#### Research Article

# Proposal of an integrated system for forecasting Harmful Algal Blooms (HAB) in Chile

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**ABSTRACT.** Harmful Algal Blooms (HAB), are natural phenomena that are produced by the proliferation of phytoplankton potentially harmful to humans and for some ecosystem services (*e.g.*, good water quality for use in aquaculture, availability of natural resources). In Chile, HAB events of *Pseudo-nitzschia* spp. and *Alexandrium catenella* are particularly relevant due to their potential toxicity. Although there are studies of the negative impact of these events, both the prediction of the occurrence of these phenomena and the associated risks are limited in the country. The proliferations have been attributed to the action of various oceanographic forcings (*e.g.*, vertical stratification, irradiation, availability of nutrients). A research has been made about the factors and processes that have been associated with the appearance, permanence and toxicity of HAB and about the methodological efforts made to study and generate HAB forecasts in other countries. Based on a compilation of the information of occurrences and localities affected by these events, the seasonal, interannual and spatial variability of the events or occurrences of HAB in Chile was constructed. Subsequently, the current monitoring system is described, as well as future prediction efforts. Finally, the configuration of a monitoring system with observations and integrated prediction for the occurrence of *Pseudo-nitzschia* spp. and *Alexandrium catenella* is suggested.

**Keywords:** phytoplankton blooms, *Alexandrium catenella*, *Pseudo-nitzschia*, HAB, southern Chile.

#### INTRODUCTION

Harmful Algal Blooms (HAB) are recurrent phenomena in aquatic ecosystems. HAB events correspond to "proliferation of microalgae in marine or brackish waters that can cause the massive death of fish, contaminate shellfish with toxins and alter ecosystems in ways that humans perceive as harmful, as it generates negative effects on public health, fishing activities, aquaculture and tourism" (Clément & Lembeye, 1994, HAB Program UNESCO/IOC, 2005). The proliferations of phytoplankton that give rise to HAB, are triggered by favorable environmental conditions in specific places, where a combination of events of different nature (biological, physical and/or chemical) determine the beginning, development and end of a bloom in a certain period. HABs can last for days and up to several months, and their spatial coverage can reach up to hundreds of kilometers (Avaria et al., 1999). Additionally, when environmental conditions are not

favorable for vegetative growth, some of these microalgae can form resistance cysts that persist long time in marine sediments (Seguel *et al.*, 2010). Thus the resuspension of sediments added to the recurrence in the triggering environmental conditions, make HABs a spatially recurrent process. The temporal and spatial scales where HABs occurs make the observation and prediction of such events a complex but inescapable task to reduce the risks in the coastal populations.

HABs have increased globally in intensity, length and geographic coverage (Villanueva, 2005). In general, the causes of this increase are not clear, but it is believed that HABs are caused by multiple factors: 1) in the case of *Alexandrium*, the expansion of cysts banks and their location would play a determining role in the magnitude of the events (F. Villanueva, *pers. comm.*), 2) the "fertilization" in closed systems such as channels (by anthropogenic action) would be contributing nitrogen and phosphorus mainly, which are the basis and substrate for many species (Smayda, 1990),

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3) the climate change would favor dinoflagellates due to their extreme conditions (Lindahl, 1993) and summer reduction in rainfall, which would result in a decrease in the contributions of silicon forcing a change in functional groups from diatoms to dinoflagellates (Torres *et al.*, 2014), and (4) the discharge of ballast water disperse contaminated water from affected regions to other areas of the country (Hallegraeff, 1993). In addition, it is possible that the higher effort of observation and detection of these events (detection by molecular tools), as well as the greater use of coastal waters (aquaculture), entails an increase in their records (Anderson, 1989).

In Chile, this increase has also been manifested. Since 1827, when the German naturalist Eduard Poeppig observed a particular coloration in Valdivia, to date more than 140 events of some harmful phytoplankton organism have been reported with more than 36 people dead (mostly in the nineties) and have been recorded around 500 poisoned persons for consumption of contaminated seafood (Silva et al., 2016). These events generated public health, economic (Martínez et al., 2008) and ecological problems (Van Dolah et al., 2001; Fire et al., 2010; Häussermann et al., 2017). As recent, a study showed that in 2015 at least 343 whales were found dead in the south of Chile, due to the consumption of the "prawn of the channels" (Munida gregaria) contaminated with HAB (Häussermann et al., 2017). Then, in early 2016, a bloom of *Pseudochattonella* spp. caused mortalities of salmonids of the order of 40,000 ton in Chiloé (41-43°S, Fig. 1b). Subsequently, in April of the same year, one of the largest blooms events of Alexandrium catenella occurred in the south of Chile. It extended about 400 km from the zone initially affected, interrupting the extractive activity and turning the Chiloé Archipelago into a zone of environmental, social, economic and health catastrophe without precedent in the region (Buschmann et al., 2016).

Historically, in Chile the main toxins have been found (Paralytic Shellfish Poisoning, PSP, Diarrhetic Shellfish Poisoning, DSP, and Amnesic Shellfish Poisoning, ASP) associated with HAB events. However, the only cases of severe poisoning and death have been generated in the south of the country by the dinoflagellate Alexandrium catenella (associated with the production of saxitoxins causing PSP). This species belongs to the complex Alexandrium tamarense/ catenella/fundyense (complex tamarensis) defined by its morphological attributes (Aguilera-Belmonte et al., 2011). Other dinoflagellate, Dinophysis acuta (associated with the DSP), has produced non-lethal intoxication cases. On the other hand, the species of Pseudo-nitzschia, associated with ASP, are diverse. In Chile, P. pseudodelicatissima (Hasle, 1965; Rivera,

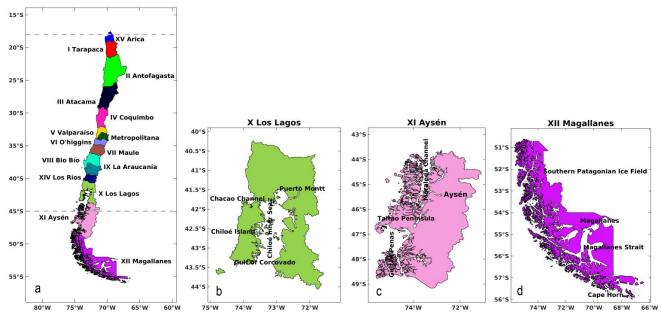
1985), P. australis (Hasle, 1972; Rivera, 1985), P. fraudulenta (Hasle, 1972), P. delicatissima (Rivera, 1985), P. pungens (Hallegraeff, 1994), P. seriata (Cassis et al., 2002), P. calliantha and P. subfraudulenta (Álvarez et al., 2009) have been identified. In various parts of the world, these species have presented Domoic Acid (DA), a neurotoxin associated with ASP. Currently, it has been discussed that P. calliantha, P. australis, P. seriata and P. pseudodelicatissima show presence of DA in Chile (Ferrario et al., 2002; Álvarez et al., 2009; Seguel et al., 2010; Gil, 2014). However, none of these species has been related to human poisoning or mass mortalities of marine organisms, but they have severely impacted shellfish production during some periods and constitute a potential permanent threat to public health (Suárez et al., 2002; López-Rivera et al., 2009).

The scientific literature on this subject is essentially descriptive for inland waters of southern Chile, with emphasis on the regions of Los Lagos, Aysén and Magallanes (Figs. 1b-1d), thanks to various studies and reports from the Instituto de Fomento Pesquero (IFOP) in the period 2006-2016 (Guzmán et al., 2007, 2009, 2010, 2011, 2012, 2013, 2014, 2015), in the context of the project "Manejo y monitoreo de las mareas rojas en las regiones de Los Lagos, Aysén y Magallanes". The area south of ~45°S is where most of the studies have been carried out, because there these phenomena began to be observed in the country, being this area the best characterized in this matter (see IFOP reports). In Los Lagos Region and to the north of the Aysén Region (40-45°S, Fig. 1a), the different natural phenomena that affect the frequency, intensity and geographical distribution of HABs are beginning to be understood, as well as their possible synergistic effects with anthropogenic factors that could be interacting (Buschmann, 2005).

However, there is currently no study that integrates all available information on the spatio-temporal distribution of HAB events in Chile, neither the discussion of study methodologies within the framework of ocean observing systems in the world, raising future challenges regarding HAB forecast in Chile. This study aims to perform a historical reconstruction of HAB events for the species *Alexandrium catenella* and *Pseudo-nitzschia* spp. and its spatial variability, to categorize the physical, chemical and biological factors that determine the bloom of both species. Finally, the state of the art for HAB study and a methodology for forecasting HABs events in the country are discussed.

#### MATERIALS AND METHODS

The information compiled from events or occurrences of these phenomena ranges from 18° to 45°S approximately (Fig. 1a). However, in the characterization of factors related to HAB in Chile and government pro-



**Figure 1**. a) Regions of Chile. Dotted lines limit the study area for HAB records. Note that they are the simplified names of the regions of Chile and does not include the recent Nuble, Region between 36° and 37°S approximately. In b), c) and d) a zoom to the three regions most affected by HAB phenomena is shown; Los Lagos, Aysén and Magallanes respectively.

grams for monitoring, mention is also made of the southernmost areas of the country (south of 45°S). It should be noted that, as will be seen below, the three regions most affected by these phenomena are the three to the south (Los Lagos, Aysén and Magallanes, Figs. 1b-1d).

### Historical reconstruction and spatial variability of events

A historical reconstruction and study of the variability of HAB events was made based on the review and compilation of the information of occurrences or events and localities of all the reports (12) of the Programa de Vigilancia de la Marea Roja in Chile of the Instituto de Salud Pública (ISP), (2002, 2012) scientific articles and IFOP reports (8). The analyzed information consists of 34 documents during the period 1993-2012 and from 18° to 45°S approximately (Fig. 1a). These records identify the presence of the PSP and/or Alexandrium catenella and ASP and/or Pseudo-nitzschia spp. either in water samples or in seafood meat. It must be noted that the species of this last genus have similar characteristics, which makes identification difficult, so that all the species of the genus were grouped for their study (Bates et al., 1998; Anderson et al., 2002; Marchetti et al., 2004; Kudela et al., 2010).

