

A SIMPLE SHORT RANGE MODEL FOR THE PREDICTION OF HARMFUL  
ALGAL EVENTS IN THE BAYS OF SOUTHWESTERN IRELAND

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## Abstract

A simple model is described which predicts harmful algal events in the bays of southwestern Ireland. Fundamental to the model is the nature of the physical forcing of circulation in these bays in summer. The predominant hydrographic feature at this time of year is a wind-driven two-layer oscillatory flow acting in a thermally stratified water column. These flows exchange substantial proportions of the volume of the bays and harmful algal events arise due to the associated transport of harmful phytoplankton populations into them. The model is therefore based on the criterion that wind driven water exchanges will result in exchanges of phytoplankton, which, if the time of year is correct, result in a toxic event. Utilising Bantry Bay as an example, hindcasting showed that the model has a high degree of success using a wind index based on the sequence of wind directions which would generate water exchange; high wind indices indicate water exchange. A predictive model was developed by estimating wind indices from the five-day weather forecast. The model was trialled in 2005 and results were published on the web in real time. During the trial, a predicted water exchange event in mid-June was accompanied by an influx of *Dinophysis acuminata* into Bantry Bay, with subsequent contamination of shellfish with Diarrhetic Shellfish Poisoning (DSP) toxins.

## Introduction

The considerable economic losses which can derive from harmful algal events, often referred to as Harmful Algal Blooms or HABs, makes their prediction desirable in order to develop or activate mitigation procedures. In the case of finfish culture in cages, primary mitigation actions include aeration, translocation of the cages or the physical prevention of bloom encroachment. Practical options include the use of skirts around the cage perimeter, airlift pumping or removal of the bloom through flocculation resulting from the spreading of clay, activities which are summarised and described in detail in Anderson et al. (2001). Mitigation procedures for the contamination of farmed shellfish with algal biotoxins, however, are not so direct. This is probably because, unlike finfish, the effects of HABs on shellfish do not usually involve mortalities of stock and the producer is only aware of its contamination after the time required for toxin testing. The whole stock in a location is usually affected, and the common option to the shellfish farmer involves simply waiting, sometimes for long periods (months), for the product to naturally depurate the toxin. A forewarning of a HAB event would be extremely beneficial here, and would enable mitigation measures such as carrying out a small harvest for shellfish processing if markets for fresh stock are not available at that time, or moving marketable stock to clean tanks on shore.

The southwest of Ireland is one of the most important national regions for shellfish culture. Approximately 80% of national rope mussel (*Mytilus edulis*) and 50% of Pacific oyster (*Crassostrea gigas*) is produced here annually (Parsons, 2005). The industry is located in the large bays of Bantry, Dunmanus and Long Island (Figure 1) which give Ireland its characteristic coastal outline in the southwest. These bays are

drowned river valleys, or rias. They exhibit limited estuarine characteristics and thermally stratify in summer (Raine et al., 1990a). Tidal currents are weak ( $<5 \text{ cm s}^{-1}$ ) in these bays and the circulation is essentially driven by the wind (Edwards et al., 1996) due to the geometry of the bays: they are axially aligned to the predominant wind direction which comes from the southwest quarter. When the bays are thermally stratified, variations in the axial wind vector cause two layer oscillatory flows which generally result in the import of water from the near coastal continental shelf together with the phytoplankton within it (Edwards et al., 1996). Substantial fractions of the bay volume are exchanged during these events, which discourage the development of indigenous phytoplankton populations within the bays (Raine et al., 1993).

It has long been suspected that toxic algal events in the bays of southwestern Ireland arise due to the transport of populations of harmful species from the near coastal shelf. Import of potentially harmful species such as *Karenia mikimotoi* have been shown to result from wind-forced water exchange in Bantry Bay (Raine et al., 1993), as well as other non-toxic phytoplankton populations (Goward and Savidge, 1993). Contamination of shellfish with algal toxins which cause Diarrhetic Shellfish Poisoning (DSP) is one of the biggest problems caused by HABs in the region (Silke et al., 2005). This paper demonstrates wind-driven import of *Dinophysis* spp. and consequent contamination of shellfish with DSP toxins and investigates the use of the short range weather forecast to predict harmful events. The prediction model is based on water and plankton exchanges resulting from variations in wind speed and direction, coupled with a simple probability approach based on the time of year when harmful blooms are most likely to occur.

## Methods

In 2001 a pilot study was carried out in Bantry Bay (Figure 1). Water movements were monitored through the deployment of a bottom mounted RDI 300 KHz ADCP current meter within the middle of the bay off Shot Head (Figure 1b) in a water depth of 40 m. The instrument was deployed on 1 August 2001 and recovered on 7 November 2001. These measurements were supplemented by the prior deployment of a thermistor chain comprising four in situ temperature sensors (TidbiT, Mass.) sited at the same distance along the bay as the current profiler. The temperature sensors were deployed at 1 m below the surface, 1 meter off the seabed, and two distributed evenly along the mooring line over a depth of 1-25 m. The thermistor chain has since been maintained at this location. Meteorological data (wind speed and direction) were routinely derived from measurements made by the national meteorological service (Met Éireann) at the Valentia weather station, a coastal station sited approximately 30 km north of the study region (Figure 1). Wind measurements made within the bay have shown a high correlation with those measured at Valentia (Raine et al., 1993b). Data for 2005 and subsequently were also obtained from the M3 weather buoy deployed at 51° 13' N 10° 33' W (Fig.1)

