RolE of Mixing on phytoplankton bloom initiation, maintEnance and DIssipatiOn in the galician ríaS (REMEDIOS)

The fertilization of phytoplankton by the Iberian upwelling is responsible for the production of \sim 250,000 t year⁻¹ of blue mussels in the Galician Rías. This amount represents 95% of the Spanish and 50% of the European production, respectively. This production is jeopardized every year by toxic phytoplankton blooms. Turbulent mixing is a key process as it controls water renewal time, which in turns determines the rate of exchange of nutrients, organisms and pollutants in the water column. Our current knowledge states that phytoplankton blooms occur when mixing provides the right levels of light and nutrients. However, due to methodological limitations, only very recently we have been able to quantify turbulence in the field. One of the most fascinating implications of this progress is the possibility to revisit classic models of phytoplankton ecology. In 1953 Sverdrup proposed a simple conservation mass model which used the depth of the mixed layer to predict the onset of the North Atlantic spring bloom. This model assumed a thoroughly mixed layer where turbulence was strong enough to distribute the phytoplankton cells evenly through the layer. Following trials, either to verify this hypothesis or to use its theoretical background, have generally forgotten this assumption and used the mixed layer, defined as a layer homogenous in density, as the equivalent of a turbulent or mixing layer. Despite evidence suggesting that vertical mixing controls the annual cycle of biomass and composition of the phytoplankton community in the Galician Rías, its importance has been inferred from hydrographic conditions. So far, a specific study relating mixing and phytoplankton bloom formation is lacking. For the first time we propose to use the theoretical framework of the Sverdrup hypothesis to investigate phytoplankton bloom initiation, maintenance and dissipation in NW Spain. Special attention will be devoted to blooms of species from the genera Dinophysis and Pseudonitzschia, responsible for lengthy shellfish harvesting closures due to accumulation of diarrhetic (DSP) and amnesic (ASP) shellfish poisoning toxins, respectively, above regulatory levels. Previous studies in this region and others in Europe indicate that these species frequently aggregate forming "thin layers". Less than five meters thick and up to several km in horizontal extension, these layers have important implications for the management of molluscan shellfish safety. Despite this, the frequency of occurrence of thin phytoplankton layers in the Galician Rías, and the role of mixing conditions in their formation and persistence remains unknown. This proposal combines field observations in the Galician Rías, time series analysis, and empirical and numerical modeling with the aim of 1) describing the seasonal variability in the sources of turbulence and the magnitude of mixing, 2) investigating the role of mixing on resource availability and phytoplankton bloom initiation, maintenance and dissipation, 3) describing the frequency and spatial distribution of thin layers of phytoplankton (TLP), and 4) investigating the mechanisms responsible for the formation of TLP. The determination of the mechanisms responsible for phytoplankton bloom formation is fundamental to improve our capabilities to predict the toxic events, and contribute to the management and mitigation of their socio-economic impacts in the region.

Keywords: turbulence, mixing, plankton, harmful algae blooms, *Dinophysis, Pseudo-nitzschia,* Galician Rías

La importancia de los procesos de mezcla en el inicio, mantenimiento y declive de floraciones de fitoplancton en las Rías Gallegas (REMEDIOS)

La fertilización del fitoplancton por el afloramiento Ibérico es responsable de la producción de ~250,000 t año⁻¹ de mejillón en las Rías Gallegas. Esta cifra representa el 95% de la producción española y el 50% de la europea. Esta actividad se ve amenazada cada año por floraciones de fitoplancton tóxico. La mezcla es un proceso clave que determina la tasa de renovación del agua, que a su vez controla el intercambio de nutrientes, organismos y contaminantes. En la actualidad se asume que las floraciones de fitoplancton tienen lugar cuando la mezcla proporciona los niveles adecuados de luz y nutrientes. No obstante, debido a limitaciones metodológicas, sólo recientemente ha sido posible cuantificar la mezcla en el medio natural. Una de las implicaciones más apasionantes de este progreso es la posibilidad de revisar modelos ecológicos clásicos. Sverdrup propuso en 1953 un modelo sencillo de conservación de masas que, a partir de la profundidad de la capa de mezcla, predice el inicio de la floración primaveral en el Atlántico Norte. Una de las asunciones del modelo es la existencia de una capa de mezcla activa donde la turbulencia es lo suficientemente intensa para distribuir uniformemente las células en la vertical. Los intentos por verificar esta hipótesis, y por utilizar su marco conceptual, han olvidado en general esta asunción y han utilizado la capa de mezcla, definida como una capa de densidad homogénea, como el equivalente a una capa turbulenta o de mezcla activa. A pesar de las evidencias que sugieren que la mezcla controla el ciclo anual de la composición y biomasa del fitoplancton en las Rías Gallegas, su importancia se ha inferido a partir de condiciones hidrográficas. Por el momento no se ha realizado ningún estudio específico que relacione la formación de floraciones fitoplanctónicas con la mezcla. Por primera vez, proponemos investigar el inicio, mantenimiento y declive de las floraciones de fitoplancton en el NO de España dentro del marco conceptual de la hipótesis de Sverdrup. Nos centraremos en las floraciones de especies de los géneros Dinophysis y Pseudo-nitzschia, responsables de cierres prolongados de extracción de mejillón en las Rías Gallegas por acumulación de toxinas diarreicas (DSP) y amnésicas (ASP), respectivamente. Estudios previos en la región y en otras partes de Europa han mostrado que estas especies se agregan con frecuencia formando "capas finas". Estas capas, con un espesor < 5m y una extensión horizontal de varios km, tienen importantes implicaciones para la gestión de la salubridad de los moluscos bivalvos. A pesar de ello, se desconoce la frecuencia de aparición de capas finas en las Rías Gallegas y el papel desempeñado por los procesos de mezcla en su formación y persistencia. Combinando observaciones de campo en las Rías Gallegas, análisis de series temporales, y diferentes aproximaciones de modelado proponemos: 1) describir la variación estacional de las fuentes de turbulencia y la magnitud de la mezcla 2) investigar el papel de la mezcla en la disponibilidad de recursos y en el inicio. mantenimiento y declive de floraciones de fitoplancton, 3) describir la frecuencia y distribución espacial de capas finas de fitoplancton (TLP), y 4) investigar los mecanismos responsables de la formación de TLP. La determinación de estos mecanismos es fundamental para mejorar la capacidad de predicción de los eventos y contribuir a la gestión y mitigación de su impacto socioeconómico en la región.

C.1. JUSTIFICATION FOR THE COORDINATION

Coastal regions have an importance which is disproportionate to the relatively small area of the global ocean they occupy. They are among the most productive parts of the ocean and support most of the world's major fisheries. As the result of the strength and the variety of the physical forcing, they are considered the most dynamic regions in the ocean. These facts turn coastal regions into a major research challenge which requires interdisciplinary collaboration between physical, chemical and biological disciplines. The main goal of this proposal is to investigate phytoplankton bloom dynamics in the Galician Rías by using the theoretical framework of the Sverdrup hypothesis. This model, formulated more than 60 years ago, and its legacy of more than 50 cites per year, represents one of the best examples of the importance of interdisciplinary collaborations.

Our proposal represents a joined effort of a multidisciplinary team with proved expertise on characterizing turbulence and mixing processes in the ocean, the description of the activity and composition of phytoplankton and zooplankton communities, and the integration of physical and biological observations through empirical and numerical models. The different nature of the ecosystem compartments involved in this proposal, as well as the distinct methodological approaches used to characterize them, motivated us to organize our research team in two groups. The first group, led by UVIGO (Team 1; IP: Beatriz Mouriño), will be responsible for characterizing the physical and chemical environment, and the modeling work. The second team, led by IEO-Vigo (Team 2; IP: Enrique Nogueira), will be focused on characterizing the plankton community, including microphytoplankton and mesozooplankton, using a combination of traditional and new sampling and analytical methods. Both teams will participate on the database analysis, and the integration of physical and biological observations with numerical models, which will promote feedback and collaboration between participants. Both research groups have a broad expertise in their specific tasks. The international collaborations will reinforce the multidisciplinary character of the proposal. The participation of Alberto Naveira-Garabato (SOC, UK), who has a broad experience in the study of ocean mixing processes, will be very valuable to interpret the turbulence processes in the Rías. High vertical resolution sampling of biological properties will be achieved with the advanced instrumentation provided by Marc Sourisseau (IFREMER, France). Finally, the expertise of Peter Franks (SCRIPS, USA), a Biological Oceanographer specialized in physical-biological interactions, will substantially reinforce the modeling team.

In order to coordinate the project activities, three meetings (one per year) are planned. The meetings will be held at the UVIGO videoconference room to facilitate remote online participation. Attendance of foreigner participants is planned for the final meeting at the end of the project.

C.2. SCIENTIFIC PROPOSAL

1. BACKGROUND

1.1 Turbulence, mixing and stratification: related but not the same

Turbulence is the dominant process involved in the transfer of momentum and heat, and in dispersing small organic and inorganic particles in the ocean (Thorpe 2007). One of its most important properties, commonly used to characterize it, is that by generating large gradients of velocity at small scales, it promotes conditions in which viscous dissipation transfer the kinetic energy of turbulent motion into heat. Turbulent fluctuations contribute to the transport of properties by turbulent mixing, at rates far higher than those due to molecular processes alone.

In a stable stratified ocean, when mixing occurs light water is pushed down and heavy water brought up, both moving against buoyancy. This mixing raises the centre of gravity of the water column, and increases its potential energy at the expenses of kinetic energy, which is dissipated when the water is moving up and down. In order to turbulence to happen, a source of kinetic energy strong enough to overcome the existing density stratification is required. Thus, turbulence can be considered as a trade-off between the kinetic (and sometimes potential, see below) energy available to drive the turbulence, and the density stratification that can suppress it (Naveira Garabato, 2009).

Turbulence in the ocean is driven by a combination of buoyancy inputs from surface heat exchange and freshwater runoff, and direct mechanical forcing by the atmosphere and tides. Heat and freshwater exchange do not inject momentum directly, instead they cause changes in density. If these changes are great enough, the surface water become denser than the water below, and thus gravitationally unstable. This process converts the potential energy to kinetic energy generating convectively driven turbulence that, due to the sinking of dense water to the equilibrium depth, is very efficient as a mixing process. Surface heat exchange in the seasonal cycle is generally the predominant buoyancy source, except in estuaries where freshwater discharge may outcompete heat exchange, and tides are the dominant mechanical forcing.

Because turbulence has been extremely difficult to measure in the past, related terms, as for example stratification, have been frequently used to infer the mixing conditions of the water column (Mouriño-Carballido et al. 2016a). However, high stratification does not necessarily mean low mixing. For example, internal waves travelling along the stratified pycnocline can produce a burst of enhanced turbulence and mixing (Villamaña et al., submitted). Only recently, thanks to the development of commercial microstructure turbulence profilers, we have been able to measure turbulence in the field on a regular basis. One of the fascinating implications of this progress is the possibility to revisit classic models in phytoplankton ecology.

1.2 Revisiting a classical model in phytoplankton ecology: the Sverdrup hypothesis

Phytoplankton growth is limited by light and nutrients, and the availability of both resources is strongly dependent on the mixing conditions of the water column. Thus, turbulence and mixing have been frequently invoked in model formulations to explain the behavior of individual phytoplankton cells or collective functional groups.

Based upon his observations in Ría de Vigo, Margalef (1978) proposed that turbulent mixing and nutrient concentration were the two main factors controlling the succession of the main microphytoplankton groups. According to his famous mandala, high turbulence and massive and intermittent nutrients inputs favour large-sized phytoplankton (diatoms), whereas motile species (dinoflagellates) and those with high affinity for nutrients (coccolithophores) dominate in nutrientpoor stratified conditions. If turbulence decays but nutrients remain abundant, an alternative successional route leads to the species forming harmful algal blooms. A number of limitations were noted when applying this approach to the field. First, the Margalef's mandala describes only the succession of vegetative phases of larger-sized microphytoplankton (Wyatt 2014). Moreover, due to the methodological difficulties in quantifying mixing in the field, the validation of Margalef's model was traditionally limited to studies where indirect estimates of nutrient supply were used.