From this information, a database was generated where the following variables were recorded; latitude, longitude, year, month and region of occurrence of these events. After this discretization of the variables in space and time, the information was presented in two ways, in 1) histograms by region, month and year to analyze the variability interannual and within a year and between regions; and 2) by a statistic for each species of the percentage of occurrence of HAB in each season of the year (summer, fall, winter, spring) for 16 groups of latitudes. This statistic is presented in pie charts that allow seeing the intraseasonal and spatial variability, and Hovmöller graphs where the seasonal and interannual variability for each one of the aforementioned groups is evaluated. Finally, all the references collected are summarized in tables by region for a better understanding of the references used.

# HABs determinant factors and forecasting methodologies in ocean observing systems

An analysis of the information of the main HAB species associated with the most relevant toxin (PSP) and the one of potential danger (ASP) in the country was elaborated. This compilation was generated on the basis of historical records (articles, reports) and foreign scientific literature.

A synthesis and compilation of the above information was carried out, according to the following criteria; toxin producing species, factors and environmental conditions associated with the species, forecasting methodologies and systems in which they develop, both in Chile and overseas. In addition, the historical and

current situation of Chile is described in the various programs dedicated to monitoring, vigilance and possible prediction of HABs.

#### **RESULTS**

# Historical reconstruction and spatial variability of events

Below, the distribution of events or appearances of HAB by region, month, and year for each of the species considered is presented (Fig. 2).

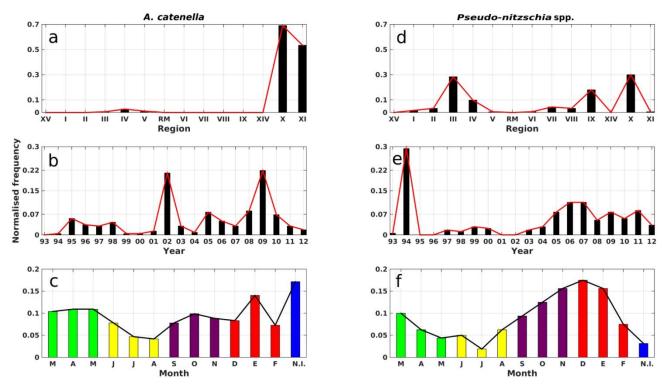
The main affected regions (Fig. 2a) by Alexandrium catenella are Los Lagos (Fig. 1b) and Aysén (Fig. 1c), where it was present in the period 2002 and 2009 (Fig. 2b, Table 1). The presence of these species can occur throughout the year, but less in late fall and winter (Fig. 2c). The records in these last stations are also due to the fact that toxins can last several months in mollusks. In general, for A. catenella the available antecedents (Fig. 3, Guzmán et al., 2002, 2007, 2009, 2010a, 2010b), show that the periods with higher probabilities of occurrence of A. catenella blooms and PSP outbreaks for Los Lagos Region (Fig. 1b), are in late spring or more likely in the summer, mainly in the south of Chiloé. While, in the Aysén Region (Fig. 1c) the blooms are most likely in summer, although there are precedents that indicate that they can also occur in fall and spring (2009, Fig. 3). In the case of Magallanes (not shown) three fields emerge: a) northern during springsummer season and fall, b) central area during springsummer and occasionally fall season, and c) southern during late spring and early summer (Guzmán et al., 2002, 2007, 2009, 2010a, 2010b). Additionally, the information gathered supports the inter-annual variability of the distribution and levels reached for the toxic complex as well as for the relative abundance and microalgae density (Fernández & Tocornal, 2000; Guzmán et al., 2002, 2007, 2009). Thus, the years without blooming are 2007-2008, 2010-2011, 2011-2012, 2012-2013, 2013-2014 y 2014-2015 and the years with blooming are 1994, 2002, 2006-2007, 2008-2009 (Fig. 2b) and 2015-2016 (Table 1). Since the periods 2013-2014 and 2014-2015 it has been observed that blooms have been increasing in both extension and permanence in comparison to previous years, even covering winter season (June-August, Fig. (Guzmán et al., 2015).

Potentially harmful species of *Pseudo-nitzschia*, *P. cf. pseudodelicatissima* and *P. cf. australis* are common in the channels and fjords of Chilean Patagonia, with important contributions in some sectors, being more important quantitatively *P. cf. pseudodelicatissima* than *P. cf. australis*, except in some stations of Aysén

and Magallanes (Guzmán *et al.*, 2015). *Pseudonitzschia* has been presented mainly in the regions of Los Lagos, Antofagasta and Biobío (Fig. 2d), with the years 1994 and 2006 being the most important (Fig. 2e, Table 2), preferably in spring-summer (Fig. 2f). According to available antecedents (Guzmán *et al.*, 2002, 2007, 2009, 2010a, 2010b) that are presented in part in Fig. 3, in Los Lagos Region DA detections are manifested during the summer (January-February), and in Aysén during fall-winter (April-September), decreasing in spring (October-December, Fig. 3). Meanwhile, in the Magallanes Region (not shown, Guzmán *et al.*, 2009), DA detection is concentrated in the fall months (May and June).

Figure 4 presents the percentage of occurrence of HAB for each season (summer, fall, winter, spring) in the years analyzed for the different latitudes. As will be discussed later, contrasting spatial patterns of bloom are observed. *Pseudo-nitzschia* spp. a) has a more latitudinal distribution with apparitions in the north, center and south of the country that have been increasing in recent years. For its part, *Alexandrium catenella* b) began in the southernmost part of the country (Martínez *et al.*, 2008) and has gradually extended to the northern sector of Chiloé (Los Lagos Region, Fig. 1b). It has remained more constant over the years and like *Pseudo-nitzschia* spp., has higher probabilities of occurrence in spring and summer.

The detection of subtoxic levels of PSP in northern Chile (28°-34°S, Fig. 4, Table 1), may not be caused by A. catenella, but rather by Alexandrium ostenfeldii, whose distribution north of 41°S (up to ~27°S) has been recently analyzed (Salgado et al., 2012b). However, A. ostenfeldii has been associated with PSP and/or saxitoxin (STX) in toxic episodes in temperate and subpolar waters in the oceans, but there have been no STX records in Chile associated with this species (Salgado et al., 2012b). Another source of income of PSP to the northern system comes from the transport/commercialization of marine resources from the southern part of the country (Puerto Montt, Fig. 1b; Aysén, Fig. 1c) or ballast water from maritime transport vessels (Avaria, 1999; Robles et al., 2003). It should be mentioned that the technique used for the detection of these toxins is highly vulnerable to false positives (Mouse Bioassay). In the southern zone of the country, the presence of A. catenella has presented sporadic occurrences since 1991 in Magallanes (Martínez et al., 2008) although the first poisoning by this in the area was in 1972 (Guzmán et al., 1975). Since 1991 it has spread to regions further north, reaching up to Aysén in 1992 (Muñoz et al., 1992) and later, to Los Lagos Region in 1998 (Avaria et al., 1999; Salgado et al., 2012). It is still present in the area with magnitude, intensity and duration not known until



**Figure 2.** Histograms by a) region, c) years, and e) months, for *Alexandrium catenella*, and *Pseudo-nitzschia* spp. (b, d, and f). In the graphs e) and f) N.I. means No Information about the month in which the event occurred. The colors in the lower graphs (c and f) are matched with the colors shown in the percentage graphs in Fig. 2.

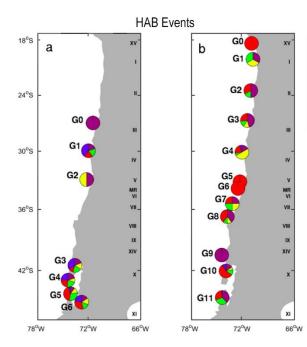
**Table 1.** Information on occurrences and locations in the reports in the different sources of records for PSP and/or *Alexandrium catenella*. The Santiago Metropolitan Region, the Araucania and Los Rios regions (see Fig. 1a) did not present cases. The Magallanes Region (see Fig. 1d) is outside the study range.

Region	Year	Reference
Atacama	2002	[10]
Coquimbo	2002 a 2003	[10, 11]
	2010	[18]
Valparaíso	2009	[17]
Bíobio	2007	[15]
Los Lagos	1998 a 1999	[2, 23, 29]
	2002 a 2012	[1, 3, 6, 7, 8, 9, 11, 12, 13, 14, 15,
		16, 19, 20, 21, 22, 23, 24, 26, 27, 29, 30, 31, 32, 34, 35]
	2015 a 2016	[4]
Aysén	1994 a 1998	[22, 23, 33]
	2000 a 2010	[6, 11, 13, 15, 18, 19, 22, 23, 29, 35]
	2013	[26]
	2015 a 2016	[4]

[1] Arriagada et al. (2003); [2] Avaria et al. (1999); [3] Buschmann (2005); [4] Buschmann et al. (2016); [5] Clement & Lembeye (1994); [6] Guzmán et al. (2007); [7] Guzmán et al. (2009); [8] Guzmán et al. (2010a); [9] Guzmán et al. (2011); [10] Informe ISP 2002; [11] Informe ISP 2003; [12] Informe ISP 2004; [13] Informe ISP 2005; [14] Informe ISP 2006; [15] Informe ISP 2007; [16] Informe ISP 2008; [17] Informe ISP 2009; [18] Informe ISP 2010; [19] Informe ISP 2011; [20] Informe ISP 2012; [21] Mardones et al. (2010); [22] Martínez et al. (2008); [23] Molinet et al. (2003); [24] Murillo et al. (2008); [25] Pizarro et al. 2011; [26] Rivera (2013); [27] Robles et al. (2003); [28] Suárez et al. (2002); [29] Salgado et al. (2012); [30] Seguel & Sfeir (2003); [31] Seguel et al. (2005); [32] Suárez & Clement (2002); [33] Uribe et al. (1995); [34] Valenzuela & Avaria (2009); [35] Vidal et al. (2006).