Water samples for phytoplankton analysis were taken each week in 2001 from a depth of 0 and 10 m at the thermistor chain site using 5 l Van Dorn water sampling bottles (Hydrobios, Kiel). The sample set was supplemented by phytoplankton samples (also from 0 and 10 m) taken at the same site as part of the National Phytoplankton Monitoring Programme (NMP) (Silke et al., 2005). All phytoplankton samples were preserved in Lugol's Iodine, and counted using a modified Utermohl's method (Raine

et al., 1990b). The NMP data archive was also analysed for the period 1993-2004. Data derived from discrete water bottle samples only, as from 2003 an increasing amount of data in the archive derived from integrated water samples using 10 m length tubes (Lindahl 1986). Data on the presence of (DSP) biotoxins in mussel flesh from rope cultured mussel samples from sites around Bantry Bay were taken from the national biotoxin monitoring programme archive.

## Results

*Field results.* The results of the analysis of phytoplankton samples for *Dinophysis* spp. content from 2001 are shown in Figure 2a, where increases in cell densities occurred on or around 26 July preceding a bloom event. Thermistor string data clearly show the co-incidence of this event with water exchange. (Figure 2b). The increase in cell counts corresponded with an influx of warm surface layer water into the bay. The relationships between bottom currents and wind, and bottom temperature with wind, shown in Figure 2c and 2d, demonstrate the predominant effect that wind forcing has on water exchange events, and that the axial wind acts as a very good proxy for water exchange in the bays. Winds blowing down the bay (seawards) are associated with an efflux of surface water and influx of bottom water. Currents reverse when the wind subsequently blows in the opposite direction. The data set shown in Figure 2 is in clear agreement with the hypothesis of Raine et al. (1993) that HAB events in the bays of southwestern Ireland are caused by the import of harmful populations.

*Model development.* The two layer, wind-driven oscillatory flow exchange model for the bays of southwestern Ireland is sketched in Figure 3a. It originates from studies carried out in the early 1990s (Raine et al., 1993b; Edwards et al, 1996). The predominant situation is portrayed in the upper diagram, as approximately 80% of the wind is from the southwest quarter. It is fluctuations in this component which promote water exchanges in Bantry Bay. Exchange events can therefore be deduced if there is a particular sequence in the axial wind vector.

The prediction model was developed on the simple hypothesis that in the first instance

harmful algal events in Bantry Bay primarily arise in summer, a time of year when the water column is thermally stratified. Implicit in this statement is that dinoflagellates are the group of organisms that are the primary cause of HAB events, due to observed general relationships between stratification levels, which have their seasonal maximum in summer in temperate regions such as Ireland, and phytoplankton group (Margalef, 1978; Jones and Gowen 1990). Of course this is by no means true in the global sense, and ignores the occasional contamination of shellfish with, for example, ASP toxins derived from the diatom genus *Pseudo-nitzschia*. Nevertheless the biggest HAB problems around southwestern Ireland arise from contamination with DSP toxins derived from populations of *Dinophysis acuta* and *D. acuminata*. Analysis of the data archive for the region derived from the national phytoplankton monitoring programme shows quite clearly that these *Dinophysis* species occur principally between June and September (Figure 3b), a period when >97% of samples containing *Dinophysis* at cell densities  $>100 \text{ cells l}^{-1}$ , a level close to that considered high enough to contaminate shellfish (Botana et al., 1996), were obtained (Table 1).

A certain sequence in wind direction, axially negative (blowing out of the bay) followed by axially positive (blowing into the bay) will cause an exchange event in the bays of southwestern Ireland in summer. Relating this sequence to time of year allows a fuzzy logic style approach to the prediction of harmful events. This is depicted in Figure 3c, which relates the relatively high probability of HABs occurring within the summer temporal window with the wind-driven hydrodynamic model of water exchange in the southwestern Irish bays. What was required was the establishment of a numerical value, derived from the wind data, which signifies the likelihood of an exchange event.



The numerical value derived was based on the magnitude and sign of the wind vector that is axially aligned to the bays ( $060^{\circ} - 240^{\circ}$ ). First, the daily average components of the wind blowing out of the bay (seawards) for three consecutive days, in  $\text{m s}^{-1}$ , were added together. This value was then added to the daily average axial component blowing landwards for the subsequent two days. Emphasis is placed on the atypical condition when the southwesterly wind component is zero or negative through the choice of a three day period. This treatment is shown schematically in Figure 3d, and the value obtained is hereafter referred to as the wind index.