Twenty-five years before Margalef's mandala, Sverdrup (1953) formulated the "critical depth hypothesis" (CDH) to predict the onset of the North Atlantic spring bloom, one of the most dramatic large-scale features in the ocean (Figure 1A). The CDH united concepts of physical and biological oceanography to formulate a quantitative relationship between the initiation of phytoplankton blooms, mixing conditions and resource availability. Sverdrup proposed a simple conservation mass model based on four main premises: 1) phytoplankton is uniformly distributed in the mixed layer, 2) phytoplankton growth at the onset of the bloom is only limited by light (and not by nutrients), 3) cellular growth rate is proportional to the available light which decreases exponentially with depth, and 4) phytoplankton loss rate is independent of depth. The CDH proposed that deep mixed layers during winter keep phytoplankton in an unfavourable light environment and therefore limit production. Each phytoplankton cell receives the average amount of light over the depth of the mixed layer, which (because light decays exponentially with depth) is lower than if phytoplankton was restricted to the euphotic zone. A "critical depth" was defined as the bottom of the layer in which depth-integrated production of organic matter is equal to its destruction by respiration. When surface heating thins the non-stratified mixed layer above the critical depth, phytoplankton has the potential to bloom because growth outweighs looses. Conversely, if phytoplankton is mixed evenly to depths that exceed the critical depth, loss exceeds production and there is a net loss of biomass.

This hypothesis has provided a working and testable framework for understanding the various factors responsible for phytoplankton bloom formation across different aquatic systems worldwide (Fischer et al. 2014). Since its formulation, several studies have attempted to verify the CDH in the field with mixed results. By using mixed-layer climatologies and satellite data, several studies found evidence supporting the CDH hypothesis (Siegel et al. 2002; Brody et al. 2013; Chiswell et al. 2013; Brody and Lozier 2014, 2015), whereas other studies rejected it based on the observation that phytoplankton growth rate was positive during deep winter mixing (Acuña et al. 2010; Behrenfeld 2010; Boss and Behrenfeld 2010; Behrenfeld et al. 2013; Behrenfeld and Boss 2014). The observation that phytoplankton blooms sometimes occur in the apparent absence of water column stratification (Townsend et al. 1992; Ellertsen 1993; Backhaus et al. 1999; Körtzinger et al. 2008) led to propose alternative bloom formation processes. Nutrients have been frequently invoked as a factor controlling spring bloom formation (Franks 2002; Moore et al. 2006). In recent years, the combination of new technologies and observational tools, and the synthesis of previous ideas have produced new hypothesis. Consistent with the Sverdrup hypothesis, Mahadevan et al. (2012) showed that the onset of the phytoplankton bloom south of Iceland occurs with the onset of stratification. However, stratification development is due to mesoscale eddies pushing well-mixed water into a shallow zone,

rather than by warming, as proposed by the CDH. This results in patchy blooms beginning ca. 30 days earlier than would have occurred by warming. The Dilution Recoupling Hypothesis proposed by Behrenfeld (2010) argues that, contrary to the CDH, bloom initiation takes place in the winter because deep mixing dilutes phytoplankton cell density, thus reducing the encounter rate between predator and prey. Finally, the Critical Turbulence Hypothesis (CTH, Huisman et al., 1999) proposed that if turbulence is low enough, phytoplankton in the well-lit surface layer could bloom independently of the thickness of the mixed layer (Figure 1B).



Figure 1A. Schematic of Sverdrup's Critical Depth Hypothesis: when mixing is deeper than the critical depth, there is net loss, so a bloom is not expected (left ellipse); if the mixing depth is shallower than the critical depth, there is excess production and a bloom can occur (right ellipse). The "critical depth" is the bottom of the layer within which the integrated production equals integrated respiration (dashed line). B) The Critical Turbulence Hypothesis: even if mixing is deeper than the critical depth, a bloom can form if the rate of mixing is slow enough that phytoplankton are retained in sunlit waters for suitably long periods (right ellipse). Modified from Fischer et al (2015).

1.3 The Critical Turbulence Hypothesis: mixed but not mixing

Sverdrup (1953) was aware of the distinction between a mixed layer, defined by a vertical density or temperature criterion, and the intensity of vertical diffusivity (K) in that mixed layer. However, by explicitly assuming it was large enough to be ignored, he avoided the need to include K in his model. According to the CTH, a bloom can develop in a mixed water column if the timescale for phytoplankton growth is less than the cell's residence time in the portion of the water column where net growth can be achieved. The decrease in turbulence has been associated with a reduction in air-sea fluxes that generally precedes mixed-layer shoaling at the end of the winter (Taylor and Ferrari 2011), and lowered wind stress (Chiswell 2011; Chiswell et al. 2013). Moreover, Brody and Lozier (2014) proposed that changes in the depth, rather than in the time scale, of turbulent mixing, could be a better predictor for the onset of the bloom. According to these authors, the decrease in mixing length scale is a better predictor for bloom initiation than the decrease in mixed-layer depth, the onset of positive heat fluxes, or decreases in wind strength. They also found that the shift from buoyancy-driven to wind-driven mixing in late winter creates the decrease in mixing length scale, and thus the conditions necessary for blooms to begin (Brody and Lozier 2015).

The residence time of a particle in a layer is inversely proportional to K. Thus, a vertical gradient in the residence time of phytoplankton would result from a vertical gradient in K or turbulence, influencing the availability of light for the cells. Franks (2015) emphasizes that testing the CDH, or using its theoretical background, requires measurements of the actual turbulence, rather than the hydrographic results of the mixing, i.e., the mixed layer. He also notes that it is crucial to determine, not only the intensity of turbulence, but also its vertical structure and temporal variability, which are strongly dependent on the source of energy that drives the turbulence, and the sources of stratification that can suppress it. The several mechanisms responsible for the generation of turbulence have specific vertical distribution of turbulence intensity (see Figure 1 in Franks (2015)), what means that phytoplankton cells would have different residence times through the water column. Moreover, heat-driven vertical density gradients (Long et al. 2012) can form on time scales of hours and spatial scales of kilometers. Due to local heating, rainy storms and river discharge, or slumping of horizontal gradients due to wind-forcing and the vertical shear of near-inertial waves (Hosegood et al.

2006), the turbulent layer can shoal tens of meters providing a quiescent environment for phytoplankton growth.

The only way to accurately determinate the existence of a thoroughly mixed-layer is by using measurements of microstructure turbulence *in situ*. For this reason, except for one study (Mahadevan et al. 2012), the CDH has not been comprehensively verified in the field (*Franks*, 2015)).

1.4. Role of mixing on the formation of phytoplankton blooms in the Galician Rías

The Galician Rías are located in the northern boundary of the Canary Current upwelling system, one of the main coastal eastern boundary upwelling ecosystems (Arístegui et al. 2009). During the springsummer months this region is characterized by intense and intermittent upwelling events (Fraga 1981). The prevailing northerly winds cause Ekman transport of surface water away from the coast, and the rise of cold nutrient-enriched subsurface waters which stimulate phytoplankton growth and support a highly productive food web (Fréon et al. 2009). By contrast, southerly winds and downwelling conditions predominate in winter.

The annual cycle of phytoplankton biomass corresponds to a typical temperate shelf sea, with the development of the spring and autumn blooms (Nogueira et al. 1997; Cermeño et al. 2006). Short-term and interanual variability of the upwelling regime and runoff pulses induce considerable variability on this average seasonal pattern (Nogueira et al. 2000; Nogueira and Figueiras 2005). In general, vertical mixing and low irradiance levels during winter preclude the accumulation of phytoplankton biomass, and cause low surface chlorophyll vales (<1 mg m⁻³). Increased irradiance and water column stabilization, induced by haline stratification and the progression of the seasonal thermocline, cause enhanced phytoplankton biomass in early spring (~10 mg chl-*a* m⁻³). Chlorophyll values stay ~5 mg m⁻³ during summer upwelling. The autumn bloom usually exhibits higher values than the spring maximum, and its formation has been attributed to advection of phytoplankton inside the Ría, due to the reversed estuarine circulation characteristic of downwelling periods (Figueiras et al. 2008). Both maxima are also characterized by different species composition (see below). The upward trend observed in phytoplankton biomass from the onset of thermal stratification (around May) until early autumn frequently manifests as a series of peaks (Nogueira and Figueiras 2005).

The role of the seasonal cycle of vertical mixing on the composition of the phytoplankton assemblage is well documented in this region (Figueiras and Rios 1993; Nogueira and Figueiras 2005; Figueiras et al. 2008; Pitcher et al. 2010). Large diatoms are dominant during early upwelling events in spring, when stratification is weak and nutrient availability high. During summer, when stratification and the relevance of regenerated nutrients increase, smaller-sized diatoms and heterotrophs coexist. Dinoflagellates, some of them potentially producers of harmful algae blooms (HAB), characterize the autumn-downwelling transition. Winter is characterized by small flagellates and benthic diatoms. Superimposed on the described seasonal variability is smaller temporal and spatial scale dynamics, primarily driven by wind forcing and circulation reversals, that creates smaller-scale successions in the dominance of phytoplankton groups.

Several potentially harmful microalgae are common in the Galician Rías under suitable hydrographic conditions (Trainer et al. 2010). More than one species of Pseudo-nitzschia spp. may produce domoic acid, which cause Amnesic Shellfish Poisoning (ASP) in humans, birds and marine mammals. The impact of high biomass blooms of *Pseudo-nitzschia* spp. is specially hard on scallops, due to their low depuration rate for domoic acid and related compounds (Salgado et al. 2003). Eight potentially toxic species of *Pseudo-nitzschia* have been reported in the Galician Rías, but short-lasting ASP outbreaks have been mainly associated with high concentrations of *Pseudo-nitzschia australis* (Míguez et al. 1996; Fraga et al. 1998). Hydrodynamic and meteorological conditions have been identified as main factors responsible for the short-term temporal and spatial variability in the distribution of Pseudo-nitzschia spp. (Velo-Suárez 2008; Velo-Suárez et al. 2010; Díaz et al. 2014), and the interannual variability of ASP outbreaks (Álvarez-Salgado et al. 2011). ASP outbreaks caused by Pseudo-nitzschia australis (Míguez et al. 1996), coinciding with or followed by Diarrhetic Shellfish Poisoning (DSP) outbreaks caused by Dinophysis acuminate (Reguera et al. 2003), are frequently associated with thermohaline stratification during the early upwelling season (March-June). Low biomass (< 10^3 cells L⁻¹) blooms of *Dinophysis* species pose the main threat for the Galician mussel industry. Blooms of Dinophysis acuminata followed some years by Dinophysis acuta may cause shellfish harvesting closures up to 9 months long in "hot spots" of Ría de Vigo and Ría de Pontevedra (Reguera et al. 2014). Their growth season and advection is tightly coupled to the upwelling season, their cell maxima being associated with relaxation and downwelling (Escalera et al. 2010; Velo-Suárez et al. 2014).

Despite evidence that vertical mixing controls the annual cycle of phytoplankton biomass and community composition in this system, its role as a driving mechanism has been inferred so far from hydrographic (temperature and salinity) conditions, and thus a specific study relating turbulence, stratification and mixing to phytoplankton bloom formation is lacking.

1.5 Thin layers of phytoplankton: concept, formation mechanisms and evidence of occurrence in the Galician Rías

Thin layers of phytoplankton (TLP) are a particular case of phytoplankton blooms that occur when large numbers of photosynthetic cells are located within a narrow depth interval. These layers may extend horizontally for several kilometers and persist for days (Durham and Stocker 2012) (Figure 2). A number of independent criteria have been proposed for their definition, most of which share three requirements (Dekshenieks et al. 2001; Sullivan et al. 2010b): a) the cells aggregation must be spatially and temporally persistent (e.g., observed in two subsequent vertical profiles), b) the vertical extension must be lower than a threshold (e.g. 5 m), and c) the maximum concentration must exceed a threshold (e.g., three times the background).



Figure 2A. Thin layers observed in 1967 off La Jolla, California. The black line shows the continuous vertical chlorophyll concentration profile measured using a submersible pump and a ship-based fluorometer. The red dashed line shows the profile obtained using values from discrete depths, mimicking what would be obtained from bottle casts. (b) Thin layers of chlorophyll (Chl), likely dominated by the flagellate Akashiwo sanguinea, observed at night in Monterey Bay using an autonomous underwater vehicle. (c) Concurrent measurements revealing that the upper portion of the water column was depleted of nitrate. Modified from Durham and Stocker (2012).

TLP influence ecological processes in the ocean in many ways. As trophic hotspots, they can mediate the survival and reproduction rates of organisms belonging to higher trophic levels (Lasker 1975). Many toxic species, including *Pseudo-nitzschia australis* (McManus et al. 2008) and *Dinophysis* spp. (Farrell et al. 2012), form TLP. Thin layers are believed to play an important role in the long-term maintenance and sudden formation of HAB. The transport of subsurface thin layers of harmful species, located in the mouth of the Rías, to inshore surface areas by upwelling events has been proposed as one of the seeding processes responsible for the formation of HAB in the region (Sellner et al. 2003). Monitoring programs based on oceanographic-bottle samples taken at specific depth intervals, or integrated water-column samples collected by hoses, might sampled inadequately the water column and miss thin layers (McManus et al. 2008; Velo-Suárez 2008; Escalera et al. 2012), offering little time to alert shellfish managers.