NI Summer Fall

Winter Spring

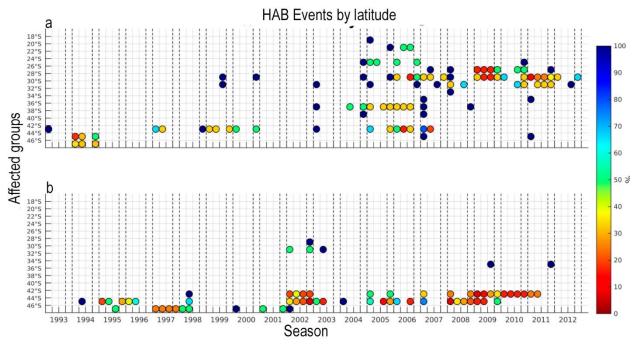


**Figure 3.** Spatial distribution by regions (in Roman numerals, see Fig. 1) of: a) *Alexandrium catenella*, and b) *Pseudonitzschia* spp. The groups (G) are ordered by appearances with common distance and are represented in graphs that indicate the percentage of occurrence of an event in the period 1993-2012. MR: Metropolitan Region of Santiago. NI: No information about the month in which the event occurred.

**Table 2.** Information on occurrences and locations in the reports in the different sources of records for ASP and/or *Pseudonitzschia* spp. The Santiago Metropolitan Region, the Araucanía and Los Rios regions (see Fig. 1a) did not present cases. The Magallanes Region (see Fig. 1d) is outside the study range.

D '	3.7	D. C.
Region	Year	Reference
Arica y Parinacota	2005	[13]
Tarapaca	2005-2006	[13, 14]
Antofagasta	2004-2006	[12, 13, 14]
	2010-2011	[18, 19]
Atacama	1999	[28]
	2004-2012	[12, 13, 14, 15, 16, 17, 18, 19, 20, 26]
Coquimbo	1999	[28]
	2003	[11]
	2005-2012	[13, 14, 15, 16, 17, 18, 19, 20]
Valparaiso	2007	[15]
O'Higgins	2011	[19]
Maule	2004	[12]
	2006	[14]
Bíobio	2003-2006	[11, 12, 13, 14]
	2008	[16]
Los Lagos	1993.	[5]
	1997-2000	[28, 33]
	2003	[11]
	2005-2009	[13, 14, 15, 16, 17]
	2012	[20]
	2016	[4]
Aysén	1995	[33]
	2007	[15]
	2008	[25]
	2010-2012	[18, 19, 26]
	2016	[4]

[1] Arriagada et al. (2003); [2] Avaria et al. (1999); [3] Buschmann (2005); [4] Buschmann et al. (2016); [5] Clement & Lembeye (1994); [6] Guzmán et al. (2007); [7] Guzmán et al. (2009); [8] Guzmán et al. (2010a); [9] Guzmán et al. (2011); [10] Informe ISP 2002; [11] Informe ISP 2003; [12] Informe ISP 2004; [13] Informe ISP 2005; [14] Informe ISP 2006; [15] Informe ISP 2007; [16] Informe ISP 2008; [17] Informe ISP 2009; [18] Informe ISP 2010; [19] Informe ISP 2011; [20] Informe ISP 2012; [21] Mardones et al. (2010); [22] Martínez et al. (2008); [23] Molinet et al. (2003); [24] Murillo et al. (2008); [25] Pizarro et al. 2011; [26] Rivera (2013); [27] Robles et al. (2003); [28] Suárez et al. (2002); [29] Salgado et al. (2012); [30] Seguel & Sfeir (2003); [31] Seguel et al. (2005); [32] Suárez & Clement (2002); [33] Uribe et al. (1995); [34] Valenzuela & Avaria (2009); [35] Vidal et al. (2006).



**Figure 4.** The "y" axis presents different groups (represented by mean latitude) for both species: a) *Pseudo-nitzschia* spp. and b) *Alexandrium catenella*. On the "x" axis, the different seasons are observed, indicating spring with a dotted line from 1993 to 2012. Finally, the colors indicate the percentage of probability of occurrence.

now (Silva et al., 2016). It should be noted that recent sediment studies indicate that the appearance of A. catenella in Los Lagos may corresponds to a process of recolonization, since there are records from the beginning of the 20<sup>th</sup> century (Salgado, 2011). For its part, the first appearance of Pseudo-nitzschia was recorded in 1995 in Magallanes (Uribe et al., 1995). However, the first detections of DA were recorded in 1999, the first in June in Tongoy Bay (Coquimbo Region) and the second in Chiloé (Los Lagos Region), affecting Chilean mussel cultures (Gil, 2014). It has been disscused in diverse localities of the country with stable toxin concentration values but increasing its frequency and spatial extension (Suárez et al., 2002; López-Rivera et al., 2009). There are records of occurrence in Los Lagos, Biobío and Coquimbo regions (Fig. 1a) in mollusks, such as semelidae, oyster and clam (Gil, 2014). This has forced the Ministry of Health to initiate a program to monitor this phenomenon.

# Factors and environmental conditions that determine HAB events

Information is presented on environmental factors, as well as their ranges, during HABs events that occurred mainly in Chile. Due to the small number of studies that integrate records of environmental factors in Chile (Table 3), information on foreign scientific articles is included (Foreign Literature, FL, and Table 3). It is

observed that these two species in general have some contrasting characteristics (*e.g.*, temperature, upwelling conditions, and salinity) and others in common (*e.g.*, some nutrients, pCO<sub>2</sub>, favorable seasons, Figs. 2-4). These characteristics are typical of the natural succession of phytoplankton, presenting differences between dinoflagellates and diatoms, the ability to mobilize and develop in different environments. For example, in turbulent environments (*e.g.*, influenced by upwelling, kinetic energy), diatoms are developed because turbulence prevents sedimentation. In more stable environments (*e.g.*, decrease in salinity, high temperatures in summer) dinoflagellates are favored because they have movement capacity that allows them to reduce sedimentation (Margalef *et al.*, 1979).

There is a great difference between the three regions most affected (Figs. 1b-1d), where different phytoplankton species and environmental conditions can be found (Guzmán *et al.*, 2015). These environmental conditions are related to the latitudinal difference of each zone, with different geomorphologies characteristics and land-sea interactions (*e.g.*, water run-off, river discharges and melting of glaciers), as well as exceptional irradiation conditions, calm and environmental temperature (Arriagada *et al.*, 2003). Despite this, the study areas show a strong interaction between oceanographic, meteorological, and biological variables in relation to the behavior of phytoplankton abundance. Although the

**Table 3.** Oceanographic, biogeochemical and meteorological variables that are associated with the presence of HAB species such as *Pseudo-nitzschia* spp. and *Alexandrium* spp. FL: indicates that it was obtained from foreign literature. NH: North Hemisphere, pCO<sub>2</sub>: CO<sub>2</sub> Partial Pressure RUV-B: UV-B radiation (280-320 nm), DON: Dissolved Organic Nitrogen, DOC: Dissolved Organic Carbon, ACC: Antarctic Circumpolar Current, ACW: Antarctic Circumpolar Wave, SST: Sea Surface Temperature, QBO: Quasi Biennial Oscillation.

Variable	Alexandrium spp.	Pseudo-nitzschia spp.
Favorable season	<ul> <li>End of spring and summer [69, 84, 61, 34, 28, 29, 30, 31, 32].</li> <li>It can occur throughout the year but is more frequent in February and April [84].</li> </ul>	➤ Spring to summer [69, 79, 34]. ➤ ASP would be during the period when densities are lowest, between mid-fall and mid-winter (end of May to end of August) [30].
Temperature	<ul> <li>Positive thermal anomalies [84, 28, 79, 46, 47, 65, 61, 34, 83 21, 19, 10, 35, 63, 29, 30, 32, 27, 36]. T from low values of 4.82-4.91°C [28], up to as high as 10-15°C [36, 12, 24, 5, 21, 31].</li> <li>PSP range 5-17°C [30, 31].</li> <li>Notorious surface thermocline [36].</li> <li>Influences concentration and increases toxicity [79, 83].</li> <li>Related to the temperature at 5 m [34].</li> </ul>	<ul> <li>➤ FL: Negative thermal anomalies [8, 70, 26, 4, 1]</li> <li>➤ T between 2 and 28.5°C [37, 12, 79].</li> <li>➤ Higher concentrations between 9-14°C [82].</li> </ul>
Salinity	<ul> <li>FL: Negative salt anomalies [38, 45].</li> <li>S as low as 8.15 but high as 33 [36, 59, 84, 24, 28, 5, 21, 31, 36]. PSP range 15 to 32 [30, 31, 27].</li> <li>Marked halocline at superficial levels [36].</li> <li>It limits distribution and abundance, but does not exclude presence during certain periods [31].</li> </ul>	<ul><li>➤ FL: Positive saline anomalies [8, 4].</li><li>➤ 30 to 36 [12, 37, 82].</li></ul>
Winds	➤ It is favored by the decrease in intensity and variation of a normal wind condition [84, 61, 39, 10].	> FL: It is favored by intense winds, mainly favorable to upwelling [70].
Large-scale climate variability	<ul> <li>➤ Hydrographic-climate interaction every 10 years [78, 28, 67]. Favored by El Niño [9, 79, 28, 61, 33, 35, 19, 10, 39, 58, 14, 84, 31, 34].</li> <li>➤ ACC, ACW, interdecadal extratropical anomalies of SST and QBO [61].</li> </ul>	➤ It has been seen in an El Niño event [60, 10].
Turbulence	<ul> <li>It prefers less turbulence [84].</li> <li>The mixing implies lower cell concentrations [81].</li> </ul>	FL: It prefers more turbulence [70].
Precipitation	<ul> <li>It prefers a decrease in precipitation [63, 10, 20].</li> <li>Relative abundance related to cloudiness [34].</li> </ul>	➤ FL: Not relevant. Higher nutrient carryover by precipitation [8, 43].
Water stratification	<ul> <li>More stable water columns [36, 84, 27, 10, 63].</li> <li>Marked thermohaline stratification [28, 79, 5, 82, 21].</li> <li>High stability values, especially between surface and 10 m depth [36].</li> </ul>	> FL: Thermal stratification [8].
Upwelling	> Relaxation of the upwelling [10].	FL: It favors upwelling and potential cold eddy (anticyclonic, NH) [75, 8, 3, 70, 44, 43].
Radiation	<ul> <li>Abnormal period of insolation [36, 27, 28, 5, 83, 84, 63, 10, 29, 30, 32].</li> <li>Tolerates increases in UV-B [57].</li> </ul>	➤ FL: High luminosity [8, 26].
Climate change	<ul> <li>It may be favored [84, 62].</li> <li>It is favored by the occurrence of exceptional. high-intensity El Niño events [63].</li> </ul>	➤ It may be disfavored [74].
Depth	> 0-15 m depth [61, 49].	➤ FL: 0-36 m depth [75].
Nutrients	➤ It develops best in environments with few nutrients. Micronutrients from freshwater inputs [32].	> FL: Important contribution of macro [23] and micro [3, 43] nutrients [75], especially of organic substances [26].
Phosphorus (P)	<ul> <li>FL: Phosphorus is a limiting factor [25, 15, 16, 17, 42].</li> <li>Phosphate is relevant [10, 60].</li> </ul>	<ul> <li>FL: Phosphate is important [75, 4, 75, 43]. P/Si radio is a limiting factor [26].</li> <li>Regulates the production of DA [2].</li> <li>ASP would be in limiting phosphate periods [30].</li> </ul>
Ammonium (NH4 <sup>+</sup> )	<ul><li>FL: Ammonium supplement [15, 16, 68, 18].</li><li>Favorite nutrient [60].</li></ul>	> FL: Ammonium supplement [26, 77, 68].