*Model hindcasting.* The phytoplankton data sets available for the summer of 2001 and 2002 were chosen to initially test the prediction model as these were two of the few early occasions when data was obtained with a suitable high frequency at this time of year for adequate hindcasting. Results are shown in Figures 4a and 4b. The timing of the *Dinophysis* bloom of 2001 (Figure 2) correlated with an exchange event which is indicated by wind index values of the order of  $10 \text{ m s}^{-1}$ . In 2002, *Dinophysis acuminata* cell densities rose from 40 cells  $\text{l}^{-1}$  taken on the 10<sup>th</sup> and 25<sup>th</sup> July up to 4600 cells  $\text{l}^{-1}$  on 1 August in samples taken at the Roancarrig monitoring point in Bantry Bay. Over the same period, levels of okadaic acid, the DSP toxin associated with *D. acuminata*, rose from 0.03 and 0.05  $\mu\text{g g}^{-1}$  on 15 and 22 July respectively to 0.20  $\mu\text{g g}^{-1}$  on 29 July. This increased concentration was above the action level of 0.16  $\mu\text{g g}^{-1}$  when harvesting is prohibited. These events spanned a high wind index of  $12 \text{ m s}^{-1}$  on 26 July, and a rapid increase in bottom (15 m) water temperature from  $12.1^{\circ} \text{C}$  on 20 July to  $14.9^{\circ} \text{C}$  on 29 July, indicating that a substantial exchange of water and phytoplankton had taken place.

The predictive model occasionally in hindcast resulted in positive indications of a toxic event but without a corresponding increase in *Dinophysis* levels or toxicity. One such ‘false positive’ indication occurred on 1 July 2002 (Figure 4b) when a high wind index (and water exchange) was not accompanied by an increase in *Dinophysis* or toxicity in shellfish tissue. The significance of ‘false positives’ is discussed later.

Prior to 2000, the biological record is not as comprehensive. This is due to an inadequate regularity in both sampling and toxin testing, as well as a problem in interpretation of toxicity test results due to changes in methods of analysis. Nevertheless, between 1991 and 2000 shellfish closures can be correlated with wind-driven exchange events as indicated by high wind indices (Blauw et al., 2006). In this period, however there were two bloom events for which suitable data sets exist for hindcasting. One of these is for a DSP event in Bantry Bay which occurred in 1994 and is described in McMahon et al. (2002). On this occasion an influx of *Dinophysis acuta* clearly coincided with a high wind index (Figure 4c). The second is for a bloom of *Karenia mikimotoi* described by Raine et al. (1993) which advected into Bantry Bay at the beginning of August 1991. Both events occurred in Bantry Bay and both can be hind cast using the model approach.

*Model implementation.* The predictive model was implemented in 2005 by downloading a daily feed of a 5-day weather forecast for Bantry Bay ([www.bbc.co.uk/weather](http://www.bbc.co.uk/weather)) and calculating the wind index. Negative values for the predicted wind index were ignored and designated zero. Negative values imply prolonged southwesterly winds, a situation which would not produce water exchange.

The wind index data were updated and published daily on the web ([www.marine.ie](http://www.marine.ie)). The accuracy of the prediction was tested against weather data from both Valentia and the M3 Weather Buoy, which had been recently deployed in 2004. The comparison is shown in Figure 5.

It can be seen that through the summer the prediction based on weather forecast indicated that within the high probability window indicated in Figure 2b exchange events should have occurred on 16 and 30 June, and 16 and 30 July. The bottom water temperature data indicates that exchange events occurred on each of these occasions (Figure 6). The forecast exchange of 30 July (and an exchange also indicated by the temperature) did not yield a wind index above  $10 \text{ m s}^{-1}$  from the actual record, either from the M3 buoy, which consistently gave higher values due to its location out to sea than at Valentia. Nevertheless the numerical threshold of  $10 \text{ m s}^{-1}$  for the (forecast) wind index appears to be satisfactory. Use of regression statistics on the forecast data is diminished, when applied to the practical success of the model, as the regression on actual data (between Valentia and M3 weather stations) gives an  $r^2$  value of only 0.44 ( $n=36$ ; positive values only). Of more practical relevance is that the model was able to predict every exchange event during the trials (Figure 5). On only the one occasion did the model produce a wind index  $>10 \text{ m s}^{-1}$  which was not matched by the actual record on 29 July.

*2005 Harmful Events.* In terms of potentially harmful phytoplankton, the year 2005 was marked by an extensive bloom of *K. mikimotoi* which extended along the entire western seaboard of Ireland (Silke et al., 2006). Three integrated (10 m) water column samples yielding 4000-10,000 cells  $\text{l}^{-1}$  of *Karenia mikimotoi* were sampled

between 31 May and 6 June. This population was most likely advected in to the bays with the exchange event of 2 June (Figure 5). Substantial increases in *Dinophysis* cell densities (in integrated water samples) were observed in samples taken on 21 June at Castletownbere, toward the mouth of Bantry Bay. This result was not replicated at Gearhies until 5 July. Both timings however corresponded to an influx of surface water after a forecast exchange event. Virtually all mussel harvesting sites in Bantry Bay were closed due to contamination with DSP toxins during the week 21-24 June 2005 (Clarke et al 2007). These results show that an influx of DSP causative organisms had occurred during the exchange event forecast for 16 June of that year.

## Discussion

The frequency and length of harvest closures of shellfish aquaculture around southwestern Ireland (see e.g. McMahon et al., 1996) demand some form of their prediction. On the basis that contamination with *Dinophysis*-derived DSP toxins is one of the principal causes of closures in the area, the use of relatively sophisticated biological and physical coupled predictive models requires an intimate knowledge of the behaviour of these species to enable the models to be successful. At present we know very little of the life cycle and behaviour of this species, except that they are known to occur in thin sub-surface layers in very high density. We still know nothing however of the maintenance and behaviour of the populations in these sub-surface layers (GEOHAB 2008). Recent advances in ability to culture the organism may improve this situation (Park et al., 2006). However, the accurate prediction of these layers is still impossible, and their origin remains unknown.

