intense wind-induced phenomena lead to cross-shore transport of *Sardinella aurita* larvae from the spawning/recruiting area of Capo Passero to Malta. This is the case of 2010, where we observed from the ichthyoplanktonic dataset a large larvae concentration off Malta, also marked by a wide dimensional range. The pairing of Lagrangian runs and the analysis of environmental parameters measured from remote sensing (i.e., SST, Chl, and wind stress) confirms, for this here, the presence of a cold, chlorophyll-rich filament that delivered the larvae to Malta from the Sicilian coast.

To give a mechanistic explanation to these correlations and to provide a diagnostic tool for the understanding of the role of Mistral wind in such a dynamics we make use of a PV theory for the evolution of surface cold filaments. Our application demonstrates

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**Table 1.** Satellite products that are used in this work.  $\Delta t$  and  $\Delta x$  indicate temporal and spatial resolutions, respectively. SST: sea surface temperature Pathfinder V5.2 (PFV52) AVHRR data interpolated through an Optimal Interpolation algorithm (Pisano et al., 2015); Chl: sea surface chlorophyll concentration computed by applying the MedOC4 algorithm (Volpe et al., 2007) to the ESA-CCI remote sensing reflectance (Rrs) spectra (ESA-CCI Rrs results from the merging of SeaWiFS, MODIS-Aqua and MERIS sensors); Ocean Wind: Cross-Calibrated, Multi-Platform Ocean Surface Wind Velocity Product (multi-sensor, made of SeaWinds su QuikSCAT e ADEOS-II, AMSR-E, TRMM TMI, SSM/I); Sea surface geostrophic velocity: multi-mission altimeter products (Saral, Cryosat-2, Jason-1&2, T/P, Envisat, GFO, ERS-1 & 2 and even Geosat).

Parameter	$\Delta t$	$\Delta x$	Data source
SST – Pathfinder V5.2 (PFV52) AVHRR L4 data	daily	4 × 4 km	<a href="http://www.myocean.eu.org/">http://www.myocean.eu.org/</a>
Chl – ESA-CCI-L4 data	daily	4 × 4 km	<a href="http://www.myocean.eu.org/">http://www.myocean.eu.org/</a>
Ocean Wind	daily	25 × 25 km	<a href="http://podaac.jpl.nasa.gov">http://podaac.jpl.nasa.gov</a>

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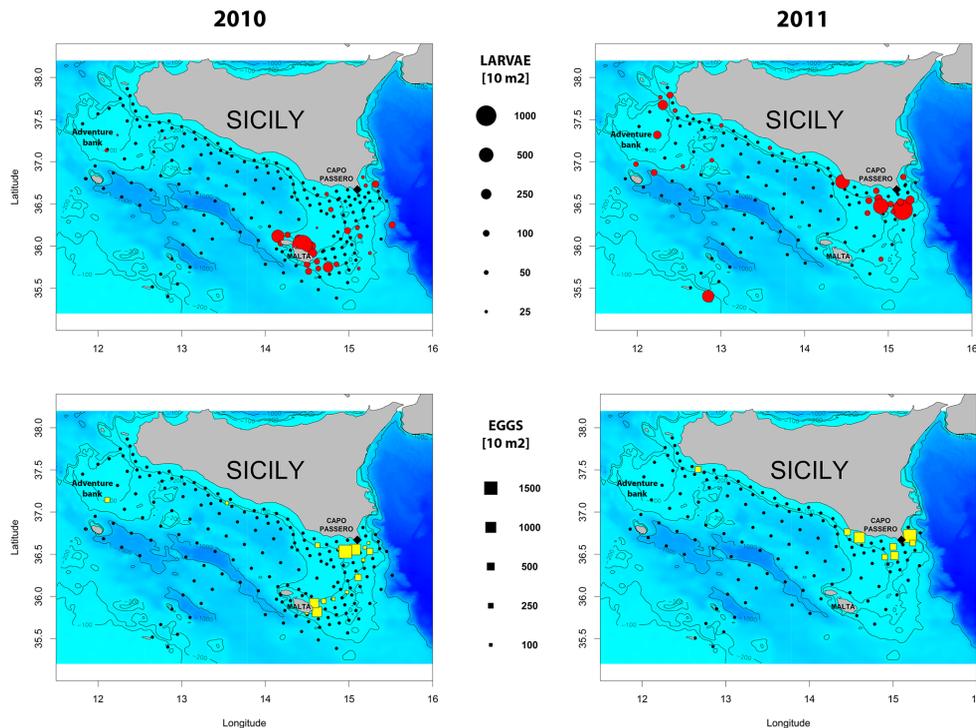
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**Figure 1.** Map of the study area (i.e., Sicily Channel) showing the sampling stations. Bathymetry are indicated contours and by background colors, from cyan (shallower) to blue (deeper). The isobaths of 100, 200 and 1000 m are shown. Red circles and yellow squares represent larval (up) and eggs (down) density of *Sardinella aurita* during 2010 (left) and 2011 (right) surveys, respectively. The main points of interest are indicated (i.e., Adventure Bank Malta, and Capo Passero).