#### Continuation

Variable	Alexandrium spp.	Pseudo-nitzschia spp.
Nitrogen (N)	<ul> <li>FL: Inorganic and organic nitrogen supplement [13, 17, 18], high nitrate [10, 73, 7, 80, 11, 60] and urea [68].</li> <li>Limitation by nitrogen increases toxicity [25].</li> </ul>	<ul> <li>➤ FL: Nitrate supplement [40, 75, 8, 75, 54, 43, 26], Low N:P radio [4]. DON is a source of N [51].</li> <li>➤ Increase in nitrate values decreases cell concentration [6].</li> </ul>
Silicon (Si)	➤ Dissolved Silicate Deficit relative to Nitrate [10, 60, 73, 74].	<ul> <li>FL: Silicate supplement [75, 76, 43, 3, 64, 70, 26] and a low Si/N radio [54].</li> <li>Silicate limitation increases toxicity [72, 2].</li> <li>ASP would be in limiting silicate period [30].</li> </ul>
pH and pCO2	<ul> <li>FL: Low pH; 7.5 [41].</li> <li>Increase in pCO<sub>2</sub> increases toxicity [72].</li> <li>Optimum growth rates specific to the species at pH ~ 8.1 [62].</li> </ul>	<ul> <li>➤ FL: High pCO₂ increases growth and toxicity [71].</li> <li>➤ High pH: 8.7-9.8 [26, 52].</li> </ul>
Others	<ul> <li>Correlated with the air temperature [83].</li> <li>Changes in phytoplankton structure [79, 5, 34, 83, 27].</li> <li>Advection by circulation and winds [61, 22, 55, 10].</li> <li>Cysts retaining areas (cyst banks) [61, 82, 56, 34, 48].</li> <li>More associated with chlorophyll-a [29, 30, 32].</li> </ul>	<ul> <li>➤ FL: Iron is a limiting agent [66, 43, 26, 53]. Limitation by iron increases toxicity [2].</li> <li>➤ Importance of Lithium [43].</li> <li>➤ DOC Supplement [4].</li> <li>➤ It was found with a water transparency of 3m and a cloud cover of four eighths [82].</li> <li>➤ "Excluded" when a bloom of A. catenella occurs in Aysén and almost simultaneously in Magallanes [34].</li> </ul>

[1] Almandoz et al., 2007; [2] Anderson et al., 2002; [3] Anderson et al., 2006, [4] Anderson et al., 2009; [5] Arriagada et al., 2003; [6] Avaria et al., 2003; [7] Avila et al., 2015; [8] Bates et al., 1998; [9] Braun et al., 1993; [10] Buschmann et al., 2016; [11] Carlsson & Granéli, 1998; [12] Cassis et al., 2002; [13] Chapelle et al., 2010; [14] Clement et al., 2016; [15] Collos et al., 2004, [16] Collos et al., 2007; [17] Collos et al., 2009; [18] Collos et al., 2014; [19] Cornejo et al., 2016; [20] Díaz et al., 2013; [21] Espinoza et al., 2016; [22] Espinoza-González & Bosaín, 2016; [23] Fehling et al., 2006; [24] Fuentes et al., 2006; [25] Garrido et al., 2012; [26] Granéli & Flynn, 2006; [27] Guzmán et al., 1975; [28] Guzmán et al., 2002; [29] Guzmán et al., 2007; [30] Guzmán et al., 2009; [31] Guzmán et al., 2010a; [32] Guzmán et al., 2010b; [33] Guzmán et al., 2014; [34] Guzmán et al., 2015; [35] Guzmán et al., 2016; [36] Guzmán et al., 2016; [40] Howard et al., 2007; [41] Hwang & Lu, 2000; [42] Jauzein et al., 2010; [43] Kudela et al., 2003; [44] Kudela et al., 2010; [45] Laabir et al., 2011; [46] Lembeye, 1981a; [47] Lembeye, 1981b; [48] Lembeye, 2006; [49] Lembeye, 1998; [51] Lourein et al., 200; [52] Lundholm et al., 2004; [53] Maldonado et al., 2002; [54] Marchetti et al., 2004; [55] Mardones et al., 2010; [56] Mardones et al., 2016; [63] Núñez & Letelier, 2016; [64] Pan et al., 2006; [65] Pizarro et al., 1997; [66] Rue & Brulan, 2001; [67] Salgado et al., 2012; [68] Seeyave et al., 2009; [69] Seguel et al., 2010; [70] Shin, 1999; [71] Sun et al. (2011); [72] Tatters et al. (2012), [73] Torres et al., 2011, [74] Torres et al., 2014, [75] Trainer et al., 2000; [82] Vidal et al., 2006; [83] Vidal et al., 2012; [84] Villanueva, 2005.

variables are treated separately for clarity, it is evident and it is demonstrated that there are interactions or have synergistic effects between them (Buschmann *et al.*, 2016).

#### Temperature, salinity, and stratification

The positive relationship of temperature with the higher abundance of phytoplankton (Lembeye, 1998; Vila *et al.*, 2001), the easy obtaining and recording from the first events (Guzmán & Lembeye, 1975) has constituted the temperature as the factor of greatest knowledge and perhaps the most important in the study of HABs (Uribe *et al.*, 1995; Vidal *et al.*, 2012). For *A. catenella* the increase in populations mainly in spring-summer occurs in optimal temperature windows (Itakura & Yamahuchi, 2001) or in relation to its increase (Lembeye, 1998; Vila *et al.*, 2001); therefore, it is estimated that certain temperature thresholds must be reach for the *A. catenella* cysts to germinate. As for *pseudodelicatissima* distribution is favored by the wide tolerance to temperatures (2-28°C), which helps

especially in the lower part of the range where other species do not seem to compete. In turn, a decrease in *P. cf. australis* with a slight increase in temperature in the Strait of Magellan (52°58'S, Fig. 1d) and Cape Horn (55°59'S, Fig. 1d) (Avaria *et al.*, 2003) has been identified.

Like temperature, salinity is a variable recorded from the first events and reflects the different effects of rainfall, thaws, and contribution of continental waters in different regions (Uribe *et al.*, 1995). Salinity is associated on the one hand with the characteristics of phytoplankton (number and/or type of species; Valenzuela & Avaria, 2009; Vidal *et al.*, 2012) and, on the other, with the importance in density (stratification) (Cornejo *et al.*, 2016) and the aggregation of cells, due to the formation of zones of convergence by saline oceanic waters and estuarine fresh waters (Valenzuela & Avaria, 2009; Espinoza *et al.*, 2016). However, for *A. catenella* it is not clear whether salinity should be a positive anomaly (*e.g.*, Guzmán *et al.*, 2002) or negative (Uribe *et al.*, 1995; Diaz *et al.*, 2013). For

diatoms, a decrease in salinity is related to a decrease in *P. cf. australis* (Avaria *et al.*, 2003).

Finally, stratification (caused by various mechanisms) seems to be a preponderant factor to trigger HAB events, especially in the ability to influence the decrease of the mixing (Valenzuela & Avaria, 2009) and generate possible particle retention zone (Guzmán et al., 1975; Espinoza et al., 2016). In this way, the stratification can regulate the vertical and horizontal distribution of A. catenella blooms (Espinoza et al., 2016), which affect the distribution of their possible triggering factors (e.g., temperature, salinity) and drive (or inhibit, Braun et al., 1993; Guzmán et al., 2002) the transport of harmful species to the photic layer (Guzmán et al., 2015).