It is now understood that virtually all harmful algal bloom events that occur in the bays of southwestern Ireland arise as a result of the transport of harmful phytoplankton populations from the continental shelf via wind driven exchange. The Irish coastal current runs clockwise around the southwest corner (Raine & McMahon 1998). The principal feature of this flow is a relatively fast (ca.  $25 \text{ cm s}^{-1}$ ), narrow (<10 km) density driven coastal jet which results from the increasing effect of tidal mixing on a stratified water column as one nears the coast (Fernand et al., 2006). There is now direct evidence showing the importance of these jets in transporting potentially harmful populations towards the mouths of the bays (Farrell et al., 2008). However, the critical point is that until physical models describing the continental shelf dynamics attain a sufficiently high resolution to incorporate mesoscale processes

such as these jets, use of biological physical coupled models to predict harmful events originating over the continental shelf will remain unsatisfactory.

An exchange event will occur if the wind vector axial to the bays is negative for a short period, ca. 2-3 days, and then positive. This sequence would arise, for example, from the passage of an atmospheric low pressure across the south of Ireland, or even a shift in the position of a high pressure region, usually located over Ireland and Britain at this time of year. The anticyclonic (clockwise) currents around the southwest corner of Ireland are typically quite small, of the order  $5 \text{ cm s}^{-1}$ , but variable dependant on the wind direction (Raine and McMahon 1998). The flow can increase substantially to  $20\text{-}25 \text{ cm s}^{-1}$  under easterly wind conditions (Raine and McMahon, 1996). Under strong southwesterly winds the flow is severely restricted and even the coastal jet does not operate (Brown et al., 2001). Nevertheless when the wind has a negative component which is axial to the bays of the southwest, the coastal flow is promoted. It takes 2-3 days for water to flow from a region off Galley Head (Figure 1) around to the mouth of Bantry Bay. This coastal shelf area of the south of Ireland is the area where high densities of *Dinophysis* are found in summer. For example a population of  $124\,000 \text{ cells l}^{-1}$  *D. acuminata* was found here in 1992 (Raine and McMahon 1996), and one of  $55\,000 \text{ cells l}^{-1}$  of *D. acuta* in 2007 (Raine 2008). On both occasions the populations were directly observed (2007) or inferred (1992) as existing in a sub-surface thin layer. The simplicity of the model presented here masks more complex physical processes occurring on the continental shelf that are wind dependant. The three day period of negative axial winds not only promotes an exchange event but also allows the correct transport of a harmful population to the mouth of the bays, prior to them being advected in when the axial wind component

becomes positive.

The simplicity of the model is its main advantage and optimises its operational value. Certain aspects of physics have been ignored. For example the effect of wind on surface currents has a cube function, although a direct linear relationship has been applied in the model. The approach used would also be readily applicable to other bays where wind driven water exchanges occur. Certain modifications will be necessary for bays where freshwater input contribute to water exchange with the shelf through processes such as entrainment. The fact that the model is site specific, as opposed to species specific, is however an advantage. Physical processes which affect the transport of *Dinophysis* will also affect other harmful species. This has been shown for *Karenia* in Bantry Bay but would also apply to other toxin producing species.

The model has a very high accuracy. For the period 1996-2000 when still at the developmental stage using fuzzy logic, the onset of toxic events correlated extremely well with water exchanges predicted in hindcast mode with a high wind index (Blauw et al 2006). Occasional closures not predicted were observed prior to these events but these were restricted to single sites: there are eight shellfish quality testing sites around the bay. Uncertainty in the toxin contamination data series increases with the age of the record, whether this is due to false positive assays or the type of bioassay applied, and bioassays used at the time would not indicate the precise nature of the contamination. Nevertheless, subsequent to 2000, the model has worked with high level of accuracy, indicating times for the onset of toxicity.

All predictive models have an in-built probability which will on occasion generate false positive results, the frequency of which will rely on the statistic used. In this case, the key criterion was the temporal window of June to end September, outside of which little or no *Dinophysis* was present. Within this risk period, false positives have occurred, that of 2002 being repeated in 2007. In the case of 2007, it is now known that a *Dinophysis* population was present in the northern Celtic Sea, but was not in the correct position to be transported into Bantry Bay when water exchange occurred (Farrell et al., 2008). A false positive in 1998 which did not result in *Dinophysis* import or DSP toxicity was however associated with a bloom of *Karenia mikimotoi* (Blauw et al 2006). By far the more important criterion of the quality of the model is if it generates false negatives, i.e. does not predict exchange event yet *Dinophysis* blooms arise. This the model has not done over the period 1992-2002 (Blauw et al. 2006) or subsequently..