Diverse biophysical mechanisms can be responsible for the formation of TLP (Durham & Stocker 2012). Straining of phytoplankton patches by shear occurs because horizontal gradients of scalars can be transformed into vertical gradients, due to vertical gradients in the horizontal velocity field (Osborn 1998; Birch et al. 2008). Shear can favor thin layer formation via straining or gyrotactic trapping (see below), but it can also trigger hydrodynamic instabilities and turbulence that dissipate layers. Thin layer formation can be induced by an active swimming response toward a preferred nutrient (Ryan et al. 2010), or life prey in the case of mixotrophic dinoflagellates such as *Dinophysis* spp. (Velo-Suarez et al. 2014), light (Sullivan et al. 2010a) and salinity level (Harvey and Menden-Deuer 2011). Nonmotile phytoplankton can also control their vertical position by regulating their buoyancy through gas vacuoles (Walsh 1971), and carbohydrate ballasting (Villareal and Carpenter 2003). The gyrotactic trapping proposed by Durham et al. (2009) describes that vertical gradients in shear trigger the formation of thin layers of motile phytoplankton by disrupting their vertical migration. Layer formation by in situ growth occurs when growth is stimulated over a small depth interval due to favourable light and nutrients levels (Birch et al. 2008). Finally, intrusion can generate TLP through the horizontal transport of nutrients or phytoplankton into adjacent waters (Steinbuck et

al. 2010). This mechanism has been attributed to the formation of thin layers in estuarine systems where saltwater, containing nutrient-limited marine phytoplankton, mix with nutrient-replete freshwater (Kasai et al. 2010). Each mechanism produces distinctive layer characteristics and correlations with the physical conditions that can help to diagnose the processes at play in the field. However, identification of the mechanisms responsible for the formation of TLP is rarely achieved exclusively by observations. Theoretical models are crucial to identify candidate mechanisms and to rule out those that are incompatible with field data.

A limited number of studies have described the occurrence of TLP in the Galician Rías so far. During May-June 2005 in Ría de Pontevedra, Velo-Suárez et al. (2008) described the formation of a TLP of toxin producing *Pseudo-nitzschia* spp. *and Chaetoceros socialis* associated with steep pycnoclines developed after upwelling events, and their displacement toward the bottom during downwelling. The fact that the location of the layer was associated with maxima levels of shear and stratification is consistent with the mechanism of straining by shear playing a major role on its formation (Velo-Suárez et al. 2010). A later study carried out at the same station in May-June 2007 noted the relevance of considering the role of short-term variability, as the tidal cycle was observed to modulate the formation and dissipation of TLP of *Pseudo-nitzschia* populations (Díaz et al. 2014). Despite this evidence, the frequency of occurrence of TLP in the Galician Rías, and the role of mixing conditions in their formation and persistence remains unknown.

1.6 Recent results from the research group relevant for the present proposal

During the last ten years members of the Team 1 have collected observations of microstructure turbulence in contrasting hydrographic regimes, including open-ocean and coastal domains, with the motivation of understanding different physical and biogeochemical processes, and their influence on plankton communities. Measurements collected in the tropical and subtropical regions of the Atlantic, Indian and Pacific oceans allowed us to describe the regional variability in the magnitude of turbulent mixing, and to identify the mechanical and convective sources of turbulence in the upper boundary layer and the ocean interior (Fernández-Castro et al. 2014). Knowing the magnitude of turbulence mixing and the origin of the different sources was crucial to quantify the amount of nitrate pumped into the euphotic zone through diffusive processes, and to evaluate the relevance of biological N_2 fixation as a source of new nitrogen in different ecosystems (Mouriño-Carballido et al. 2011; Fernández-Castro et al. 2015).

This valuable dataset of microturbulence observations allowed us to investigate, for the first time, the influence of mixing and nutrient supply on smaller-sized picoplankton groups in the context of Margalefs mandala (Otero-Ferrer et al. 2015; Mouriño-Carballido et al. 2016a). Our results indicate that, in agreement with Margalef's work, picophytoplankton groups exhibit contrasting responses to changes in nitrate supply. Chemostat experiments confirmed that these differences are the result of differential nutrient uptake ability by picoplankton groups (Mouriño-Carballido et al. 2016b).

Observations carried out in the Galician Rías, in the framework of previous projects (CHAOS IP: B. Mouriño, STRAMIX IP: M. Gil-Coto, ASIMUTH), unveil the large range of variability scales influencing turbulence and mixing conditions, and their role on the accumulation, composition and vertical distribution of phytoplankton communities. The higher internal wave activity observed during spring tides on the shelf off Ría de Vigo causes a significant increase, compared to neap tides, in mixing and nitrate diffusive fluxes (Villamaña et al., submitted). This nitrate supply could contribute to explain the continuous dominance of large-sized phytoplankton during the upwelling favorable season (April-September). Evidence for the seasonal variability in mixing conditions, and their role on the formation of phytoplankton blooms, are provided by data collected inside Ría de Vigo (Figure 3A, unpublished results). Over the annual cycle mixed layers, identified as homogenous layers in density, did not correspond to active and vertical homogenous mixing layers. Vertical gradients in both temperature and salinity contributed to the variability in stratification, which exhibited minima values in February. As a result of the variability in vertical stratification and microstructure turbulence, maxima values of mixing were observed in February through the water column, and at the bottom during intense upwelling events (July). Maxima values of chlorophyll were observed in July, probably due to the increase in nutrients supply by diffusive and advective processes driven by upwelling (Cermeño et al., submited), and at the surface in May, probably related to an increase in vertical stability and the availability of light. Preliminary analysis of these data points out to the role of mixing in the vertical distribution of phytoplankton cells. In Ría de Vigo chlorophyll maxima were in general located at depths where mixing was low (Figure 3A). A thin layer of chlorophyll was observed at ca. 20 m in March, just below a region of minimum mixing. The role of mixing in the persistence and destruction of a TLP was also clearly observed from the data collected in Ría de Pontevedra in June 2013 by both teams (Figure 3B, unpublished results). A TLP was observed during about 36 hours in close association with the minimum of mixing observed at the seasonal pycnocline. The layer was destroyed between 22:00 and 7:00 when an increase in mixing was observed, and reformed again after that.



Figure 3A. Temperature, salinity, squared Brünt-Väissälä frequency, dissipation rates of turbulent kinetic energy, vertical diffusivity and chlorophyll concentration sampled by a microstructure profiler in Ría de Vigo between 12/02/2012 and 24/01/13. The black line represents the mixed layer depth computed as the depth where sigma-t differs 0.125 from the 10 m value. B) Vertical distribution of fluorescence, squared Brünt-Väissälä frequency, and inverse Ozmidov scale at station P2 in Ría de Pontevedra in June 2013.

In the framework of the REIMAGE project, members of Team 1 have implemented a four grid configuration of the Regional Ocean Modeling System (ROMS-AGRIF), coupled to a biogeochemical model to Rías of Pontevedra and Vigo and adjacent shelf. The model reproduces successfully seasonal variability of observed vertical distribution of temperature, salinity, horizontal currents, inorganic nutrients and phytoplankton biomass (unpublished results).

Members of Team 2 have conducted several studies focused on bloom dynamics (Alvarez et al. 2009; Acuña et al. 2010), and the relationship between environmental factors and phytoplankton community structure (Nogueira et al. 2000; Nogueira and Figueiras 2005) and zooplankton dynamics (Buttay et al. 2015). The team has contrasted experience in the use of traditional and new technologies to characterize the plankton community. Semi-automatic methods have been applied for the characterization of phytoplankton (Álvarez et al. 2011, 2012, 2016; Álvarez et al. 2013) and mesoplankton communities (Nogueira et al. 2004; Cabal et al. 2008; Vandromme et al. 2014). Studies on autoecology and bloom dynamics, with special focus on *Dinophysis* and *Pseudo-nitzschia* species have been carried out, led by B. Reguera, within the framework of EU projects ASIMUTH and HABIT, national projects PHYCODISIS and TURECOTOX and the ongoing RETOS2013 DINOMA. Observations during previous cruises using high resolution instruments in collaboration with IFREMER-Brest have confirmed the formation of thin layers of *Pseudo-nitzschia* in the Galician Rías (Velo-Suárez 2008; Díaz et al. 2014) and of Dinophysis in Galicia, Bay of Biscay and Celtic Sea (Velo-Suárez 2008; Farrell et al. 2012, 2014). Preliminary results suggest TLP of Pseudo-nitzschia and the haptophytes are controlled by physics (optimal depth for shear rate, maximum density gradient...) whereas biological factors and behaviour (phototactism, search of ciliate prey, active aggregation for mating purposes) are more likely to be the driving mechanisms within active swimmers, such as mixotrophic *Dinophysis* spp. requiring life prev and inorganic nutrients (Gonzalez-Gil et al. 2010; Reguera et al. 2012). Results from seasonal and interannual studies provided evidence on the close association between *Dinophysis* cell maxima and stability, but responses need to be analyzed at species-specific level (Diaz et al. 2013, 2016).

REFERENCES (*Indicate publications from the research group)

*Acuña, J., M. López-Alvarez, E. Nogueira, and F. González-Taboada. 2010. Diatom flotation at the onset of the spring phytoplankton bloom: an in situ experiment. Mar. Ecol. Prog. Ser. 400: 115– 125.

- *Álvarez, E., Á. López-Urrutia, and E. Nogueira. 2012. Improvement of plankton biovolume estimates derived from image-based automatic sampling devices: Application to FlowCAM. J. Plankton Res. 34: 454–469.
- *Álvarez, E., Á. López-Urrutia, E. Nogueira, and S. Fraga. 2011. How to effectively sample the plankton size spectrum? A case study using FlowCAM. J. Plankton Res. **33**: 1119–1133.
- Álvarez, E., X. A. G. Morán, Á. López-Urrutia, and E. Nogueira. 2016. Size-dependent photoacclimation of the phytoplankton community in temperate shelf waters (Southern Bay of Biscay). Mar Ecol Prog Ser **in press**: 73–87.
- *Alvarez, E., M. Moyano, A. Lopez-Urrutia, E. Nogueira, and R. Scharek. 2013. Routine determination of plankton community composition and size structure: A comparison between FlowCAM and light microscopy. J. Plankton Res. **36**: 170–184.
- *Alvarez, E., E. Nogueira, J. L. Acuña, M. Lopez-Alvarez, and J. A. Sostres. 2009. Short-term dynamics of late-winter phytoplankton blooms in a temperate ecosystem (Central Cantabrian Sea, Southern Bay of Biscay). J. Plankton Res. **31**: 601–617.
- *Álvarez-Salgado, X. A., S. Beloso, I. Joint, E. Nogueira, L. Chou, F. F. Pérez, S. Groom, J. M. Cabanas, a. P. Rees, and M. Elskens. 2002. New production of the NW Iberian shelf during the upwelling season over the period 1982-1999. Deep. Res. Part I Oceanogr. Res. Pap. 49: 1725–1739.
- Álvarez-Salgado, X. A., F. G. Figueiras, M. J. Fernández-Reiriz, U. Labarta, L. Peteiro, and S. Piedracoba. 2011. Control of lipophilic shellfish poisoning outbreaks by seasonal upwelling and continental runoff. Harmful Algae 10: 121–129.
- *Álvarez-Salgado, X. A., F. G. Figueiras, F. F. Pérez, S. Groom, E. Nogueira, A. . Borges, L. Chou, C. . Castro, G. Moncoiffé, A. . Ríos, A. E. . Miller, M. Frankignoulle, G. Savidge, and R. Wollast. 2003. The Portugal coastal counter current off NW Spain: new insights on its biogeochemical variability. Prog. Oceanogr. 56: 281–321.
- Arístegui, J., E. D. Barton, X. A. Álvarez-Salgado, a. M. P. Santos, F. G. Figueiras, S. Kifani, S. Hernández-León, E. Mason, E. Machú, and H. Demarcq. 2009. Sub-regional ecosystem variability in the Canary Current upwelling. Prog. Oceanogr. 83: 33–48.
- Backhaus, J. O., H. Wehde, E. N. Hegseth, and J. Kämpf. 1999. "Phyto-convection": The role of oceanic convection in primary production. Mar. Ecol. Prog. Ser. 189: 77–92.
- Behrenfeld, M. J. 2010. Abandoning Sverdrup's Critical Depth Hypothesis on phytoplankton blooms. Ecology **91**: 977–89.
- Behrenfeld, M. J., and E. S. Boss. 2014. Resurrecting the Ecological Underpinnings of Ocean Plankton Blooms. Ann. Rev. Mar. Sci. 6: 167–194.
- Behrenfeld, M. J., S. C. Doney, I. Lima, E. S. Boss, and D. A. Siegel. 2013. Annual cycles of ecological disturbance and recovery underlying the subarctic Atlantic spring plankton bloom. Global Biogeochem. Cycles 27: 526–540.
- *Birch, D. A., W. R. Young, and P. J. S. Franks. 2008. Thin layers of plankton: Formation by shear and death by diffusion. Deep Sea Res. Part I Oceanogr. Res. Pap. 55: 277–295.
- Bjornsen, P. K., and T. G. Nielsen. 1991. Decimeter scale heterogeneity in the plankton during a pycnocline bloom of Gyrodinium aureolum. Mar. Ecol. Prog. Ser. **73**: 263–267.
- Boss, E., and M. Behrenfeld. 2010. In situ evaluation of the initiation of the North Atlantic phytoplankton bloom. Geophys. Res. Lett. **37**: n/a–n/a.
- Brody, S., and M. S. Lozier. 2015. Characterizing upper-ocean mixing and its effect on the spring phytoplankton bloom with in situ data. ICES J. Mar. Sci. **72**: 1961–1970.
- Brody, S. R., and M. S. Lozier. 2014. Changes in dominant mixing length scales as a driver of subpolar phytoplankton bloom initiation in the North Atlantic. Geophys. Res. Lett. 41: 3197– 3203.
- Brody, S. R., M. S. Lozier, and J. P. Dunne. 2013. A comparison of methods to determine phytoplankton bloom initiation. J. Geophys. Res. Ocean. **118**: 2345–2357.
- *Buttay, L., A. Miranda, G. Casas, G.-Q. Rafael, and E. *. 2015. Long-term and seasonal zooplankton dynamics in the northwest Iberian shelf and its relationship with meteo-climatic and hydrographic variability. J. Plankton Res. **in press**: 1–16.
- *Cabal, J., G. González-Nuevo, and E. Nogueira. 2008. Mesozooplankton species distribution in the NW and N Iberian shelf during spring 2004: Relationship with frontal structures. J. Mar. Syst. 72: 282–297.
- Cermeño, P., E. Marañón, V. Pérez, P. Serret, E. Fernández, C. G. Castro. 2006. Phytoplankton size structure and primary production in a highly dynamic. coastal ecosystem (Ria de Vigo, NW-Spain): Seasonal and short-time scale variability. Estuar. Coast. Shelf Sci. 67: 251–266.