#### Climate-oceanographic interaction or disturbance

The evidence seems to point out that climatic disturbances and interactions lead to trigger events of blooms of A. catenella and Pseudo-nitzschia spp. They have been related from local factors like atmospheric disturbances in winter (Suárez & Clément, 2002) or after a strong storm (Arriagada et al., 2003) in Chiloé, to large scale factors (Uribe, 1988; Benavides et al., 1995; Salgado et al., 2012). In the latter case, Molinet et al. (2003), for example, hypothesizes that the A. catenella blooms would originate between mixed subantarctic surface waters and subantarctic surface waters. In addition, it states that the appropriate conditions for the massive germination of cysts come from oscillations (and possibly their interactions) of the ACC, ACW, El Niño Southern Oscillation (ENSO), interdecadal extratropical anomalies of the SST and the QBO (acronyms in the Table 3). With respect to these interactions of climate-hydrographic type of low frequency (Uribe, 1988; Guzmán et al., 2002), the most cited are the teleconnections with the ENSO (see authors Table 3). This phenomenon shows a prolonged maintenance of the South Pacific anticyclone with clear positive anomalies of temperature and atmospheric pressure, high radiation, absence of precipitation, less runoff from rivers, high water temperatures, a weakening of force and wind direction, a strong anomaly of less cloudiness and probably melting of glaciers (Guzmán et al., 2014, 2016). In this way, a triggering factor is suggested on a macro scale that regulates the distributions and abundances of harmful species (Salgado et al., 2012), but later local factors would regulate these biotic variables (Guzmán et al., 2009, 2010a, 2014, 2016). In addition, climate change is likely to favor the occurrence of exceptional highintensity El Niño events, which would increase the probability of occurrence of HAB events (Collins et al., 2010; Vecchi & Wittenberg, 2010; Power et al., 2013; Cai et al., 2014, 2015; Sun et al., 2017).

#### **Upwelling, retention, and transport**

HABs in the world are frequently associated with upwelling fronts where passive accumulation or concentration of cells occurs in the sea (Suárez & Guzmán, 1992). These fronts are generated by marked differences in density, by the presence of currents or by a combination of these factors. If the blooms are initiated near the coast, they can be advected along these lower density water plumes (Molinet *et al.*, 2003). When they are trapped on the coast, physical aggregation and vertical migration of A. catenella are the mechanisms that seem to promote the formation of a HAB (Espinoza-González & Bosaín, 2016). On the other hand, the characteristics of the general circulation, the transport of water masses (Mardones et al., 2010) and the dispersion between 0 and 15 m depth (where it registers its greatest abundances) allow to increase the spatial distribution, which in turn, it is influenced by the drift caused by the winds (Molinet et al., 2003; Buschmann et al., 2016).

#### Biological and biogeochemical factors

As other agents that initiate or support the maintenance of a harmful bloom can be considered biological factors such as resistance cysts, changes in the structure of the phytoplankton community (phytoplankton taxocenosis), and chemical factors such as nutrients or ocean acidification.

Studies infer that, for a wide spatial scale, the periods where the highest values of PSP are recorded, are related to certain environmental conditions that allow differences in the composition, distribution and abundance of phytoplankton, going from a predominance of diatoms (in qualitative terms) to a predominance of dinoflagellates (Uribe et al., 1995; Arriagada et al., 2003; Vidal et al., 2012). These mechanisms are manifested due to the constant competition for resources, where in the presence of an environment without nutrient limitations and high availability of silicon, diatoms tend to predominate (M. Vergara, pers. comm.). They live and reproduce quickly but have low efficiency of resource use and when there are not enough, they begin to lose competitiveness and longerlived species such as dinoflagellates appear which tolerate lower concentrations of certain nutrients better (Margalef et al., 1979; Smayda & Reynolds, 2001; Glibert et al., 2016; D. Cassis, pers. comm.).

On the other hand, the dynamics of *A. catenella* blooms is highly dependent on the existence of cyst banks (Molinet *et al.*, 2003). The formation of this state is favored by the increases of sedimentation in areas of channels with little circulation (<5 cm s<sup>-1</sup>, Vidal *et al.*, 2006), as well as conditions of low temperature (10-12°C), high salinity (30), depletion of N and/or P and low irradiation (20 µE m<sup>-2</sup> s<sup>-1</sup>) (Mardones *et al.*, 2015).

Finally, among the chemical variables, a decrease in dissolved oxygen concentrations was observed for the A. catenella bloom in Chiloé in 2002 (Arriagada et al., 2003), as well as functional adaptation responses to variations in pCO<sub>2</sub>/pH (Table 3) in the laboratory (Müller et al., 2016). With respect to the latter, it is proposed that the Chilean strains of A. catenella and other species of coastal phytoplankton are highly adapted to the spatio-temporal fluctuations of pCO<sub>2</sub>/pH in marine surface waters, becoming resilient winners in the expected effects of the climate change and ocean acidification (Müller et al., 2016). In addition to this, it can be expected that ocean acidification combined with nutrient limitation or temperature changes may increase the toxicity of HABs (Sun et al., 2011; Tatters et al., 2012).

# HAB monitoring programs in Chile and forecasting methodologies

In Chile, there are different institutions that have funded research with various funds, as well as government projects and programs devoted to the study, monitoring, detecting, and control of HABs. Since 1995, the MINSAL, the ISP, and the SEREMIS (see acronyms in legend Fig. 5) formally implemented the Programa de Vigilancia de la Marea Roja for all country (Ord. 4B/6518 y Ord. 9B/3557). Subsequently, was reinforced to the Programa Nacional de Vigilancia y Control de las Intoxicaciones por Fenómenos Algales Nocivos (R. Ex. N°24) in 2008, part of the PNIMR coordinated by ACHIPIA Since 2010 together with SERNAPESCA and the Undersecretary of Public Health in the context of the PNIA in 2009 (Fig. 5). The general objective is to prevent intoxications of the population derived from the consumption of marine resources contaminated with toxins. This program contemplates the monthly sampling of coast bivalves from the stations (227) and its subsequent sending for analysis (Villanueva, 2005). This has allowed monitoring these toxins in order to detect HABs in a timely manner; there are online reports from 2002 to 2013. In December 2005, REPLA was approved (Fig. 5) by Supreme Decree N°345. The purpose of this regulation is to establish protection and control measures to prevent the introduction of species that they constitute hydrobiological plagues, isolate their presence, prevent the spread and move towards its eradication. In this context, through a monitoring and vigilance program of A. catenella (executed by IFOP), SUBPESCA prepares technical reports that contain the foundations for the declaration of HAB area of A. catenella, with the contribution of an advisory committee and national experts. The last Exempt Resolution N°1770 of May 29, 2017, extended that of 2014 (R. Ex. N°3575) until December 31, 2017, which declared as a HAB area the

macrozone that extends from the south of Chiloé to the south end of the Magallanes Region, dividing it into 2 zones with certain restrictions; the sector between the Taitao Peninsula and the Gulf of Penas (Aysén Region, Fig. 1c), as well as south of the 55°S (Magallanes Region, Fig. 1d), will be considered undeclared areas as to the date does not exist information that confirms the presence of A. catenella. Additionally, a PVCAc (R. Ex. N°529 of 2009, Sernapesca), R. Ex. N°2004 of 2017 was established, which implements a qualitative monitoring for A. catenella in Moraleda Channel and Gulf of Corcovado (Fig. 1b) for the control of wellboats. In addition, SERNAPESCA, in agreement with the United States, has been developing the PSMB since 1989 for exports to the European Union. Since 2002, the PSMB passed into the hands of private laboratories, with the ISP remaining as the reference laboratory for this program. The PSMB works on the classification and monitoring of the production areas of bivalve molluscs and other resources susceptible to being affected by marine toxins within the culture centers (~121). In this program, the environmental consulting company Plancton Andino is positioned as one of the most far-reaching, as well as in the POAS. The POAS was born in 1998 and consists of the execution of an active and systematic monitoring with different companies of the salmon industry, in order to inform opportunely in relation to the spatio-temporal distribution of the presence and concentration of harmful algae in the salmon farms in the southern Chile. Today, POAS is in the strengthening stage (POAS 2.0) and working on a HAB index. Finally, as mentioned above, the IFOP has been monitoring the XII Region since 1994 (Fig. 1d) first with FIP projects (Program 1, Fig. 5) and now between May 2006 and until 2018 with the Program 2 (Fig. 5) (Guzmán et al., 2007, 2009, 2010a, 2011, 2012, 2013, 2014, 2015). In addition, since 2015. the Biobío Region has been included. This program is based on the collection of transvector shellfish for a toxicity analysis by the SEREMI of Health, along with registering oceanographic variables (temperature, salinity, density, transparency and oxygen of the water column). To date, it is the largest database of concentration of harmful plankton and toxins in these regions. It is executed with the support of the SUBPESCA, having a total of 251 stations distributed in representative areas of each region.

The effectiveness of these monitoring systems depends on the frequency of sent samples to laboratory by the regions involved and the financing available and obtained by the various public tenders; it must be kept in mind that samplings at sea (carried out by ISP or IFOP) have a limited budget, and therefore, there are a number of specific sampling stations at strategic locations.

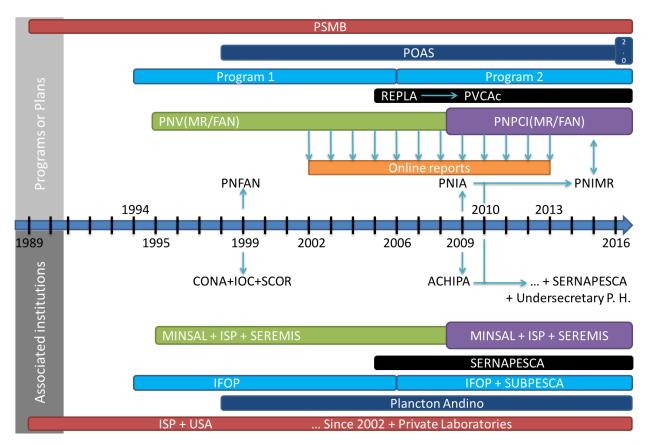


Figure 5. Diagram of the different monitoring programs in Chile. The length of the bar corresponds to the length of time in the timeline. PSMB: Programa de Sanidad de Moluscos Bivalvos, ISP: Instituto de Salud Pública, USA: United States of America, Program 1: Monitoreo de Marea Roja en la Región de Magallanes y Antártica Chilena, Program 2: Manejo y Monitoreo de las Mareas Rojas en las regiones de Los Lagos, Aysén y Magallanes, PNV(MR/FAN): Programa de Vigilancia de la Marea Roja/Floraciones Algales Nocivas, MINSAL: Ministerio de Salud, SEREMIS: Secretarias Ministeriales Regionales, PNPCI(MR/FAN): Programa Nacional de Prevención y Control de las Intoxicaciones por Marea Roja/Floraciones Algales Nocivas, PNFAN: Plan Nacional sobre Floraciones Algales Nocivas, CONA: Comité Oceanográfico Nacional, IOC: Intergovernmental Oceanographic Commission, SCOR: Scientific Committee on Oceanic Research, PNIA: Programa Nacional de Inocuidad de los Alimentos, PNIMR: Programa Nacional Integrado de Marea Roja, ACHIPA: Agencia Chilena de Inocuidad Alimentaria, SERNAPESCA: Servicio Nacional de Pesca y Acuicultura, Undersecretary of Public Health. POAS: Programa Oceanográfico y Ambiental en Salmónidos PVCAc: Programa de Vigilancia, detección y control de plaga *Alexandrium catenella*, REPLA: Reglamento de Plagas Hidrobiológica.