The model will not predict when the contamination of shellfish will dissipate, information highly desirable to the shellfish producer. Another disadvantage of the model lies in its forecast range. This is restricted to that of the meteorological forecast, which at present is accurate for approximately five days. Obviously predictions which have a 30+ day range would be much more desirable and practical, allowing the activation of suitable mitigation measures by shellfish producers. For systems such as the bays of southwest Ireland which are driven by wind speed and direction, this is at present unattainable. However, given recent observations confirming the transport of *Dinophysis* within the coastal jet off the south of Ireland (Farrell et al 2008), one practical measure which could lead to an extended prediction would be the deployment of offshore plankton observatories. These utilise modern,



sophisticated *in situ* monitoring techniques, and are only now beginning to be developed (Greenfield et al., 2006). Their placement at key locations upstream in the known paths of harmful blooms would provide information which would improve the prediction capability of the model presented here, as well as those for other areas such as the Gulf of Maine (Keafer et al., 2005) where offshore harmful blooms impact onshore as a direct result of weather patterns.

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- Figure 1. Map of the bays of southwest Ireland showing locations mentioned in the text.
- Figure 2. Field data for 2001; a) *Dinophysis* record for Gearhies. Note the increase in cell densities of *D. acuminata* from 28 July; b) contoured temperature data from thermistor string located at Gearhies. Note the influx of warm surface water occurring between 26 July and 7 August; c) comparison of wind vector axial to Bantry Bay (060°) with near bottom (33 m) currents measured with a bottom mounted ADCP located off Shot Head; d) comparison between axial wind vector and bottom temperature measured at Gearhies. Locations are marked in Figure 1.
- Figure 3. The basis of the predictive model described in the text. a) schematic of wind driven two layer oscillatory flows described in Edwards et al (1994); b) analysis of the *Dinophysis* record for Bantry Bay, 1993-2003. Note the significant increase in samples with levels considered potentially harmful (>150 cells litre<sup>-1</sup>) between weeks 25 and 39 (~ mid June to end September); c) the fuzzy logic style approach to prediction of harmful events in the bays of southwestern Ireland; d) generation of the wind index used to predict wind-driven exchange events.
- Figure 4. Hindcasting the predictive model using wind indices to predict exchange events. a) wind indices for 2001. The high wind index >10 m s<sup>-1</sup> observed on 16 July (arrowed) was followed by a sharp increase in *Dinophysis* cell numbers at Gearhies (see Figure 2a); b) wind indices for 2002. An initial high wind index on 30 June was not accompanied with a toxic event. Shellfish harvest closures did, however, follow a second exchange event indicated by the high wind index of 16 July (arrowed); c) wind indices measured over a toxic event in 1994 shown in d). Increases in both densities of *D. acuta* and associated toxin (DTX-2) levels in Bantry Bay. Maximum observed cell densities have been

plotted for clarity (adapted from McMahon et al., 2002).

Figure 5 Predicting harmful algal events in Bantry Bay. The wind index obtained from 5-day weather forecast is plotted against actual values measured at both Valentia and the M3 Weather Buoy (see Figure 1 for respective locations). Exchange events are predicted for 18 May, 16 and 30 June, and 16 and 30 July. On each occasion, water exchange as shown in the bottom temperature record took place. The event of 16 June (arrowed) was followed by an increase in cell densities of *D. acuminata*. And shellfish harvest closures were in operation in Bantry Bay from this date. See text for details.

Figure 6. Temperature record from Gearhies, Bantry Bay, 2005. Arrows indicate the forecast water exchanges for 17 May, 2 June, 16 June, 30 June, 16 July and 30 July respectively, which can be compared with those predicted from Figure 5.



Table 1. Occurrence of *Dinophysis* spp. around the south, southwest and west coasts of Ireland, analysed by week number, over the period 1993-2004. Data are taken from the National Phytoplankton Monitoring Program of the Irish Marine Institute.

Week Number	Total Samples Analysed	No. of samples with >100 cells l <sup>-1</sup>		
		<i>D. acuminata</i>	<i>D. acuta</i>	<i>D. acuminata</i> or <i>D. acuta</i>
1-21	4570	15	7	19
22-38	8647	744	610	1117
39-52	2539	6	5	11