- *Cermeño P., P. Chouciño, B. Fernández-Castro, F. G. Figueiras, E. Marañón, C. Marrasé, B. Mouriño-Carballido, M. Pérez-Lorenzo, T. Rodríguez-Ramos, I. G. Teixeira, S. M. Vallina. Biodiversity enhances marine primary productivity through selection effect (Marine Ecology Progress Series, submitted).
- Chiswell, S. 2011. Annual cycles and spring blooms in phytoplankton: don't abandon Sverdrup completely. Mar. Ecol. Prog. Ser. **443**: 39–50.
- Chiswell, S. M., J. Bradford-Grieve, M. G. Hadfield, and S. C. Kennan. 2013. Climatology of surface chlorophyll a, autumn-winter and spring blooms in the southwest Pacific Ocean. J. Geophys. Res. Ocean. 118: 1003–1018.
- Dekshenieks, M. M., P. L. Donaghay, J. M. Sullivan, J. E. B. Rines, T. R. Osborn, and M. S. Twardowski. 2001. Temporal and spatial occurrence of thin pytoplankton layers in relation to physical processes. Mar. Ecol. Prog. Ser. 223: 61–71.
- *Díaz, P. ., M. Ruiz-Villarreal, Y. Pazos, T. Moita, and B. Reguera. 2016. Climate variability and Dinophysis acuta blooms in an upwelling system. Harmful Algae **53**: 40–52.
- *Díaz, P. A., B. Reguera, M. Ruiz-Villarreal, Y. Pazos, L. Velo-Suarez, H. Berger, and M. Sourisseau. 2013. Climate variability and oceanographic settings associated with interannual variability in the initiation of dinophysis acuminata blooms. Mar. Drugs **11**: 2964–2981.
- *Díaz, P. a., M. Ruiz-Villarreal, L. Velo-Suárez, I. Ramilo, P. Gentien, M. Lunven, L. Fernand, R. Raine, and B. Reguera. 2014. Tidal and wind-event variability and the distribution of two groups of Pseudo-nitzschia species in an upwelling-influenced Ría. Deep. Res. Part II Top. Stud. Oceanogr. **101**: 163–179.
- Durham, W. M., J. O. Kessler, and R. Stocker. 2009. Disruption of vertical motility by shear triggers formation of thin phytoplankton layers. Science **323**: 1067–70.
- Durham, W. M., and R. Stocker. 2012. Thin phytoplankton layers: characteristics, mechanisms, and consequences. Ann. Rev. Mar. Sci. 4: 177–207.
- Ellertsen, H. C. 1993. Spring blooms and stratification. Nature 363: 24.
- *Escalera, L., Y. Pazos, M. Dolores Doval, and B. Reguera. 2012. A comparison of integrated and discrete depth sampling for monitoring toxic species of Dinophysis. Mar. Pollut. Bull. **64**: 106–113.
- *Escalera, L., B. Reguera, T. Moita, Y. Pazos, M. Cerejo, J. M. Cabanas, and M. Ruiz-Villarreal. 2010. Bloom dynamics of Dinophysis acuta in an upwelling system: In situ growth versus transport. Harmful Algae **9**: 312–322.
- *Farrell, H., P. Gentien, L. Fernand, P. Lazure, M. Lunven, A. Youenou, B. Reguera, and R. Raine. 2014. Vertical and horizontal controls of a haptophyte thin layer in the Bay of Biscay, France. Deep. Res. Part II Top. Stud. Oceanogr. 101: 80–94.
- *Farrell, H., P. Gentien, L. Fernand, M. Lunven, B. Reguera, S. González-Gil, and R. Raine. 2012. Scales characterising a high density thin layer of Dinophysis acuta Ehrenberg and its transport within a coastal jet. Harmful Algae **15**: 36–46.
- *Fernández-Castro, B., B. Mouriño-Carballido, V. M. Benítez-Barrios, P. Chouciño, E. Fraile-Nuez, R. Graña, M. Piedeleu, and a. Rodríguez-Santana. 2014. Microstructure turbulence and diffusivity parameterization in the tropical and subtropical Atlantic, Pacific and Indian Oceans during the Malaspina 2010 expedition. Deep Sea Res. Part I Oceanogr. Res. Pap. 94: 15–30.
- *Fernández-Castro, B., B. Mouriño-Carballido, E. Marañón, P. Chouciño, J. Gago, T. Ramírez, M. Vidal, a. Bode, D. Blasco, S.-J. Royer, M. Estrada, and R. Simó. 2015. Importance of salt fingering for new nitrogen supply in the oligotrophic ocean. Nat. Commun. **6**: 8002.
- *Figueiras, F. G., A. Miranda, I. Riveiro, A. Vergara, and C. Guisande. 2008. El plancton de la Ría de Vigo, p. 111–152. *In* A. González-Garcés, F. Vilas, and X.A. Alvarez-Salgado [eds.], La Ría de Vigo: Una aproximación integral al ecosistema marino de la Ría de Vigo. Instituto de Estudios Vigeses.
- Figueiras, F. G., and A. F. Rios. 1993. Phytoplankton succession, red tides and the hydrographic regime in the Rı´as Bajas of Galicia, p. 239–244. *In* T.J. Smayda and Y. Shimizu [eds.], Toxic Phytoplankton Blooms in the Sea. Elservier Science Publishers.
- Fischer, A. D., E. A. Moberg, H. Alexander, E. F. Brownlee, K. R. Hunter-Cevera, K. J. Pitz, S. Z. Rosengard, and H. M. Sosik. 2014. Sixty years of Sverdrup: A retrospective of progress in the study of phytoplankton blooms. Oceanography 27: 222–235.
- Fraga, F. 1981. Upwelling off the Galician Coast, Northwest Spain. Coast. Estuarines Sci. 1.
- Fraga, S., M. J. Alavarez, A. Míguez, M. L. Fernández, E. Costas, and V. López-Rodas. 1998. Pseudonitzschia species isolated from Galician waters: toxicity, DNA content and lectin binding assay, p. 270–273. *In* Harmful Algae. Xunta de Galicia. IEO UNESCO.

- *Franks, P. 2015. Has Sverdrup's critical depth hypothesis been tested? Mixed layers vs. turbulent layers. ICES J. Mar. Sci. J. ... 72: 1897–1907.
- *Franks, P. J. S. 2002. NPZ models of plankton dynamics: Their construction, coupling to physics, and application. J. Oceanogr. **58**: 379–387.
- Fréon, P., M. Barange, and J. Arístegui. 2009. Eastern Boundary Upwelling Ecosystems: Integrative and comparative approaches. Prog. Oceanogr. 83: 1–14.
- *Gonzalez-Gil, S., L. Velo-Suarez, P. Gentien, I. Ramilo, and B. Reguera. 2010. Phytoplankton assemblages and characterization of a Dinophysis acuminata population during an upwellingdownwelling cycle. Aquat. Microb. Ecol. 58: 273–286.
- Harvey, E. L., and S. Menden-Deuer. 2011. Avoidance, movement, and mortality: The interactions between a protistan grazer and Heterosigma akashiwo, a harmful algal bloom species. Limnol. Oceanogr. 56: 371–378.
- Hosegood, P., M. C. Gregg, and M. H. Alford. 2006. Sub-mesoscale lateral density structure in the oceanic surface mixed layer. Geophys. Res. Lett. **33**: L22604.
- Huisman, J., P. van Oostveen, and F. J. Weissing. 1999. Critical depth and critical turbulence: Two different mechanisms for the development of phytoplankton blooms. Limnol. Oceanogr. 44: 1781–1787.
- Ji, R., C. S. Davis, C. Chen, D. W. Townsend, D. G. Mountain, and R. C. Beardsley. 2007. Influence of ocean freshening on shelf phytoplankton dynamics. Geophys. Res. Lett. **34**: 1–5.
- Ji, R., C. S. Davis, C. Chen, D. W. Townsend, D. G. Mountain, and R. C. Beardsley. 2008. Modeling the influence of low-salinity water inflow on winter-spring phytoplankton dynamics in the Nova Scotian Shelf–Gulf of Maine region. J. Plankton Res. **30**: 1399–1416.
- Kasai, A., Y. Kurikawa, M. Ueno, D. Robert, and Y. Yamashita. 2010. Salt-wedge intrusion of seawater and its implication for phytoplankton dynamics in the Yura Estuary, Japan. Estuar. Coast. Shelf Sci. 86: 408–414.
- Körtzinger, A., U. Send, R. S. Lampitt, S. Hartman, D. W. R. Wallace, J. Karstensen, M. G. Villagarcia, O. Llinás, and M. D. DeGrandpre. 2008. The seasonal p CO 2 cycle at 49°N/16.5°W in the northeastern Atlantic Ocean and what it tells us about biological productivity. J. Geophys. Res. 113: C04020.
- Lasker, R. 1975. Field criteria for survival of anchovy larvae: the relation between inshore chlorophyll maximum layers and successful first feeding. Fish. Bull. **73**: 453–462.
- Long, M. C., L. N. Thomas, and R. B. Dunbar. 2012. Control of phytoplankton bloom inception in the Ross Sea, Antarctica, by Ekman restratification. Global Biogeochem. Cycles **26**: GB1006.
- Mahadevan, A., E. D'Asaro, C. Lee, and M. J. Perry. 2012. Eddy-Driven Stratification Initiates North Atlantic Spring Phytoplankton Blooms. Science **337**: 54–58.
- Margalef, R. 1978. Life-forms of phytoplankton as survival alternatives in an unstable environment. Oceanol. Acta 1: 493–509.
- McManus, M. a., R. M. Kudela, M. W. Silver, G. F. Steward, P. L. Donaghay, and J. M. Sullivan. 2008. Cryptic blooms: Are thin layers the missing connection? Estuaries and Coasts 31: 396– 401.
- Miguez, A., M. L. Fernández, and S. Fraga. 1996. First detection of domoic acid in Galicia (NW of Spain), p. 143–145. *In* T. Yasumoto, Y. Oshima, and Y. Fukuyo [eds.], Intergovernmental Oceanographic Commission of UNESCO.
- Moore, C. M., M. M. Mills, A. Milne, R. Langlois, E. P. Achterberg, K. Lochte, R. J. Geider, and J. La Roche. 2006. Iron limits primary productivity during spring bloom development in the central North Atlantic. Glob. Chang. Biol. 12: 626–634.
- *Moreira-Coello, V., B. Mouriño-Carballido, E. Marañón, A. Fernández, P. Chouciño, M. M. Varela, and A. Bode. 2015. Nitrogen fixation in the upwelling region off NW Iberia. 2015 Aquatic Sciences Meeting.
- *Mouriño-Carballido, B., R. Graña, A. Fernández, A. Bode, M. Varela, J. F. Domínguez, J. Escánez, D. de Armas, and E. Marañón. 2011. Importance of N-2 fixation vs. nitrate eddy diffusion along a latitudinal transect in the Atlantic Ocean. Limnol. Oceanogr. 56: 999–1007.
- *Mouriño-Carballido, B., E. Hojas, P. Cermeño, P. Chouciño, B. Fernández-Castro, M. Latasa, E. Marañón, X. A. G. Morán, and M. Vidal. 2016a. Nutrient supply controls picoplankton community structure during three contrasting seasons in the northwestern Mediterranean Sea. Mar. Ecol. Prog. Ser., doi:10.3354/meps11558
- *Mouriño-Carballido, B., S. Susana Agustí, A. Bode, P. Cermeño, P. Chouciño, J. C. B. da Silva, B. Fernández-Castro, J. M. Gasol, M. Gil Coto, R. Graña, M. Latasa, L. M. Lubián, E. Marañón, X. A. G. Morán, E. Moreno-Ostos, V. Moreira-Coello, J. L. Otero-Ferrer, M. Ruiz-Villarreal, R.