The monitoring plan in Chile has been oriented towards the detection of HAB events from the frequent measurement of samples of potentially contaminated bivalves. However, the delivery of information is aimed at making decisions in an operational context and the management of HAB requires a study of the trigger variables, and the evolution and development of the events in an integral manner. In September 2017, the Centro de Estudios de Algas Nocivas (CREAN), located in Puerto Montt, Los Lagos Region, was inaugurated as an effort made by the IFOP with the financing of the Corporación de Fomento de la Producción (CORFO) to focus, go further and understand the processes of HAB that are happening

with intensity in recent times on the coasts of Chile. In addition, starting in 2018 IFOP will begin the study "Programa de manejo y monitoreo de las floraciones de algas nocivas y toxinas marinas en el Océano Pacífico desde Biobío a Aysén (I etapa) 2018". Therefore, studies on harmful algae under the IFOP will cover from 37° to 55°S, in order to have timely and reliable information to protect public health and minimize the impacts associated with HAB events.

In developed countries, there are ocean observation systems (OOS such as Ocean Observing System) that are oriented to make forecasts in the ocean conditions that allow predicting potential HAB events; namely: NOAA Harmful Algal Bloom Operational Forecast System (HAB-OFS), Southern California Coastal Ocean Observing System y Central and Northern California Ocean Observing System (CeNCOOS) (Harmful Algal Bloom Monitoring and Alert Program, HABMAP), Northwest Association of Networked Ocean Observing Systems (NANOOS), WHOI-New England Harmful Algal Bloom, Gulf of Mexico Coastal Ocean Observing System Regional Association (Harmful Algal Bloom Integrated Observing System, HABIOS), Alaska Harmful Algal Bloom Network (in initiation) y European Global Ocean Observing System (in initiation).

One of the most advanced systems in terms of monitoring and forecasting of HAB is the National Oceanic and Atmospheric Administration (NOAA). NOAA maintains an operational HAB observation and prediction system to assist federal, municipal, and industry organizations in managing the risks of harmful algal blooms that affect coastal regions. This is materialized through an Integrated Ocean Observing System (IOOS, www.ioos.noaa.gov). NOAA generates weekly HAB bulletins in three sectors monitored routinely; the Gulf of Mexico, the Gulf of Maine and California. Additionally, it offers a weekly experimental forecast for Lake Erie West. In addition, NOAA is in the development stage of new sensors for HAB detection, for the forecasting (seasonal and weekly) in other regions and the transition of these new systems in routine operations in observation systems.

Environmental data collected in real time by the IOOS have proven to be fundamental in assessing HAB threats. Hourly records of ocean temperature and information of ocean currents have helped to identify the conditions triggering the proliferation of toxic algae. This allows them to evaluate the risk associated with the consumption of marine resources during these periods [http://www.ioosassociation.org/]. IOOS works with partner research institutions to integrate ocean and coastal data, and make them compatible and easily available in one place and in the formats needed for each HAB forecast. This makes scientific work more efficient, so they invest more time in improving models and forecasts [www.ioos.noaa.gov].

The detection, monitoring, expansion, and intensification of events have been extensively addressed by several research groups (Anderson *et al.*, 2015). However, only some of these studies are dedicated to the prediction of events (Tables 4-5). Tables 4 and 5 (based on Table 17.2 of Anderson *et al.*, 2015) summarize some proposed models to predict different species in the two genus of this study. One observation to take into account with this information is that a single strain culture is not representative of the world population, due to the heterogeneity between same species of different localities (Müller *et al.*, 2015).

The physical and biological coupled mechanistic models are widely used to simulate the population dynamics and the production of toxins by HABs. With these models, it is possible to make predictions of blooms by investigating the various factors that influence the germination and growth of cysts (McGillicuddy et al., 2003, 2005; Stock et al., 2005), initiation and development of blooms (He et al., 2008) and the mortality of the species that leads to the decay of bloom (McGillicuddy et al., 2003, 2005). Physical models may have more or less complexity (McGillicuddy et al., 2003) and biological models may be "on-line" (Stock et al., 2005) or "off-line" type based on the individual (McGillicuddy et al., 2003). Furthermore, in these species-specific physiological models, growth can be calibrated by applying different rates of nutrient supplement (phosphorus, Chapelle et al., 2010).

On the other hand, there are approximations for the prediction of HAB based on logic and reasoning based on rules, such as fuzzy logic. This approach constructs and quantifies a conceptual model based on a review of the literature and expert knowledge, representing the relationships between input variables, intermediate variables and the probability of a HAB event (Blauw et al., 2006). With temperature thresholds and wind mixing, it is possible to represent the gradual transitions between suitable (or not) for a bloom. It is worth noting the interest that concerns A. catenella due to the ability to become cyst and remain in the sediments while waiting for favorable conditions for their vegetative growth (Molinet et al., 2003; Seguel et al., 2011). That is why statistical models that determine the location and geographic extent of abundance of sediment cysts banks to be able to define future regions and magnitudes in events, are an important step for the prediction and prevention of these phenomena (Genovesi et al., 2009, 2013; Angeles et al., 2010, 2012; Ní-Rathaille & Raine, 2011; Anderson et al., 2014).

Data sets of several years of sampling, develop predictive logistic models (Lane *et al.*, 2009) of *Pseudo-nitzschia*, as well as find thresholds of bloom values that trigger the production of toxins (Anderson *et al.*, 2009, 2010). By achieving the statistical reconstruction of biogeochemical fields through the integration of satellite data and hydrodynamic models, the latter statistical model manages to predict HAB events of this species and its toxin (DA) up to three days in advance (Anderson *et al.*, 2011, 2014). In addition, the predictive ability in toxic DA events can be improved by using a validated ecosystem model coupled to a ROMS model (Table 5) and with particle tracking of *Pseudo-nitzschia* spp. from the well-known "hot spots" of HAB training (Giddings *et al.*, 2014).

**Table 4.** Models for the prediction of Alexandrium spp. in the world, together with a brief review of the method and the variables that they use, in addition to their advantages and disadvantages. T: Temperature, S: Salinity, chl-*a*: Chlorophyll-*a*, ROMS: Regional Ocean Modeling System, MODIS: Moderate-Resolution Imaging Spectroradiometer.

Model	Method	Variable	Advantages and Disadvantages	Reference
Mechanisist	Growth and germination cyst model (determinist).	Estuarine, Coastal and Ocean Model (ECOM) coupled with a biological model of the <i>A. fundyense</i> cyst (germition and growth rates) based on environmental forcing.	Advantages: 1) Capture duration and magnitude of the bloom, and 2) reproduce the A. fundyense dynamic.  Disadvantages: 1) Biological model simplified restrict variability scales reproduced, and 2) overestimate of the cell abundance of A. fundyense.	Stock et al. (2005)
		A fundyense population dynamic model coupled to ROMS model forced by momentum and density fluxes, tides, river runoff, nutrients and benthonic cyst abundance of the species	Advantages: 1) The hydrodynamic model reproduce small scale coastal dynamic and 2) reproduce spatial distribution of bloom. Disadvantages: 1) Underestimate abundance of cells, 2) there are problems with the mortality rates, and 3) no metal trace for growth are included.	He et al. (2008)
Empiric / Statistical	Fuzzy logic	Survey data (T, S), river discharge, wind stress, surface heat flux and insolation	Advantages: 1) Provide a quantitative method to assess distribution, timing and magnitude of the bloom, and 2) provide a base to the configuration of the conceptual model for the season dynamic of <i>A. fundyense</i> .  Disadvantages: 1) Reduced data of small scale to contrast the model output, and 2) ecological pronostic require real time data fluxes and assimilation techniques to be used operationally	Mcgillicuddy <i>et al.</i> (2003, 2005, 2011)
		Water temperature, wind speed and nutrients fluxes.	Advantage: 1) Quantitative technique to model development of blooms based on a number of variables and its interactions Disadvantage: 1) Lack of understand of the species dynamics and the ecosystem functioning and, 2) difficult to implement feedback mechanisms	Blauw <i>et al.</i> (2006)
Ecosystem / Biogeochemical	Biogeochemical specie- specific model	Based on the P limitation for A. minutum and H. triquetra cells.	Advantage: 1) Provide information on impact of nutrient on the blooms of the study species.  Disadvantage: 1) Presents limitation because mathematical parametrization are valid to specific conditions and, 2) it is limited to assess nutrients, physical control and plankton dynamic.	Chapelle et al. (2010)

As we said previously (Table 3), upwelling is a relevant factor for *Pseudo-nitzschia* species. By quantifying this phenomenon (Sacau-Cuadradro et al., 2003) by generating indexes (Palma et al., 2010) and relating them to the presence of these blooms, statistical models can be constructed to assess seasonal variation and predict conditions that are likely to promote HAB of this species (Palma et al., 2010). In this sense, the combination of SST and pigment information (remote sensing) allows the identification of key processes in blooms and their relationship with physical dynamics (Sacau-Cuadradro et al., 2003). In addition to the upwelling, another factor that controls the production of DA is the limitation in Si or P that Pseudo-nitzschia spp. may present. This is evaluated by a mechanistic model (Terseleer et al., 2013).