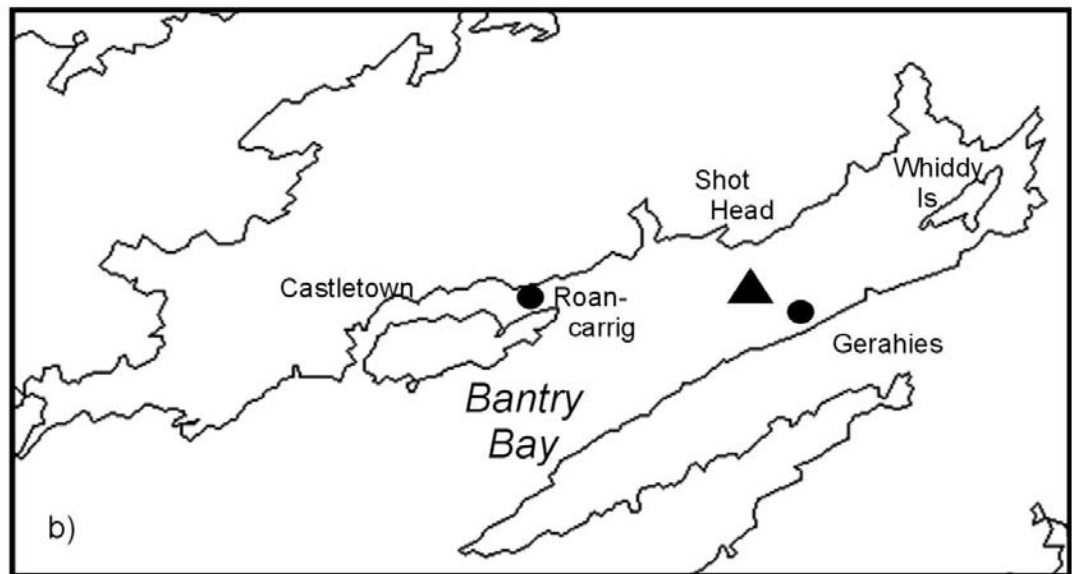
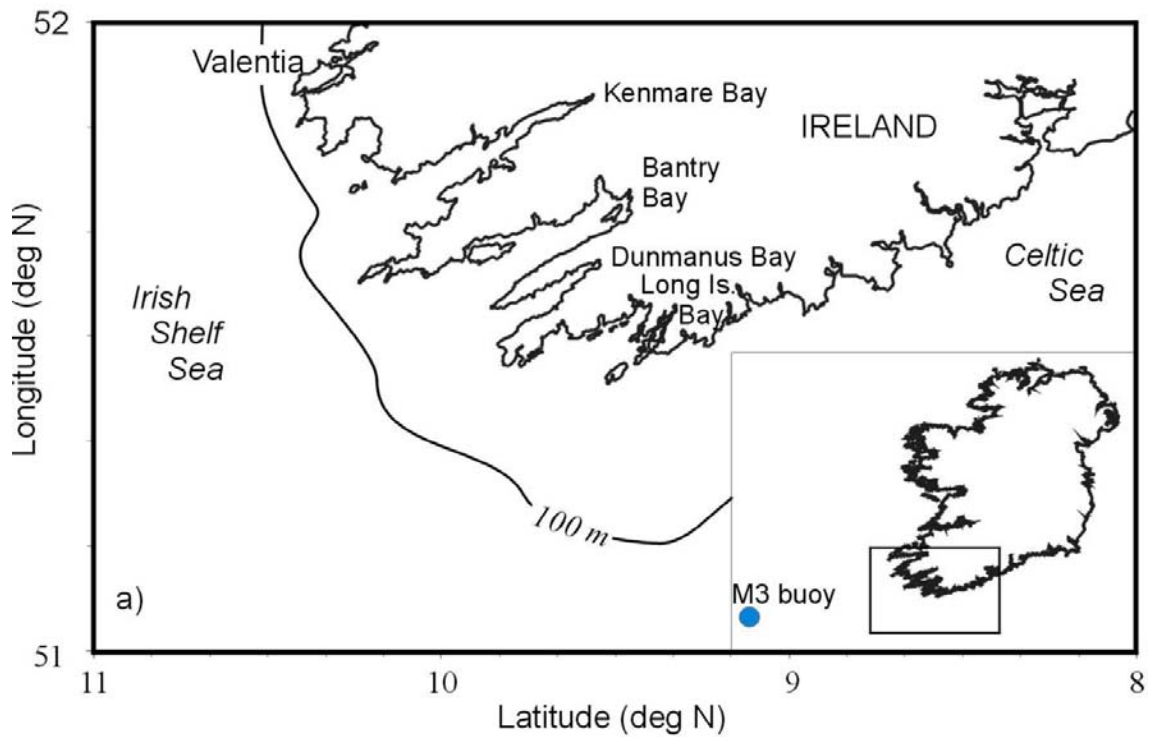


Figure 1. a) Map of the bays of southwest Ireland showing locations mentioned in the text. b) Bantry Bay southwest Ireland, showing location of the current meter mooring (filled triangle) and thermistor string (filled circle).