Scharek, S. M. Vallina, M. M. Varela, and M. Villamaña-Rodríguez. 2016b. Nutrient supply does play a role on the structure of marine picophytoplankton communities. *Ocean Sciences Meeting 2016 (New Orleans, USA)*.

- *Naveira Garabato, A. 2009. Energetic of ocean mixing. Encyclopedia of Ocean Sciences, 2nd ed. -Steele, J.H., Turekian, K.K. and Thorpe, S.A. (eds.) 261-270.
- *Nogueira, E., and F. G. Figueiras. 2005. The microplankton succession in the Ría de Vigo revisited: species assemblages and the role of weather-induced, hydrodynamic variability. J. Mar. Syst. 54: 139–155.
- *Nogueira, E., G. González-Nuevo, A. Bode, M. Varela, X. A. G. Mor??n, and L. Vald??s. 2004. Comparison of biomass and size spectra derived from optical plankton counter data and net samples: Application to the assessment of mesoplankton distribution along the Northwest and North Iberian Shelf. ICES J. Mar. Sci. **61**: 508–517.
- *Nogueira, E., F. Ibanez, and F. G. Figueiras. 2000. Effect of meteorological and hydrographic disturbances on the microplankton community structure in the Ria de Vigo (NW Spain). Mar. Ecol. Prog. Ser. **203**: 23–45.
- *Nogueira, E., F. F. Perez, and A. F. Rios. 1997. Seasonal patterns and long-term trends in an estuarine upwelling ecosystem (Ria de Vigo, NW Spain). Estuar. Coast. Shelf Sci. 44: 285–300.
- Obata, A., J. Ishizaka, and M. Endoh. 1996. Global verification of critical depth theory for phytoplankton bloom with climatological in situ temperature and satellite ocean color data. J. Geophys. Res. **101**: 20657.
- Osborn, B. T. 1998. Finestructure, microstructure, and thin layers. Oceanography 36-43.
- *Otero-Ferrer, J. L., B. Mouriño-Carballido, P. Cermeño, S. Agustí, A. Bode, P. Chouciño, B. Fernández-Castro, J. M. Gasol, M. Latasa, L. M. Lubián, E. Marañón, X. A. G. Morán, V. Moreira-Coello, E. Moreno-Ostos, M. M. Varela, and M. Villamaña-Rodríguez. 2015. Which factors control the picoplankton community structure in the ocean? ASLO Aquatic Sciences Meeting 2015 (Granada, Spain).
- Pitcher, G. C., F. G. Figueiras, B. M. Hickey, and M. T. Moita. 2010. The physical oceanography of upwelling systems and the development of harmful algal blooms. Prog. Oceanogr. **85**: 5–32.
- *Reguera, B., E. Garcés, Y. Pazos, I. Bravo, I. Ramilo, S. González-Gil, and others. 2003. Cell cycle patterns and estimates of in situ division rates of dinoflagellates of the genus Dinophysis by a postmitotic index. Mar. Ecol. Prog. Ser. **249**: 117–131.
- *Reguera, B., P. Riobo, F. Rodriguez, P. A. Diaz, G. Pizarro, B. Paz, J. M. Franco, and J. Blanco. 2014. Dinophysis toxins: Causative organisms, distribution and fate in shellfish. Mar. Drugs 12: 394–461.
- *Reguera, B., L. Velo-Suarez, R. Raine, and M. G. Park. 2012. Harmful Dinophysis species: A review. Harmful Algae 14: 87–106.
- Ryan, J. P., M. a. McManus, and J. M. Sullivan. 2010. Interacting physical, chemical and biological forcing of phytoplankton thin-layer variability in Monterey Bay, California. Cont. Shelf Res. **30**: 7–16.
- *Salgado, C., J. Maneiro, J. Correa, J. L. Pérez, and F. Arévalo. 2003. ASP biotoxins in scallops: The practical application in galicia of commission decision 2002/226/EC, p. 169–177. *In* A. Villaba, B. Reguera, J.L. Romalde, and R. Beiras [eds.], Molluscan Shellfish Safety. Xunta de Galicia Intergovernmental Oceanographic Commission of UNESCO.
- Sathyendranath, S., R. Ji, and H. Browman. 2015. Revisiting Sverdrup's critical depth hypothesis. ICES J. Mar. Sci. J. du Cons. **72**: 1892–1896.
- Sellner, K. G., G. J. Doucette, and G. J. Kirkpatrick. 2003. Harmful algal blooms: causes, impacts and detection. J. Ind. Microbiol. Biotechnol. 30: 383–406.
- Siegel, D. a, S. C. Doney, and J. a Yoder. 2002. The North Atlantic spring phytoplankton bloom and Sverdrup's critical depth hypothesis. Science **296**: 730–733.
- Steinbuck, J. V., A. Genin, S. G. Monismith, J. R. Koseff, R. Holzman, and R. G. Labiosa. 2010. Turbulent mixing in fine-scale phytoplankton layers: Observations and inferences of layer dynamics. Cont. Shelf Res. 30: 442–455.
- Stramska, M., and T. D. Dickey. 1993. Phytoplankton bloom and the vertical thermal of the upper ocean structure. J. Mar. Res. 819–842.
- Sullivan, J. M., P. L. Donaghay, and J. E. B. Rines. 2010a. Coastal thin layer dynamics: Consequences to biology and optics. Cont. Shelf Res. 30: 50–65.
- Sullivan, J. M., M. A. McManus, O. M. Cheriton, K. J. Benoit-Bird, L. Goodman, Z. Wang, J. P. Ryan, M. Stacey, D. Van Holliday, C. Greenlaw, M. A. Moline, and M. McFarland. 2010b. Layered organization in the coastal ocean: An introduction to planktonic thin layers and the

LOCO project. Cont. Shelf Res. 30: 1–6.

- Sverdrup, H. U. 1953. On Conditions for the Vernal Blooming of Phytoplankton. J. du Cons. **18** : 287–295.
- Taylor, J. R., and R. Ferrari. 2011. Shutdown of turbulent convection as a new criterion for the onset of spring phytoplankton blooms. Limnol. Oceanogr. **56**: 2293–2307.
- Thorpe, S. A. 2007. An introduction to ocean turbulence, Cambrige.
- Townsend, D. W., M. D. Keller, M. E. Sieracki, and S. G. Ackleson. 1992. Spring Phytoplankton Blooms in the Absence of Vertical Water Column Stratification. Nature **360**: 59–62.
- *Trainer, V. L., G. C. Pitcher, B. Reguera, and T. J. Smayda. 2010. The distribution and impacts of harmful algal bloom species in eastern boundary upwelling systems. Prog. Oceanogr. 85: 33–52.
- Vallina, S. M., and R. Simó. 2007. Strong relationship between DMS and the solar radiation dose over the global surface ocean. Science **315**: 506–508.
- *Vandromme, P., E. Nogueira, M. Huret, Lopez-Urrutia, G. González-Nuevo González, M. Sourisseau, and P. Petitgas. 2014. Springtime zooplankton size structure over the continental shelf of the Bay of Biscay. Ocean Sci. **10**: 821–835.
- *Velo-Suárez, L. 2008. Thin layers of Pseudo-nitzschia spp . and the fate of Dinophysis acuminata during an upwelling downwelling cycle in a Galician Ria. Limnol. Oceanogr. **53**: 1816–1834.
- *Velo-Suárez, L., L. Fernand, P. Gentien, and B. Reguera. 2010. Hydrodynamic conditions associated with the formation, maintenance and dissipation of a phytoplankton thin layer in a coastal upwelling system. Cont. Shelf Res. **30**: 193–202.
- *Velo-Suárez, L., S. Gonzalez-Gil, Y. Pazos, and B. Reguera. 2014. The growth season of Dinophysis acuminata in an upwelling system embayment: A conceptual model based on in situ measurements. Deep. Res. Part II Top. Stud. Oceanogr. **101**: 141–151.
- *Villamaña M., Mouriño-Carballido B., Marañón E., Cermeño P., Chouciño P., da Silva J., Díaz P., Fernández-Castro B., Gil Coto M., Graña R., Latasa M., Magalhaes J., Otero-Ferrer J.L., Reguera B., Scharek R. Role of internal waves on mixing, nutrient supply and phytoplankton community structure during spring and neap tides in the Ría de Vigo (NW Iberian Peninsula) (Limnology and Oceanography, submitted).
- Villareal, T. a., and E. J. Carpenter. 2003. Buoyancy regulation and the potential for vertical migration in the oceanic cyanobacterium Trichodesmium. Microb. Ecol. **45**: 1–10.
- Walsh, J. J. 1971. Relative Importance of Habitat Variables in Predicting the Distribution of Phytoplankton at the Ecotone of the Antarctic Upwelling Ecosystem. Ecol. Monogr. 41: 291– 309.
- Wyatt, T. 2014. Margalef's mandala and phytoplankton bloom strategies. Deep Sea Res. Part II Top. Stud. Oceanogr. **101**: 32–49.

2. HYPOTHESES AND GENERAL OBJECTIVES

The fertilizing effect of the Iberian upwelling is responsible for the production of more than 250.000 tons of mussels per year in the Galician Rías. This amount represents 95% of the Spanish production and about half of the mussels produced in Europe. Approximately 400,000 people make their living from fishing or performing services that help the industry. Every year blooms of harmful algae threaten the economic viability of shellfish production. The determination of the mechanisms responsible for phytoplankton bloom formation is fundamental to gaining the required predictive capacity to assist the management and mitigation strategies. The need for the proper management of shell seas is a strong motivation for understanding their functioning and developing predictive models of how they will respond to future scenarios.

The main goal of this proposal is to investigate the mechanisms responsible for the initiation, maintenance and dissipation of phytoplankton blooms in the Galician Rías by using, for the first time, the theoretical framework included in the critical depth hypothesis (Sverdrup 1953). The collection of microstructure turbulence observations will allow us to quantify the magnitude of mixing in the field, which in turn will provide accurate estimates of light and nutrient availability for phytoplankton cells. The analysis of the microphytoplankton community at different organization levels, from aggregated properties to species, and high temporal and vertical resolution will allow us to investigate plankton dynamics and parameterize the physical-biological coupling. Because of its relevance as potential producers of HAB, particular attention will be devoted to *Pseudo-nitzschia* spp. and *Dinophysis* spp., which are responsible, respectively, of ASP and DSP outbreaks in this region. Previous evidence that these groups frequently bloom in thin layers, adding complications to monitoring programs and shellfish management, lead us to specifically investigate the characteristics (i.e. frequency of occurrence, intensity, spatial distribution, horizontal extension and persistence) of TLP in the region.

Our particular hypotheses are:

H1. Mixed layers, identified as homogenous layers in density, are not homogenous layers in mixing in the Galician Rías.

H2. Mixing, through its role on nutrients and light availability, controls phytoplankton bloom dynamics (initiation, maintenance and dissipation) and community assembly in the Galician Rías.