Finally, empirical or statistical models are used to characterize the concentration of DA in strains of *Pseudo-nitzschia pungens* (Blum *et al.*, 2006). In this case, linear regression and logistic methods are employed. However, to predict parameters of a complex and dynamic environment in an autonomous way and in real time, more complex statistics such as Case Based Reasoning systems with artificial neural networks and a set of diffuse inference systems are tested (Fernández-Riverola & Corchado, 2003). Models of artificial neural networks and genetic programming are widely used in the context of HAB problem (Recknagel *et al.*, 2002; Muttil & Lee, 2005; Muttil & Chau, 2006; Velo-Suárez & Gutiérrez-Estrada, 2007; Qian & Zhang, 2009; Gu *et al.*, 2012). In addition, it

**Table 5.** Models for the prediction of Pseudo-nitzschia spp. in the world, together with a brief review of the method and the variables they use, in addition to their advantages and advantages. T: Temperature, S: Salinity, chl-*a*: Chlorophyll-*a*, ROMS: Regional Ocean Modeling System, MODIS: Moderate-Resolution Imaging Spectroradiometer.

Model	Method	Variable	Advantages and Disadvantages	Reference
-	Generalized Lineal Model	Phytoplankton abundance, water quality, fresh water discharge, chl-a, T, S, nitrate, nitrite, ammonium, orthophosphate, silicic acid, dissolved oxygen, dissolved organic carbon, Secchi disk depth.	Advantages: (1) Allow to estimate DA, and, (2), identify environmental variables related to <i>Pseudo-nitzschia</i> .  Disadvantages: (1) It cannot identify environment indicators associated to the toxic agent, DA.	Anderson et al. (2009, 2010)
	ROMS model and satellite products (MODIS)	Ensamble of high resolution ROMS configuration, Modis- Aqua (1 km) and hydrodynamic, optic and chemical data linked in a statistical threshold model.	Advantages: (1) Allow to monitor ocean perturbations to track environmental variables that trigger a bloom, and (2) satellite data provide a broad image of the chl-a synoptic variability.  Disadvantage: (1) Requires validates ROMS and biogeochemical models, and (2) the teledetection models overestimated bloom events driving to more false positives detections.	Anderson et al. (2011, 2014)
	Generalized lineal model and multiple lineal regression	Statistical model that use <i>Pseudo-nitzshchia</i> cells, chl- a, T, nutrients, upwelling index and river discharge.	Advantage: (1) First statistical model used to predict <i>Pseudo-nitzshchia</i> based on long monitoring data.  Disadvantage: (1) Difficulty to access counting data and time series of <i>Pseudo-nitzshchia</i> cells.	Lane et al. (2009)
Empirical / Statistical	Neuronal networks	Model based on buoy, satellite and monitoring data.	Advantages: (1) Provides a system allow predicting HAB events.  Disadvantages: (1) The system is place specific and cannot apply to other cases, and (2) require a lot of data.	Fernández-Riverola & Corchado (2003)
Ecosystem / Biogeochemical	Multiple linear regression	Predictor variables include nutrients ratios and <i>Pseudo-</i> <i>nitzschia multiseries</i> cell abundance.	Advantages: (1) Model capable to predict cellular DA and nutrients in the labs and field conditions, and (2) model helps to support DA monitoring.  Disadvantages: (1) Model was validated with lab transformations which does not fit de concentration values in the field.	Blum et al. (2006)
	Ecosystem model including particle tracking and wind indices	Model forced with realistic atmospheric forcing, tides, river flow and boundary conditions.	Advantages: (1) Coupled biogeochemical model that improve HAB predictivity, and (2) model reproduce well physical process.  Disadvantage: (1) Several false positives occur.	Giddings et al. (2014)
Physical indices and monitoring with Lagrangian particles	Index of upwelling, SST and wind	Upwelling indices SST and Pseudo-nitzschia concentration.	Advantages: (1) Allow to explain physical and biological interactions, and (2) reproduces annual cycles and seasonality of upwelling Disadvantages: (1) It does not reproduce different <i>Pseudo-nitzschia</i> species.	Palma et al. (2010)
		SST and chl-a satellite images	Advantages: (1) Relationship between different sources (wind, upwelling index, SST, chl-a) set the favorable conditions to develop an algae bloom.  Disadvantages (1) It is just an analysis of the different satellite images of chl-a and SST in HAB events.	Sacau-Cuadrado et al. (2003)
	Deterministic production model focused on growth- mortality and toxicity	Nutrients (Si, P, N), light, T, chl-a and DA concentration	Advantages: (1) Development of a model that allow to link toxin production and environmental variables.  Disadvantages (1) Lack of studies oriented on adaptation of <i>Pseudo-nitzschia</i> to different light regimes.	Terseleer et al. (2013)

should be noted that neural networks along with generalized and additives linear models have already been implemented in Chile (Silva *et al.*, 2016).

According to the above, there are other prediction approaches for other species such as cyanobacteria, *Dinophysis* spp., *Phaeocystis globosa*, etc. (see Table 17.2 of Anderson *et al.*, 2015) and other models that are not species-specific, but attack the problem as HAB in general (Wong *et al.*, 2007, 2009; Bisset *et al.*, 2008; Mao *et al.*, 2009; Glibert *et al.*, 2010; Park *et al.*, 2013; Park & Lee, 2014; Jeong *et al.*, 2015).

### Detection, monitoring, and prediction efforts in Chile

Currently, different detection, monitoring and possible prediction projects of these events have been carried out in the country:

- 1) The Laboratorio de Toxinas Marinas of the Los Lagos Health Service and the Mariscope company, in conversation with the Proyecto Asociativo Regional Explora of CONICYT (Comisión Nacional de Investigación Científica y Tecnológica) Los Lagos. From 2003 until today they use data from 4 satellites to develop satellite maps of phytoplankton concentration and algal metabolic activity (Rodríguez-Benito *et al.*, 2003, 2006, 2008; Grant *et al.*, 2009).
- 2) There is a project of researchers of the Centro de Biotecnología y Bioingeniería, of the Center for Mathematical Modeling (both in the Faculty of Physical and Mathematical Sciences of the University of Chile and with financing of the Programa de Investigación Asociativa of CONICYT), scientists of the Center i-Mar from the University of Los Lagos and the IFOP in Puerto Montt. They form a multidisciplinary team that seeks to analyze the viability of a biological-mathematical model that allows understanding the dynamics of HAB episodes.
- 3) The project entitled "Modelo e implementación de un sistema de seguimiento y vigilancia de marea roja al sistema de información geográfica de la Subsecretaría de Pesca y Acuicultura" Silva *et al.*, 2016), is a computational visualization system [http://mapas.sub-pesca.cl/visualizador/], with a geos-patial analysis model that relates the biological, oceanographic and meteorological variables collected by the IFOP Program 2 (see Fig. 5). This allows monitoring and vigilance of the occurrence of these events and estimating the area of influence. In 2016, FIPA N°2016-13 ID N°4728-43-LP16 was approved, which is the second stage that will continue with this project.
- 4) The Instituto Tecnológico del Salmón (INTESAL) has an online platform that seeks to follow the conditions of abundance of total phytoplankton, the presence of harmful algae and HABs, specifically of the species *Alexandrium catenella*, and the concentration

- of chlorophyll-*a* [http://mapas.intesal.cl/publico]. It is carried out thanks to the data collected by the Programa de Monitoreo de Fitoplancton (PROMOFI), which is owned by the companies associated with SalmonChile.
- 5) Taking synthetic biology tools, at the end of 2016, a group of students of the Molecular Biotechnology Engineering career at the University of Chile (UChile-Biotec) develop BiMaTox, a biosensor specifically aimed at the detection of STX. The advance that this model would make with respect to the current method of detection of paralyzing toxins (mouse bioassay), would be in a lower cost and time of reaction (~3 h) as well as higher efficiency.
- 6) In June 2017, the Universidad de La Frontera (UFRO), together with the University of Kyoto, Okayama University, the Fisheries Research Agency of Japan, the University de Los Lagos, and the University of Antofagasta were awarded a project to monitor, predict, and detect HABs in southern Chile, especially in areas where salmon are grown for export. The aim is to build a monitoring and prediction kit (1 or 2 days) that allows identifying all the microorganisms that accompany the HAB or that can predict it before it happens.
- 7) During 2017, the Fondo de Fomento al Desarrollo Científico y Tecnológico (FONDEF) called a first thematic technological research contest on aquaculture fisheries systems against HABs (FANs-IDEA). Among those awarded, the following stand out: "Análisis de riesgo y sistema de alerta temprana de Floraciones Algales Nocivas para la acuicultura y áreas de manejo en el norte de Chile" (Northern Catholic University), "Sistema masivo y de bajo costo para el monitoreo *in situ* de algas nocivas en toda la costa Chilena" (Pontifical Catholic University of Chile) and "Huella digital hiper-espectral de especies de marea roja mediante el acoplamiento de señales bio-ópticas remotas e *in situ* en Chile Austral" (CSIRO-CHILE Research Foundation).
- 8) The Programa Nacional Estratégico, Fisheries and Aquaculture component, designed a mobile application with preventive information on HAB in Chile (i~FAN). The application, designed by the company Dialecto Sur, allows knowing the updated and detailed reports on HAB that are generated by the CREAN specialists of the IFOP.

At present, there are monitoring, detection, and/or forecasting efforts to find solutions to understand these events and thus delimit areas of risk. However, these efforts correspond to studies of particular and uncoordinated research groups. This means that at present there are no available HAB prediction models in constant operation in Chile, delivered as public forecasting tools. Given the tendency of the frequency of events (Figs. 2, 4) and the spatial extension (Fig. 3, Table 1-2) of these in the country, it is urgent to

understand the processes and factors that trigger HABs to be able to have an integrated and associative system for predicting these events.