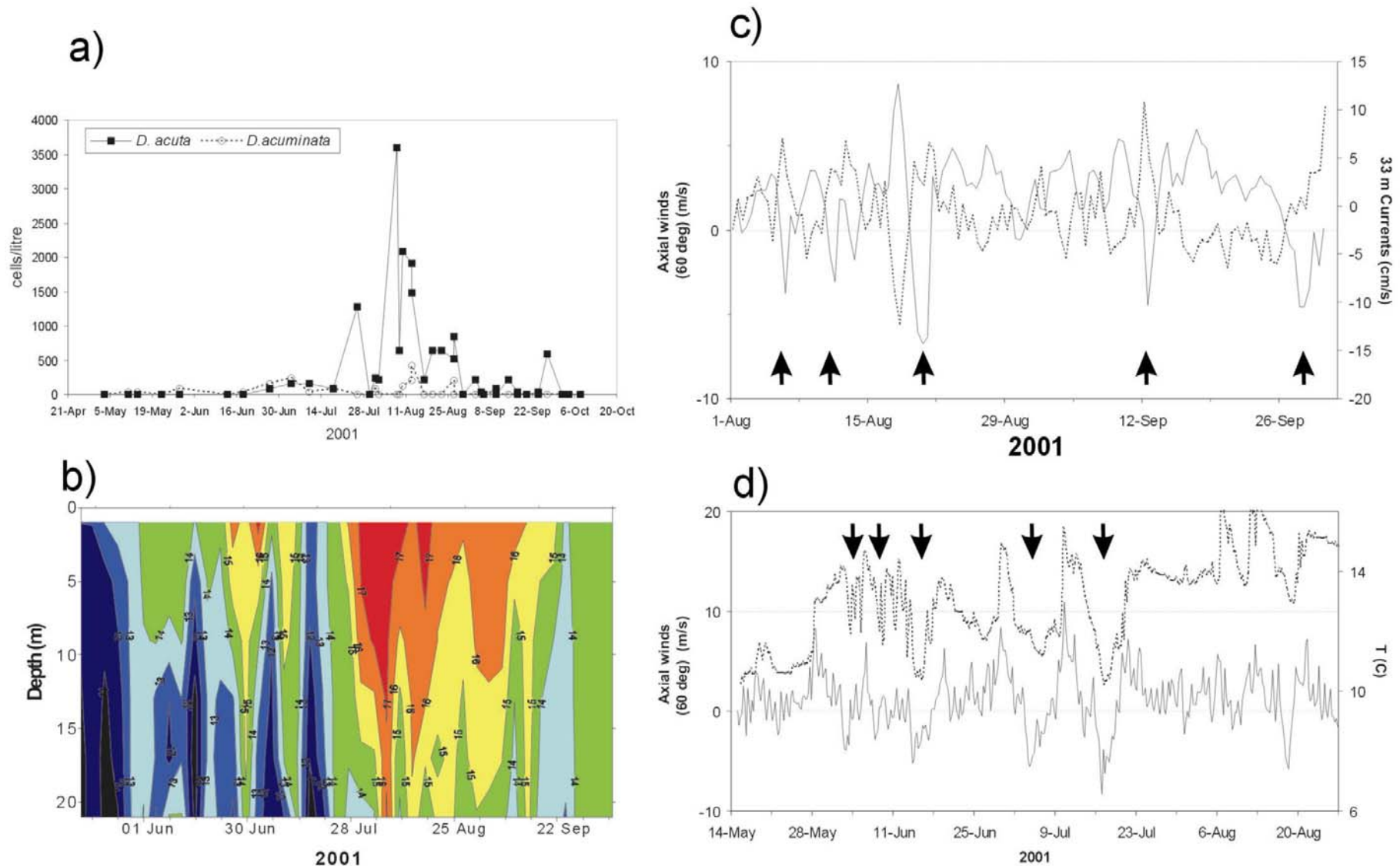


Figure 2. Field data for 2001; a) *Dinophysis* record for Gearhies. Note the increase in cell densities of *D. acuminata* from 28 July; b) contoured temperature data from thermistor string located at Gearhies. Note the influx of warm surface water occurring between 26 July and 7 August; c) comparison of wind vector axial to Bantry Bay (060°) with near bottom (33 m) currents measured with a bottom mounted ADCP located off Shot Head; d) comparison between axial wind vector and bottom temperature measured at Gearhies. Locations are marked in Figure 1.