H3. *Pseudo-nitzschia* spp. and *Dinophysis* spp. frequently bloom in thin layers in the Galician Rías. H4. The relative importance of straining and turbulent mixing controls the formation of TLP of *Pseudo-nitzschia* spp. in the Galician Rías.

H5. Adaptive ecophysiological traits, such as motility and community interactions, control the formation of TLP of *Dinophysis* spp. in the Galician Rías.

In order to test these hypotheses we propose a combination of data base analysis, field observations, and modeling techniques to address the following *general objectives*:

O1. To describe seasonal variability in the sources of turbulence and the magnitude of mixing in Ría de Vigo.

O2. To investigate the role of mixing on resource availability and phytoplankton bloom dynamics, with special interest in the autoecology of *Pseudo-nitzschia* and *Dinophysis* spp. populations, in Ría de Vigo.

O3. To describe the characteristics (timing, persistence, location in the water column, horizontal extension) of thin layers of phytoplankton, with special interest in *Pseudo-nitzschia* spp. and *Dinophysis* spp, in the Galician Rías and adjacent shelf.

O4. To investigate the mechanisms responsible for the initiation, maintenance and dissipation of thin layers of phytoplankton in the Galician Rías and adjacent shelf.

The present proposal will contribute to attain several objectives that have been given high priority by the Spanish National Plan for Scientific and Technical Research and Innovation (2013-2016) in its Challenges for the Society Research Programme. In particular, this proposal fits within the Challenge 'Seguridad y calidad alimentaria, sostenibilidad de recursos naturales, investigación marina y marítima' (Section 6.4.2 of the Plan), such as 'to improve the management of natural marine resources as well as the ecosystems of coasts, seas and oceans'. In addition, our proposal will help to attain several objectives of the Challenge 'Acción sobre cambio climático y eficiencia en la utilización de recursos', which includes within its goals (Section 6.4.5) 'to generate scientific knowledge on the effects of climate change (...) including the processes, mechanisms and interactions in the oceans and marine ecosystems'.

The proposal will also contribute to the objectives of the EU Horizon 2020 research programme as delineated in its challenge on 'Food security, sustainable agriculture and forestry, marine and maritime and inland water research and the bio-economy". This challenge highlights the need to 'optimizing the sustainable contribution of fisheries and aquaculture to food security, boosting innovation through blue biotechnologies and fostering cross-cutting marine and maritime research to harness the potential of Europe's oceans, seas and coasts for jobs and growth.'

The results obtained will be relevant for the implementation of the Marine Strategy Framework Directive (MSFD, 2008/56/EC), for those indicators of environmental status based on the plankton component of the ecosystem. In that context, the proposal will be relevant in relation to the following MSFD descriptors and their associated indicators: 1) biodiversity of pelagic habitats (descriptor D1), with associated indicators based on the dynamics of functional groups, phytoplankton and zooplankton biomass and abundance, and diversity indices; 2) food-web status (D4), with indicators related to phytoplankton bloom dynamics and the occurrence of HAB.

Finally, the scientific objectives of this project coincide with some of the research priorities identified by the International Oceanographic Commission of UNESCO (IOC, http://www.unesco.org/new/en/natural-sciences/ioc-oceans/). In particular, the Harmful Algal Bloom Program highlights the need to 'foster the effective management of, and scientific research on, harmful algal blooms in order to understand their causes, predict their occurrences, and mitigate their effects'.

3. SPECIFIC OBJECTIVES

1. Field observations: characterization of the seasonal cycle

In order to investigate the seasonal variability in the magnitude and the sources of turbulence, and the factors responsible for microphytoplankton dynamics, during 2017 we plan to visit weekly (every Wednesday) a central station in the middle part of Ría de Vigo (40 m depth, station EF from the INTECMAR monitoring programme), on board R/V Kraken (from the Marine Science Station of Toralla-ECIMAT, University of Vigo). This is an 8-m long vessel equipped with a CTD, Niskin sampling bottles and phyto-zooplankton nets, suitable for sampling in shallow coastal waters. Sampling will include the deployment of a microstructure profiler and the collection of samples for the determination of inorganic nutrients, microphytoplankton and mesozooplankton community organization, from aggregated properties to species level, and phytoplankton production. We will profit from the monitoring carried out in Ría de Vigo by the Xunta de Galicia monitoring programme -INTECMAR on Monday every week, to acquire water samples at EF for the characterization of the microphytoplankton community at three depth strata. On board R/V Kraken samples to characterize the plankton community will be initially collected at the surface and at the fluorescence maximum. Additional sampling depths will be added if observations the previous Monday (i.e. alerts of HAB outbreaks) from the INTECMAR confirm an increasing trend of the target HAB species. In order to characterize horizontal currents, an ADCP (Acoustic Doppler Current Profiler) will be moored at EF station during the sampling period (ca. 365 days).

Main specific objectives of this work package are:

O1.1. Determination of the variability in the horizontal currents in Ría de Vigo during the 2017 seasonal cycle (Team 1, IP: B. Mouriño).

O1.2. Analysis of the contribution of mechanical and convective sources of turbulence (Team 1, IP: Beatriz Mouriño).

O1.3. Characterization of the variability in the magnitude of turbulence and mixing (Team 1, IP: B. Mouriño).

O1.4. Determination of the variability in phytoplankton primary production rates and photosynthetic parameters (Teams 1).

O1.5. Determination of the dynamics of the microphytoplankton community at different organization levels (from aggregated properties to species), using a combination of traditional and novel methods (imaged-based and molecular) (Team 2, IP: E. Nogueira).

O1.6. Analysis of water-column integrated mesozooplankton community and estimation of the community grazing impact on primary production by food removal method (supported by image-based methods) (Team 2, IP: E. Nogueira).

O1.7. Characterization of the in situ vertical distribution of >100 μ m plankton derived from profiling by LOPC (laser optical plankton counter) (Team 2, IP: E. Nogueira).

O1.8. Analysis of the variability of plankton community attributes (from micro to mesoplankton), such as diversity and size-structure (Team 2, IP: E. Nogueira).

O.1.9. Estimation of growth rates of phytoplankton derived from measurements of ¹⁴C incorporation and population dynamics (image-based biovolume-to-carbon conversion -FlowCAM) (**Both Teams**).

O1.10. Determination of the role of light and nutrients availability, computed considering the mixing conditions of the water column, in phytoplankton blooms dynamics and community assembly (**Both Teams**).

2. Database analysis: characterization of thin phytoplankton layers (TLP)

Several time series databases are available to analyze the main characteristics of phytoplankton thin layers in the Galician Rías. INTECMAR (Instituto Tecnolóxico para o Control do Medio Mariño de Galicia, http://www.intecmar.org/Default.aspx?language=en) carries out weekly samplings in 43 oceanographic stations in the Rías of Vigo, Pontevedra, Arousa, Muros-Noia and Ares-Betanzos. At each station hydrographic conditions, nutrients and abundance of harmful phytoplankton species (integrated tube samples) are characterized. The high sampling frequency and spatial resolution provide a highly valuable database to characterize phytoplankton thin layers based on the CTD casts (temperature, salinity and fluorescence). The RADIALES (http://www.seriestemporales-ieo.com, Instituto Español de Oceanografía) programme has been monitoring coastal-shelf waters of NW Spain for the last 25 years. Core observations include hydrographic, biogeochemical and plankton community structure (phytoplankton and mesozooplankton) at monthly frequency in four (Ría de Vigo) and five (Ría da Coruña) oceanographic stations. These observations will be complemented with buoy and satellite information available for the region.

Main specific objectives of this work package are:

O2.1. Analysis of the characteristics (frequency of occurrence, spatial distribution and intensity) of TLP in the Galician Rías and adjacent shelf **(Both teams)**.

O2.2. Identification of favorable meteo-hydrographic conditions for the formation of TLP (Both teams).

O2.3. Analysis of the relationship between the occurrence of TLP, the abundance of *Pseudo-nitzschia* spp., and the frequency of shellfish harvesting closures (**Both teams**).

3. Field observations: dynamics of TLP observed in spring

A 21-d cruise is planned on board R/V Ángeles Alvariño (or alternatively R/V Ramón Margalef) in order to investigate the mechanisms responsible for the dynamics (i.e. formation, maintenance and dissipation) of TLP in the Galician Rías. The location of the sampling area will be decided based on the information provided by the analysis of the frequency of occurrence and spatial distribution of TLP in this region (specific objective O2.1). Ideally, the timing of the cruise should be chosen based on the information provided by the database analysis. However, due to the necessity to schedule ship-time, we request this sampling to happen during the period between mid May and mid June 2018, according to previous observations of TLP occurrence in the Galician Rías (Velo-Suárez 2008; Velo-Suárez et al. 2010; Díaz et al. 2014). Three weeks of ship time are necessary to capture a whole upwelling-relaxation or upwelling-downwelling cycle, mostly occurring within a 30 days time-window in this region (Álvarez-Salgado et al. 2002, 2003), which seems to be crucial for the evolution of TLP (Velo-Suárez et al. 2010).

The sampling design for the cruise will be as follows:

Week 1: By using a SCAN undulator we will prospect the spatial distribution of hydrographic conditions and chlorophyll distributions in the target Ría and adjacent shelf. Information from the INTECMAR monitoring on hydrographic conditions (CTD casts), phytoplankton community composition and HAB outbreaks occurrence previous to and during the cruise will be used to guide the sampling. Special attention will be paid to the vertical and horizontal distribution of fluorescence maxima. By collecting water samples at the fluorescence maximum we will determine the species composition of the phytoplankton assembly. The analysis of these data will allow us to select more precisely the location for the high vertical resolution sampling based on the presence of a TLP, ideally formed by *Pseudo-nitzschia* spp. Once we choose the location we will deploy at this spot an ADCP mooring and a CTD multiparameter autonomous profiler with fluorescence sensor included.

Week 2: During the second week we will study the temporal evolution of physical, chemical and biological properties associated to the TLP. This sampling will include high temporal and vertical resolution data of microstructure turbulence, inorganic nutrients; and micro-phytoplankton and meso-zooplankton species composition and activity (primary and secondary production). The IFREMER particle-size analyzer profiler (IPSAP) and a Fine-Scale Sampler (FSS) will be used to detect and sample fine-scale vertical distribution of microphytoplankton, particularly of *Pseudo-nitzschia* spp.

Week 3: By using again the SCAN undulator we will sample the spatial distribution of hydrographic and fluorescence conditions at the end of the sampling period. Finally, we will recover the ADCP mooring and the CTD multiparameter autonomous profiler.

Main specific objectives of this work package are:

O3.1. Mapping of the horizontal distribution of TLP in the target Ría and adjacent shelf in late spring (Team 1, IP: B. Mouriño).

O3.2. Description of the temporal variability in horizontal currents and mixing during the transition upwelling-relaxation-downwelling (Team 1, IP: B. Mouriño).

O3.3. Analysis of the variability in light and nutrients availability (Team 1, IP: B. Mouriño).

O3.4. Determination of the variability in phytoplankton primary production rates and photosynthetic parameters (Both Teams).

O3.5. Analysis of the fine-scale vertical and temporal dynamics of the microphytoplankton community, whit special attention to those species forming TPL (**Team 2, IP: E. Nogueira**).

O3.6. Analysis of the temporal variability in the fine-scale vertical distribution of the target genus *Pseudo-nitzschia* using molecular techniques and electron microscopy (**Team 2, IP: E. Nogueira**).

O3.7. Analysis of the temporal variability of *Dinophyisis spp.* and its physiological status (Team 2, IP: E. Nogueira).

O3.8. Characterization of the species composition of the mesozooplankton community and its vertical and temporal dynamics (Team 2, IP: E. Nogueira).

O3.9. Determination of estimates of mesozooplankton grazing rates (Team 2, IP: E. Nogueira).

4. Modeling

4.1 Seasonal variability of mixing conditions and phytoplankton growth

The ROMS circulation model coupled to the PISCES biogeochemical model developed, by members of Team 1, for the Rías of Pontevedra and Vigo in previous proposals will be used to investigate the mechanisms responsible for phytoplankton bloom dynamics in the Galician Rías. The current implementation of ROMS used by these researchers uses the K-profile parameterization (KPP) (*Large et al., 1994*), that may need a tuning of the closure model in estuaries and regions of freshwater influence [Burchard et al., 2008]. Recent versions of ROMS incorporate the Generic Length Scale approach (GLS) turbulence model, that may be more suitable for estuaries (*Warner et al.* 2005a, 2005b). The high frequency (once a week) observations of microstructure turbulence planned for the first year of this proposal, during a complete seasonal cycle, represent a great opportunity to validate the performance of the turbulence models. At the same time the high frequency observations of microphytoplankton and mesozooplankton activity and composition will be used to constrain the biogeochemical model compartments.