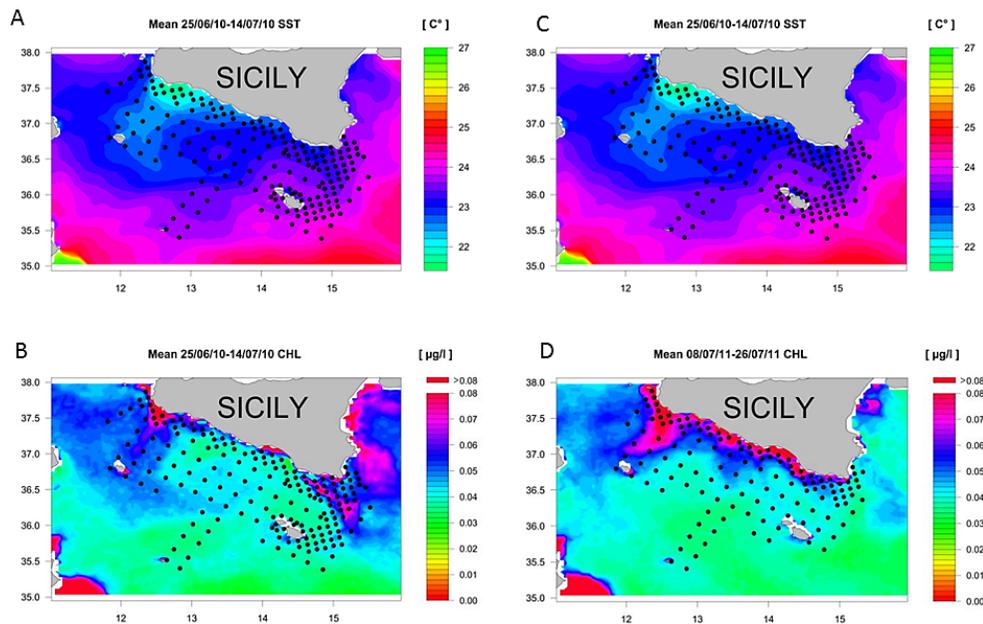






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**Figure 5.** Cruise averaged Sea Surface Temperature (**a, c**) and Chlorophyll concentration (**b, d**) for the two Bansic 2010 and 2011 cruises.

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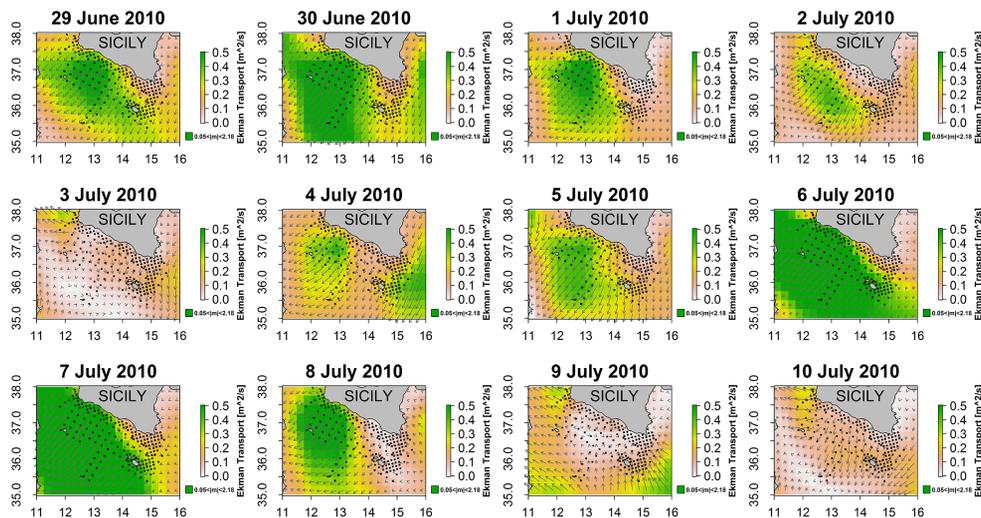
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**Figure 6.** Daily Ekman transport during the Binsic 2010 cruise, from 29 June to 10 July.

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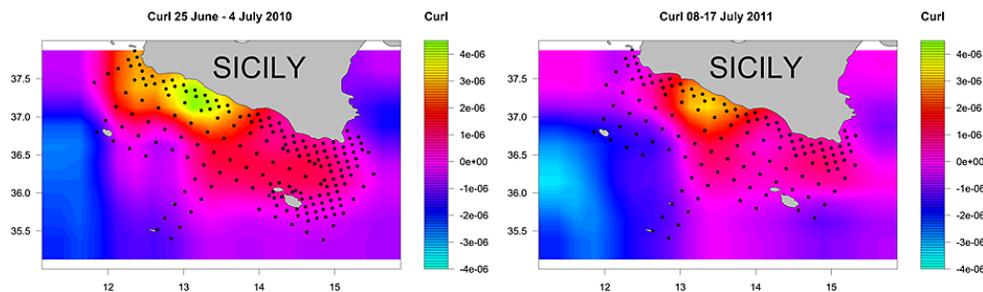
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**Figure 7.** Integral of the curl of wind stress  $\rho h \Pi$  ( $\text{Ns m}^{-3}$ ) (see Eq. 4) performed throughout the Bansic 2010 (25 June–14 July) and Bansic 2011 (8–27 July) cruises showing the more intense potential vorticity increase that occurred in 2010. Such a potential vorticity input led to the formation of the cold filament.

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