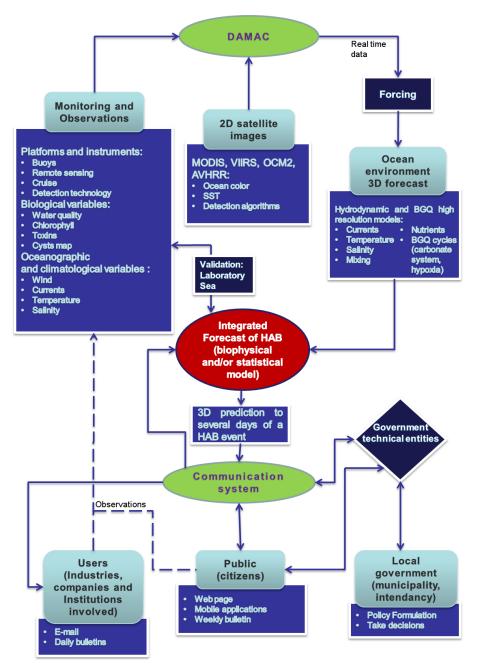
#### DISCUSSION

# The future in the problematic of HAB: System of observation and forecast

Ocean observation systems are powerful tools for the constant and integrated monitoring of the oceans in the world and have been able to respond to various environmental problems in these systems. In developed countries that have an OOS, the policy making agencies and the central government: 1) identify and prioritize areas of basic and applied strategic research to address the HAB problem, 2) identify the most important vulnerabilities of the system, and 3) focus work on mitigation measures that have the greatest impact on society (Anderson et al., 2015). On the other hand, universities and research institutions work to strengthen scientific studies and joint collaboration to address the problems associated with HAB in a more efficient way from the point of view of research and operation (Wilson, 2011). The problem of the prediction of HAB events has been addressed from the perspective of in situ observations or remote sensing, to the use of numerical models that allow projections of the HAB (see Tables 4 and 5 of this article and 17.2 from Anderson et al., 2015). Anderson et al. (2009, 2011) represent an efficient alert system that includes multidisciplinary efforts and provides quantitative predictions of the probability of HAB events, their intensity and movement or influence along the coast. CeNCOOS, a regional association of the IOOS, with this methodology generates predictions of 1 to 3 days calculating in each pixel the probability of bloom of a toxic event (maps with color scales representative of the probability of a toxic event, http://www.cencoos. org/data/models/habs). The combination of an HAB in situ monitoring system and operational forecasting models with data assimilation, determines the HAB detection system in California, currently fully operational (Anderson et al., 2015).

HABs in Chile have been approached from the point of view of monitoring programs (PSMB, PNPCI (MR/FAN), Program 2, Fig. 5) and control through laboratory studies. However, these efforts are not enough when necessary to develop a forecasting strategy. Currently, in the Biobío Region, a research initiative has been launched called "Chilean Integrated Ocean Observation System-CHIOOS" (INNOVA 15.5 -IN.IIP, http://chioos.cl/en/). This developing system provides a technological platform that collects, integrates and delivers coastal ocean information to

facilitate decision-making regarding security and resource management, the environment, maritime transport and predictions and mitigation of coastal threats in the Chilean territory. Although it is an expanding observation system, the basic components that a HAB prediction system and its requirements could have, due to their importance in the area (in particular Pseudo-nitzschia spp., A. catenella and A. ostenfeldii, Figs. 2-3), have been evaluated. These requirements are listed below: 1) Improve the current observation and continuous monitoring system by defining key observation sites ("hot spots") and incorporating the cysts distribution study, more environmental data (physical and biogeochemical), satellite information, and more and better technologies of timely detection, 2) it requires experts dedicated exclusively to the various areas that form the problem (multidisciplinary analysis). These experts must be able to integrate the exceptional combination of different conditions of these phenomena, analyze and synthesize the information, as well as conceive and validate hydrodynamic and biogeochemical models for the prediction of the oceanic environment, 3) incorporate the control and storage of data in charge of a Departamento de Almacenamiento, Manejo y Control de datos (DAMAC, Fig. 6), to generate and disseminate in a sustained manner data, information, models, products and services, 4) a comprehensive or ecosystemic understanding of these phenomena is required, understanding the physiological adaptations of these microalgae, the population growth and encystment rates (e.g., A. catenella). With this purpose, we will obtain an understanding of which are the variables that are directly related to the HAB at the national, regional and local levels. This will lead to a study of a set of variables that are basic measurements made by possible regional observation systems. The occurrence of HAB events may be conditioned for A. catenella, for example, by positive temperature anomalies, stability and stratification of the column, wind reduction, nutrients, chlorophyll-a, distribution of cysts, among others (Table 3). On the other hand, the occurrence of HAB events for Pseudo-nitzschia may be conditioned by indicators such as negative temperature anomalies, upwelling wind, presence of Equatorial Subsurface Water and turbulence indicators, chlorophylla, among others (Table 3), 5) Understanding the key biological aspects, a short-term forecasting model based on data and hydrodynamic and ecosystemic models should be developed (e.g., biophysical and/or statistical approach). This model must be validated both in laboratories and at sea in a permanent and retroactive way (T. Antezana, pers. comm.), 6) a system must be elaborated that delivers efficient operational products that are used by the community such as: mobile appli-



**Figure 6.** Diagram of the integrated forecasting system for HABs events in Chile, based on Anderson *et al.* (2014). DAMAC: Departamento de Almacenamiento, Manejo y Control de Datos, VIIRS: Visible Infrared Imaging Radiometer Suite, OCM2: Ocean Color Monitor of Oceansat-2, AVHRR: Advanced Very High Resolution Radiometer, BGQ: Biogeochemical.

cations, information displayed on the Internet (online system), newsletters, among others. This must reach a communication system with information for users, public and technical government entities that are capable of disseminating this information and making it available to local governments, responsible for formulating policies and decision makers (Fig. 6) and finally, 7) implement a feedback system between oceanographic observations and models to better adjust

and force predictive models. In addition, the community should be asked for feedback about the products provided and possible non-registered blooms/intoxications (see Fig. 6).

In response to this idea and within the framework of an integrated ocean observing system for Chile, a model of HAB event information management should incorporate: a) government participation in the planning processes and delivery of updated information to the citizens and institutions involved, and b) coordinate information between participating institutions and incorporate channels to transmit information from authorities to users and vice versa. Only in this way, we will be able to have a forecast both in the causes that trigger an event and possible extension, and in the explanation of intensity, magnitude, toxicity and eventually its permanence or recurrence. The aforementioned, it would be an important step for the strengthening of the institutionality in Chile, delivering instruments that allow to face effectively the potential events of HAB. In the Biobío Region, in June 2016, the POSAR system (Plataforma de Observación del Sistema Acoplado Océano Atmósfera, http://dgf. uchile.cl/POSAR/) anchored in front of the Itata River mouth (36°S, 72°W, Fig. 1) was installed and is currently measuring. This buoy has hourly observations of meteorological variables and physical and chemical parameters of the surface ocean relevant to detect and forecast HAB phenomena (e.g., wind, solar radiation, salinity, pH and chlorophyll in the sea). Thanks to these efforts, the creation of the monitoring program for harmful species and marine toxins in selected bays of the Biobío Region by the IFOP (18 stations) and together with other mentioned platforms (satellites, numerical models, etc.), could enhance this integrated observation system to forecast these events in the region.

The ultimate social objective of all HAB model efforts should be to mitigate negative impacts. The costs of developing an operational forecasting system are balanced by socio-economic benefits and the protection of living marine resources, or at least they should provide significant added value (Anderson *et al.*, 2015). An unique advantage of a prevention system for fishermen and shellfish cultivators is the spatial and temporal prediction of bloom or the presence and dispersion of the toxin, which would allow better definition of cultivation and resting areas, geographical changes in the fishermen's efforts, protection of health in people, the marine environment and reduction in monitoring costs and medical attention.

#### CONCLUSION

In Chile, the HAB events of *Alexandrium catenella* have been reported since 1992 in Aysén and 1998 in the Los Lagos Region to the present. These events have generated environmental, social, economic and health catastrophes mainly in the years 2002, 2006, 2009 and 2016. However, in the last time the various appearances of *Pseudo-nitzschia* spp. have had a serious impact during some periods on the production of shellfish mainly in the years 2006, 2007, and 2009. The latter

represents a risk to public health, as *A. catenella*, due to the content of ASP. The spatial and temporal patterns of both species are different in the records however, what they do have in common is that there has been an increase in the coverage and frequency of these phenomena. In addition, although both are HAB species and are part of marine plankton, they have some contrasting or antagonistic characteristics in the differences between these two functional groups: dinoflagellates (*A. catenella*) and diatoms (*Pseudonitzschia* spp.).

Although the Chilean system based on monitoring has managed to prevent fatalities in the last bloom of 2016, it has not generated mitigation solutions and contingency plans based on prediction. This generates the consequent loss of effectiveness in the management actions before a HAB. HABs are a complex problem that may depend on the interaction of many biological, physical, chemical, climatological and anthropic factors. Adequate monitoring of the column of water and sediments (resistance cysts), long time series (>30 years), understanding of the life cycles of microalgae, satellite images together with numerical and statistical models should be included in the search to forecast these phenomena and improve our understanding of the structures and processes that give rise to HABs.

In the world there are various models of prediction of these phenomena. From the simplest based on empirical relationships between predictive variables, to the most complex systems of artificial neural networks that require much expert statistical knowledge. The fact that certain technology is used in other latitudes is not ruled out for the national situation, but it must be adapted to the current Chilean situation. It is clear that the lack of records and long-term historical environmental data, as well as in real time during the HAB in Chile, is a serious obstacle to identify and understand the mechanisms that are involved in the triggering, intensity, extent and toxicity of these events. This limits our understanding of the relationship with natural and/or anthropic factors. We must be able to group the individual efforts, in order to generate a model with integrative capacity of this problem. Although, the initial costs for this model, the trained personnel and the computational requirement would be high, they would be balanced by the socioeconomic benefits that they bring, such as higher protection of living marine resources, prevention for public health, and lower economic losses. The latter because it would not be necessary to paralyze the total fishing activity, a significant decrease in the purchase of seafood products (national and international) and an increase in monitoring costs for medical care.

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