Main specific objectives of this work package are:

O4.1. Comparison of modelled and observed phytoplankton biomass and growth (Both Teams).

O4.2. Analysis of the mechanisms responsible for phytoplankton bloom dynamic (Team 1, IP: B. Mouriño).

O4.3. Comparison of diapycnal diffusivity values derived from microstructure observations and model results, by using KPP and GLS turbulence closure models, for Ría de Vigo during 2017 (Team 1, IP: **B. Mouriño**).

4.2. Mechanisms responsible for the formation of TLP

Field observations, planned for the high-frequency resolution sampling in spring, will be combined with theoretical models proposed for straining (Birch et al. 2008; Stacey et al. 2007), convergence by buoyancy or swimming (Stacey et al. 2007), gyrotactic trapping by shear (Durham et al. 2009), and biological processes to investigate the mechanisms responsible for the formation of TLP in the Galician Rías.

Main specific objectives of this work package are:

O4.4. Determination of the processes responsible for the formation of TLP in the Galician Rías by using a combination of theoretical models and field observations (**Team 1, IP: B. Mouriño**).

3. METHODOLOGY

1. Observations in the field

During the seasonal study (weekly sampling on board R/V Kraken) and/or the spring cruise (21-d sampling on board R/V Ángeles Alvariño) we will carry out the following tasks:

Task 1.1 Hydrography, irradiance and fluorescence (Team 1, IP: B. Mouriño).

Responsible: B. Mouriño (Participants: B. Fernández, J.L. Otero, M. Villamaña, V. Moreira, hired technician)

Objectives: O1.10, O3.3

Vertical casts with a high-precision CTD probe, included in the microstructure profiler, will be used to obtain the vertical variability in temperature, salinity and fluorescence. The vertical distribution of incident irradiance will be determined with a Li-Cor spherical PAR sensor connected to a datalogger.

Task 1.2. Measurements of dissipation rates of turbulent kinetic energy (ε) (Team 1, IP: B. Mouriño). Responsible: B. Mouriño, (Participants: A. Naveira, B. Fernández, J.L. Otero, M. Villamaña, V. Moreira, hired technician)

Objectives: 01.2, 01.3, 01.4, 03.2, 03.3, 04.3, 04.4

Measurements of dissipation rates of turbulent kinetic energy (ϵ) will be conducted by using a microstructure profiler (MSS, ISW Wassermesstechnik, Prandke and Stips (1998), with a vertical resolution of 1 m. The profiler is equipped with two-velocity microstructure shear sensors (type PNS98), a microstructure temperature sensor, a sensor to measure horizontal acceleration of the profiler, and high-precision CTD sensors. The profiler is balanced to have negative buoyancy and a sinking velocity of ~0.4-0.7 m s⁻¹. The data sampling frequency is 1024 Hz. The calibration of the shear sensors will be performed just before the cruises and the sensitivity checked during data processing. Due to significant turbulence generation close to the ship, data shallower than 4 m will be discarded. Data processing will be carried out with the commercial MSSpro software, which includes removal of spiky data as described in detail in Mouriño-Carballido et al. (2011).

Task 1.3 Acoustic Doppler Current Profiler (ADCP) (Team 1, IP: B. Mouriño).

Responsible: M. Gil Coto (Participants: B. Fernández, hired technician)

Objectives: 01.1, 03.2, 04.1, 04.2, 04.4

During the seasonal study, horizontal currents will be registered at EF station to characterize the hydrodynamic conditions (tidal modulations, vertical shear, and residual currents). An ADCP will be bottom deployed and upward looking, recording profiles of 3D velocities every 10-20 minutes in vertical layers of 0.75-1 m thick, from ~4 meters above the bottom till ~8 meters below the sea surface. The ADCP will be recovered every 3-4 months to change the batteries and download the stored data. An acoustic release will be used in the deployment design to facilitate the field operations. The probability of losing or damaging the equipment is not negligible during one year mooring. If something goes wrong, information about the seasonal variability of horizontal currents at this station will be obtained from the 420 days ADCP mooring deployed in the framework of the STRAMIX project. During the spring cruise, the same sampling scheme will be used for recording horizontal currents.

Task 1.4 CTD multi-parameter autonomous profiler (Team 1, IP: B. Mouriño).

Responsibles: M. Gil Coto (Participants: B. Fernández, hired technician) Objectives: O3.5, O4.2

During the spring cruise a Brook Oceans WireWalker (Rainville and Pinkel, 2001) will be deployed. This is an autonomous vertical profiler that harvest the energy from the waves, carrying a RBR-XR620 CTD that includes a fluorometer sensor (Cyclops-7). This instrument will provide profiles of temperature, salinity, density and fluorescence every 5-10 minutes during 20-22 hours before draining the batteries. Then the batteries should be replaced and the data downloaded to a computer, afterwards the instrument can be deployed again (in 20-30 minutes).

Task 1.5 SCAN-undulator (Team 1, IP: B. Mouriño).

Responsibles: M. Gil Coto (Participants: B. Fernández, hired technician) Objectives: O3.1

A Guildline Minibat undulator carrying an Applied Microsystems Ltd CTD and Wetlabs fluorescence sensor will be used during the spring cruise to characterize the spatial distribution (vertical and along the steaming course of the ship) of the thermohaline and fluorescence fields. The tow speed of under 5 knots allows saw-tooth profiles spaced at approximately 150 m.

<u>Task 1.6.</u> Nutrient concentration (**Team 1, IP: B. Mouriño**). *Responsibles: B. Mouriño (Participants: hired technician)*

Objectives: O1.10, O3.3

Samples for the determination of inorganic nutrients will be collected at 7-10 depths during the seasonal study and the spring cruise. The concentration of nitrate, nitrite, ammonium, phosphate and silicate will be measured with a Technicon autonalyser at the University of Oviedo's Research Support Service, following the protocols described in Grasshoff et al (1983).

Task 1.7. Nutrient and light availability (Team 1, IP: B. Mouriño).

Responsibles: B. Mouriño (Participants: B. Fernández, hired technician) Objectives: 01.10, 03.3

Resources (nutrients and light) availability for phytoplankton growth will be estimated considering mixing conditions of the water column derived from the microstructure profiler. Vertical diffusive fluxes of nitrate will be calculated, following the Fick's law, from the product of the nitrate gradient across the nitracline and the averaged vertical diffusivity for the same depth interval (Sharples et al. 2001, Fernández-Castro et al. 2015). The nitrate gradient will be obtained by linearly fitting nitrate concentrations in the nitracline, determined as the region of approximately maximum and constant gradient. A proxy for light availability in the photic layer will be calculated by using the expression proposed by Vallina and Simó (2007) modified according to Mouriño et al. (2016), considering the magnitude of the surface radiation, the light attenuation coefficient, and the vertical displacements due to turbulent diffusivity.

Task 1.8. Size-fractionated particulate organic carbon production and photosynthetic parameters (Team 1, IP: B. Mouriño).

Responsible: B. Mouriño (Participants: hired technician) Objectives: O1.4, O1.9, O3.4

We will use the ¹⁴C technique to measure particulate primary production, following the methodology described in detail in Marañón et al. (2004), during the seasonal study and the spring cruise. Carbon fixation rates will be measured during 2-h incubations with NaH¹⁴CO₃. Incubations will be conducted at noon in duplicate 20-ml glass vials, to which 5 μ C of NaH¹⁴CO₃ will be added, under the same irradiance and temperature conditions the samples are experiencing. Incubations will be terminated by

filtration of 5-ml subsamples onto 5 and 0.2 μ m polycarbonate filters using low (<100 mmHg) vacuum pressure. Filters will be treated with concentrated HCl fumes to remove inorganic ¹⁴C and then assayed for radioactivity using a liquid scintillation counter. In addition, the filtrates will be collected, treated with 0.1 ml 1N HCl and kept stirring overnight, in order to remove all dissolved inorganic ¹⁴C. Liquid scintillation cocktail will then be added to these filtrates, and the radioactivity will be measured on a liquid scintillation counter. In cardinal moments of the seasonal cycle (i.e. winter minimum, spring bloom, sub-surface chlorophyll maximum, autumn bloom) and during the spring cruise, photosynthetic parameters will be determined at 2 depths (surface and chlorophyll maximum) in short-term (ca. 1-2 h) photosynthesis-irradiance (P-E) experiments using the ¹⁴C technique (Morán y Estrada 2001).

Task 1.9. Flow cytometry

Responsible: B. Mouriño (Participants: hired technician) Objectives: O1.5

The abundance, biovolume and composition of picoplankton will be determined by using the flow cytometer service in the University of Vigo's Research Support Service. Water samples of 2 to 10 ml in volume will be preserved with 1% (v/v) glutaraldehyde and stored in liquid nitrogen until cytometry analysis. The size and abundance of all cells in the range 0.6 to 10 μ m of equivalent spherical diameter (ESD) will be determined by using their fluorescence and side light scatter (SLS) signals, which will be calibrated with image-analysis measurements of the volume of several species cultured in the laboratory (Rodriguez et al. 1998).

Task 1.10. Chlorophyll-a concentration (Team 2, IP: E. Nogueira).

Responsible: E. Nogueira (Participants: MA. Villamarín, hired technician) Objectives: 01.4, 01.5, 03.5

Water samples for the measurement of chlorophyll concentration will be collected, during the seasonal study and the spring cruise, in dark bottles to avoid pigment damage. For each sample, 100 mL will be filtered at low vacuum pressure (100 mm Hg) through glass-fiber filters (0.7 um nominal pore). Filters will be kept frozen for later analysis in the laboratory. Chlorophyll a will be extracted with 90% acetone for 24 h, and afterwards measured with a spectrofluorometer (Perkin Elmer LB-50s). Fluorescence measurements from the rosette fluorometer sensor will be calibrated against estimated chlorophyll a from these extracts.

Task 1.11. Functional groups, community attributes and growth estimates (Team 2, IP: E. Nogueira). Responsible: E. Nogueira (Participants: B. Reguera, MA. Villamarín, hired technician) Objectives: 01.5, 01.8, 01.9, 01.10, 03.5, 03.7

Nano- and microphytoplankton will be analyzed and quantified by FlowCAM (Álvarez et al. 2014) on fresh samples acquired from Niskin bottles during the seasonal study and the intensive cruise. FlowCAM will allow us to quantify the relative abundance of autotrophs, heterotrophs and non-living material in the size range 5-200 μ m (Álvarez et al., 2011). FlowCAM samples will be analysed by image-based methods in order to characterise the imaged particles on the basis of morphometric and texture features and to classify them using automatic classification algorithms (Álvarez et al. 2012), from which size-distribution, plankton biovolume and diversity will be estimated (Álvarez et al. 2014). Estimates of growth rates will be obtained combining the analysis of the evolution of population biomass (estimated trough biovolume-to-carbon conversion) and ¹⁴C-based primary production rates. During the spring cruise, samples at high vertical resolution from the IPSAP (particle-size analyzer profiler) device will be observed in vivo on board for physiological characterization of *Dinophysis* cells (González-Gil et al 2010), and on samples treated with different fixatives for further counts at the laboratory.

Task 1.12. Species composition (Team 2, IP: E. Nogueira).

Responsibles: B. Reguera and Y. Pazos (Participants: S. González-Gil, E. Nogueira, hired technician) Objectives: O1.5, O1.10, O3.5

Nano- and microphytoplankton identification and counting will be carried out under an inverted microscope (Nikon ECLIPSE 2000) following the Utermöhl (1931) method. Samples of 100 mL will be preserved in acid Lugol's solution and 100 ml in buffered formaldehyde solution. After sedimentation of sub-samples (10-50 ml), cells will be counted and identified to the species level whenever possible. An interactive image analysis system coupled to the microscope will be used to discriminate detritus and other non-living particles from phytoplankton cells. Individual linear measurements will be used to compute cell volume by assigning each cell to the geometric shape that most closely resembles the real shape of each species, and compare with the estimates obtained automatically from the same fixed samples by means of FlowCAM analysis (Task 1.10)

Task 1.13. Physiological state of *Dinophysis* spp. (Team 2, IP: E. Nogueira).

Responsibles: S. González-Gil (Participants: B. Reguera, E. Nogueira, hired technician) Objectives: O3.5, O3.7

Dinophysis spp. populations from phytoplankton samples taken during the spring cruise, and eventually during the seasonal study, will be analyzed following the methods described in González-Gil et al (2010) in order to obtain indicators of their physiological state. In situ division rates will be estimated from the frequency of dividing (paired) and recently divided (incomplete development of the left sulcal list) cells (Reguera et al., 2003). Characterization of the state of the population will be based on the frequency of cells containing digestive vacuoles (formalin-fixed concentrates), as an indicator of recent phagotrophic feeding, and the frequency of cells with starch deposits (Lugol's-fixed concentrates), as an indication of photosynthetic activity (González-Gil et al. 2010). The same samples will be analyzed using FlowCAM to explore the possibility of automation of the image-based methods applied to estimate physiological state indicators.

Task 1.14. Characterization of species of *Pseudo-Nitzschia* by electron microscopy (Team 2, IP: E. Nogueira).

Responsible: Y. Pazos (Participants: B. Reguera, MA. Villamarín) Objectives: O1.5, O3.6

Discrimination of species within the genus *Pseudo-nitzschia* will be conducted using TEM (transmission electron microscopy) techniques for detailed morphological characterization. Plankton samples will be collected during the spring cruise, and eventually during the seasonal study, using 20 μ m mesh plankton net. Afterwards, samples will be filtered using a 0.2 μ m nylon membrane filter and preserved with modified saline ethanol (Miller and Scholin 2000) in 50 mL centrifuge tubes. In the laboratory, samples will be rinsed by distilled water several times to remove the saline ethanol. Organic materials of the samples will be acid-cleaned by adding 96% H2SO4, saturated KMnO4, and 10% oxalic acid (Bargu et al. 2002). Cleaned samples will be mounted to a 100 square-mesh formvar-coated copper grib, and air-dried overnight. Samples will be examined using SEM and TEM techniques.

Task 1.15. Relative abundance of *Pseudo-Nitzschia* spp. by molecular analysis (Team 2, IP: E. Nogueira).

Responsible: B. Reguera (Participants: S. González-Gil, F. Rodríguez –letter of support, hired technician)

Objectives: O1.5, O3.5, O3.6

Due to the difficulties for the identification and determination of relative abundance of *Pseudo-Nitschia* spp., FISH (Fluorescence in situ hybridization) technique will be applied during the spring cruise, and eventually during the seasonal study, in selected samples during proliferations of *Pseudo-nitzschia* spp. to estimate their specific composition and relative abundance on the basis of SSU rRNA probes available in the literature.

Task 1.16. Size-fractionated mesozooplankton biomass (dry weight) (Team 2, IP: E. Nogueira). Responsible: A. Miranda (Participants: G. Casas, E. Nogueira, hired technician) Objectives: O1.6, O1.7

During the seasonal study and the spring cruise, depth-integrated plankton samples will be obtained by means of double oblique fishing hauls with a Bongo net of 200 μ m mesh size provided by flowmeter and a TD (temperature-depth) data logger for the control of sampled volume and fishing haul performance (Buttay et al. 2016). Once in the lab, samples from one cod-end will be size-fractionated through a series of sieves of 200, 500, 1000 and 2000 um mesh size, washed with filtered seawater and transferred to pre-weighted glass-fiber filters, dried (60°C, 48 h) and stored frozen until dry weight per volume will be obtained (Nogueira et al., 2004).

Task 1.17. Vertical distribution of mesoplankton (> 100 μm) (**Team 2, IP: E. Nogueira**).

Responsible: E. Nogueira (Participants: G. Casas, A. Miranda, hired technician) Objectives: 01.6, 01.7, 03.8

The vertical distribution of plankton > 100 μ m during the seasonal study and the spring cruise will be obtained by means LOPC (Laser Optical Plankton Counter) profiling (Herman et al., 2004). The LOPC is an optical instrument capable of measuring in situ the size of particles that cross a laser beam. The smallest recorded particles (living + non-living) are of 100 μ m ESD and the largest are of a few millimeters. From the records acquired with the LOPC, in combination with information obtained by means of ZooScan/ZooProcess, we will obtain the distribution of living and non-living particles in the water column and derive descriptors of the community, such as the normalized biomass size

spectrum and the ratio between living and non-living particles within the prescribed size range (Vandromme et al., 2014).

Task 1.18. Functional groups and community attributes (Team 2, IP: E. Nogueira). Responsible: E. Nogueira (Participants: A. Miranda, G. Casas, hired technician) Objectives: O1.5, O1.6, O1.8, O.3.5

Plankton samples collected with Bongo fishing hauls during the seasonal study and the spring cruise (depth-integrated), and at specific depths by means of the IPSAP (particle-size analyzer profiler) during the spring cruise, will be analyzed using the image-based semi-automatic ZooScan/ZooProcess, which is a laboratory flatbed scanning system used for the digitization of fixed samples (Gorsky et al., 2010). All scanned objects will be then visually classified into zooplankton or non-living particles, and can be further automatically classified into several categories, which are sometimes allocable to functional zooplankton groups (such as copepods, cladocerans, large and small crustaceans, chaetognaths, carnivorous gelatinous and appendicularians) (Vandromme et al., 2014). Furthermore, biovolume estimates of each scanned particle could be also obtained based on morphologic characteristic of the scanned items, and converted to carbon from literature-based conversion factors.

Task 1.19. Species composition (>200µm) (Team 2, IP: E. Nogueira).

Responsible: A. Miranda (Participants: G. Casas, hired technician) Objectives: O1.6, O3.8

Taxonomic identification and counting will be carried out under the stereoscopic binocular from samples acquired weekly by means of the Bongo net. During the spring cruise, besides fishing hauls, samples will be pumped from prescribed depths by means of the IPSAP device (Velo-Suárez et al. 2008). The zooplankton sample will be preserved in 4% sodium tetraborate-buffered formaldehyde. Subsamples will be taken until at least 1000 zooplankton organisms per sample were identified to the lowest possible taxonomic level. Counts in the subsamples will be converted to full-sample number per cubic meter for both total abundance and abundance within individual taxa (e.g. Buttay et al., 2015).

Task 1.20. Grazing rates (Team 2, IP: E. Nogueira).

Responsible: A. Miranda (Participants: G. Casas, hired technician) Objectives: O3.9

Zooplankton grazing will be estimated during the spring cruise by the radiotracer method based on uptake rate of ¹⁴C labeled phytoplankton (Harris et al. 2000). Water collected at a prescribed depths by means of the IPSAP (particle-size analyzer profiler), filtered through a 100 μ m gauze, will be dispensed in to 1L polycarbonate bottles and inoculated with ¹⁴C-bicarbona (50 μ Cu·L⁻¹). The bottles will be incubated on board for 1 hour at light conditions on the sampling depth. Mesozooplankton collected by short tows in the mixed layer will be added to the incubation bottles. After 30, 60, and 90 min, replicate bottles will be removed, the zooplankton concentrated though a 200 μ m mesh, filtered onto pre-weighted glass-fiber filter, washed with 10% HCl, dry weight determined and the isotope activity measured. Community grazing impact on primary production will be estimated at cardinal periods of the seasonal cycle, using food removal methods supported by image-based FlowCAM analysis.

2. Data base analysis (Both Teams).

Responsibles: B. Mouriño and E. Nogueira (Participants: Y. Pazos, B. Reguera) Objectives: O2.1, O2.2, O2.3

Task 2.1. Characteristics of TLP and favorable conditions

Fluorescence profiles from the data base (IEO and INTECMAR time-series) will be analyzed in order to determine the frequency of occurrence of TLP at the sampling stations in the Galician Rías and the adjacent shelf. TLP will be identified using the following thresholds: 1) the vertical extension must be lower than 5 m, and 2) the maximum concentration must exceed three times the background. Based on this information, we will describe the frequency of occurrence, the spatial distribution and the intensity of TLP. Additional information from temperature and salinity CTD casts, meteorological stations, and satellite information will be used to infer the physical favourable conditions for TLP formation.

Task 2.2. Relationship between TLP, Pseudo-nitzschia spp., and shellfish harvesting closures

Information about the occurrence of TLP in the Galician Rías (Task 2.1) will be combined with data provided by the INTECMAR in order to investigate the relationship between the occurrence of TLP, *Pseudo-nitzschia* spp. abundance, and shellfish harvesting closures at each sampling site.

3. Mathematical modeling (Team 1, IP: B. Mouriño).

Task. 3.1. Seasonal variability of mixing and phytoplankton biomass and growth

Responsibles: C. Souto (Participants: R. Reboreda, M. Gil, B. Fernández, B. Mouriño, E. Nogueira) Objectives: 01.1, 01.2, 01.3, 01.4, 01.5, 01.6, 01.9, 04.1, 04.2, 04.3

The ROMS circulation model, with AGRIF nesting capabilities (Haidvogel et al., 2008; Penven et al., 2006; Shchepetkin and McWilliams, 2005), coupled to the PISCES biogeochemical model (Aumont and Bopp, 2006) will be used to model seasonal variability in mixing conditions and phytoplankton bloom dynamics in Ría de Vigo during 2017. ROMS is a 3D ocean circulation model with freesurface, vertical terrain-following coordinates, and horizontal orthogonal curvilinear coordinates. It is based in the Boussinesq and hydrostatic approximations to solve the incompressible primitive equations. It is coupled with advection/diffusion schemes for potential temperature, salinity and biological tracers, as well as a nonlinear equation of state. PISCES has twenty-four compartments that include five modeled limiting nutrients for phytoplankton growth, two phytoplankton size-classes (nanophytoplankton and diatoms), two zooplankton size classes (microzooplankton and mesozooplankton) and three non-living compartments (semi-labile dissolved organic matter, small and big sinking particles). In addition to the ecosystem model, PISCES also simulates dissolved inorganic carbon, total alkalinity and dissolved oxygen. The model will be forced with meteorological data from Meteogalicia's forecasting WRF model, available to download with an hourly frequency and up to 1.3 km resolution in the area, and river flux from SWAP model (daily frequency). Boundary conditions of velocity, salinity and temperature will be interpolated from IBI-ROOS data (http://www.ibiroos.eu/Access-to-products/Spain/Mercator-Ocean-Puertos-Del-Estado), also available to download. Data from the model corresponding to the EF station will be collected with a 5-10 minutes frequency. Initial and boundary conditions for the biogeochemical (PISCES) variables will be obtained from the Global Ocean Analysis and weekly forecast Product produced by Mercator Ocean in Toulouse, France. This is a global Ocean Biogeochemical analysis product at 1/2°, providing a 7-days mean global forecast updated weekly, as well as 3D global ocean biogeochemical weekly mean analysis for the past 2 years updated every week.

Task. 3.2. Mechanisms responsible for thin layer formation

Responsibles: B. Mouriño (Participants: B. Fernández, M. Villamaña and P. Franks) Objectives: O4.4

A 1D advection-diffusion equation will be used to model competition between convergence and turbulent dispersion, and determine the ideal characteristics and life expectancy of a layer formed by a candidate process:

$$\frac{\partial c}{\partial t} = \frac{-\partial (cW)}{\partial z} + \frac{\partial}{\partial z} \left(K z \frac{\partial c}{\partial z} \right) (1)$$

where z is depth, c is the cell concentration, Kz is vertical diffusivity derived from observations, and W is a convergent vertical velocity. The form of W depends on the process considered. For convergent swimming, W will be modelled as using the simple mechanistic model proposed by Stacey et al. (2007). Information about the characteristics of the plankton groups found in the thin layer will be used to test whether cell velocity diagnosed from the steady state solution of this model is realistic (Sullivan et al. 2010). Swimming velocities required for thin layer formation will be also estimated by using the model proposed by Durham et al. (2009). To test if the layer is formed by non-motile cells sinking to their neutral density depth, cell diameter will be diagnosed by using the buoyancy model proposed by Stacey et al. (2007), whereby $W = \Delta \rho g D 2/(18\rho 0v)$, where ρ is density, D is the cell diameter, g is the gravity constant and v is viscosity. Finally, the growth rates needed to form the thin layer by in situ phytoplankton growth will be diagnosed from the 1D model and the Kz measurements by adding a growth term to equation 1.

The role of intrusions by horizontal advection will be estimated from the hydrographic observations following a similar approach described by Steinbuck et al. (2010). Thin layers formed by a plankton patch straining have particular characteristics that can be used to identify this mechanism. In particular, these layers are tilted with respect to isopycnals and they cannot reach a steady state balance between convergence and turbulent dispersion, because the patch intensity decreases as it is strained by shear and diffused by turbulence. A strained path can reach a minimum width of $H_{min} \sim (Kz L_0/S)^{1/3}$ (Birch et al. 2008) where L_0 is the original patch horizontal size before straining occurs. Hence, original patch size can be calculated from its minimum as long as Kz and S are known. Unrealistic L_0 values will be used to rule out this process as candidate for the thin layer formation.