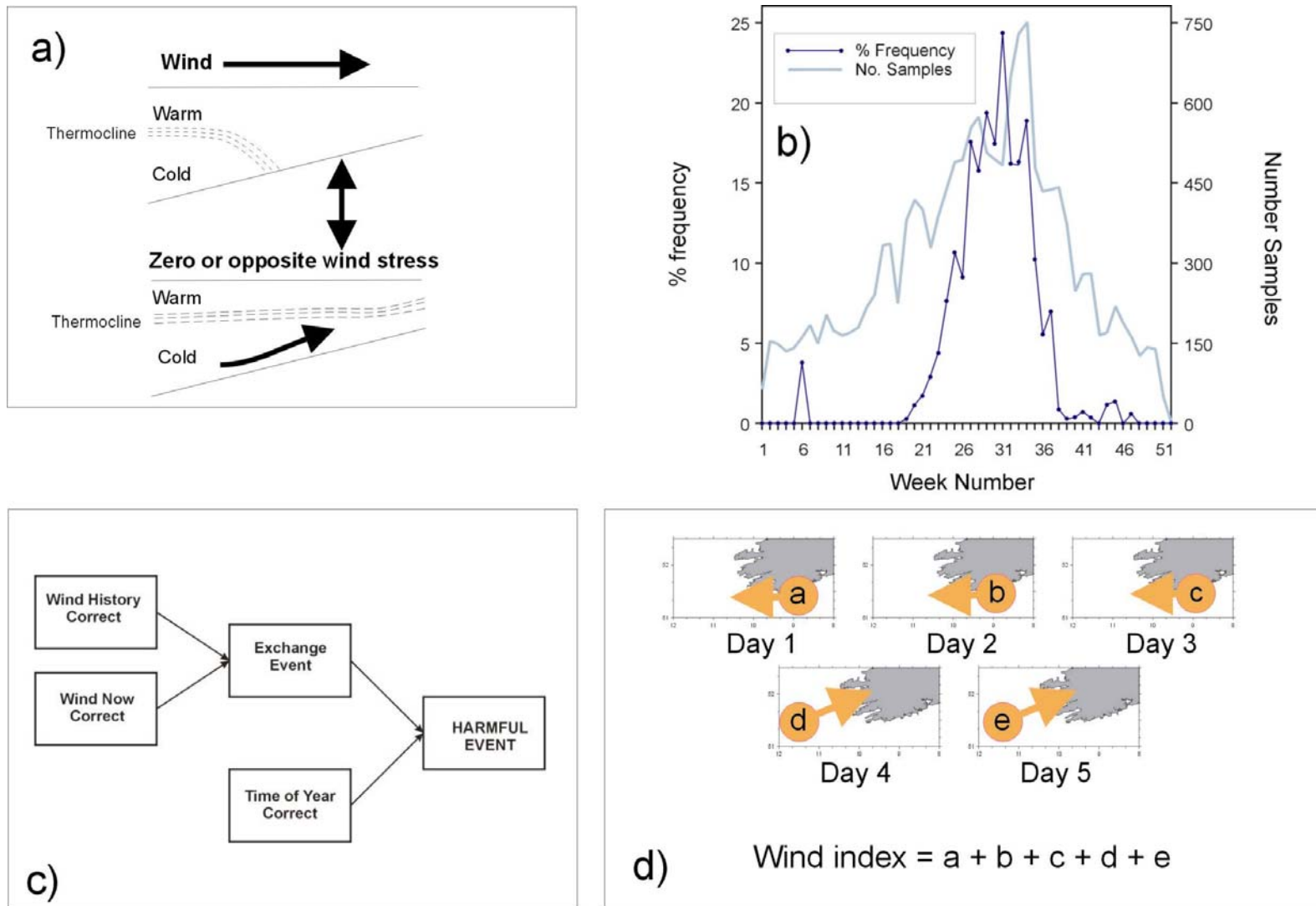


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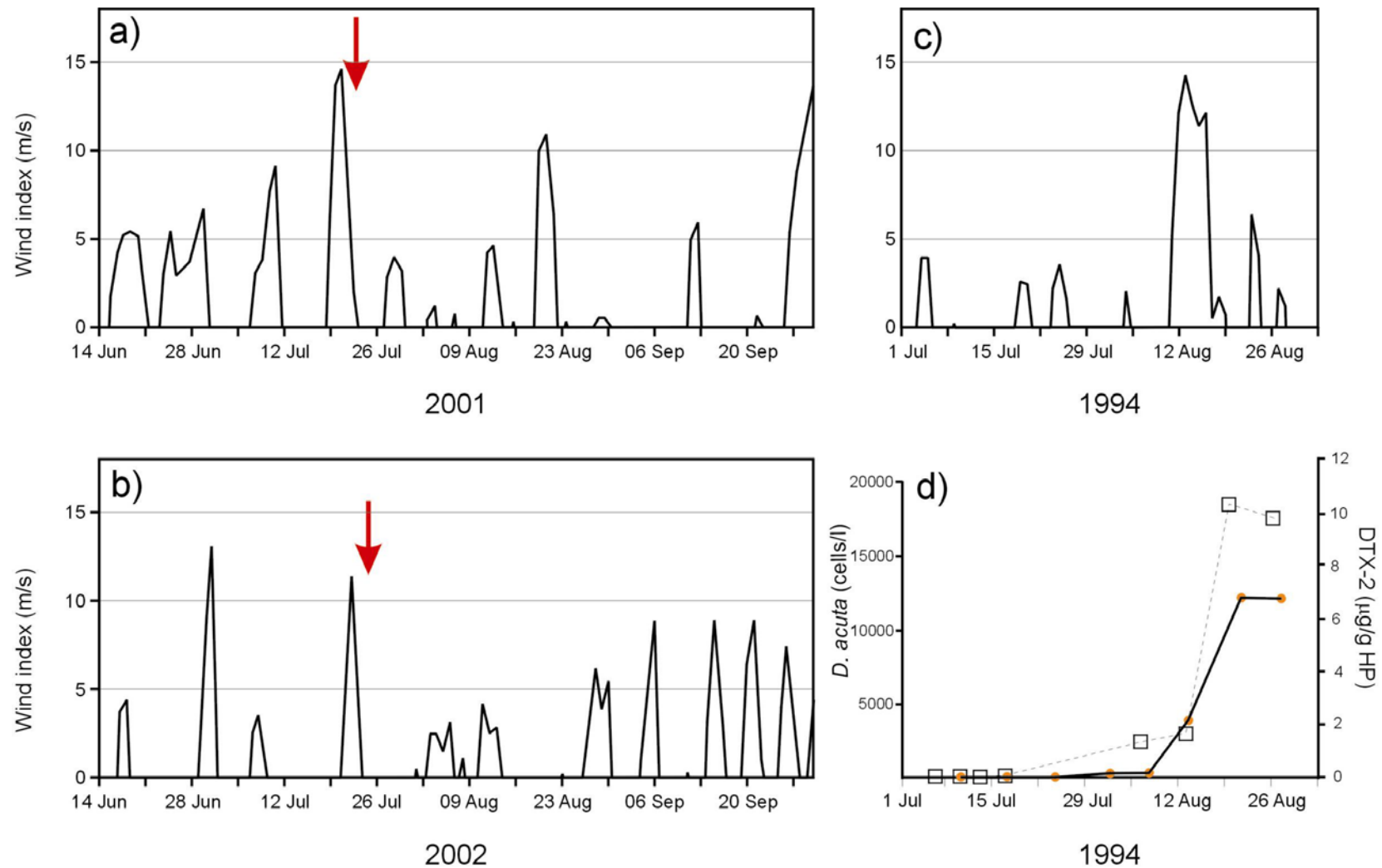


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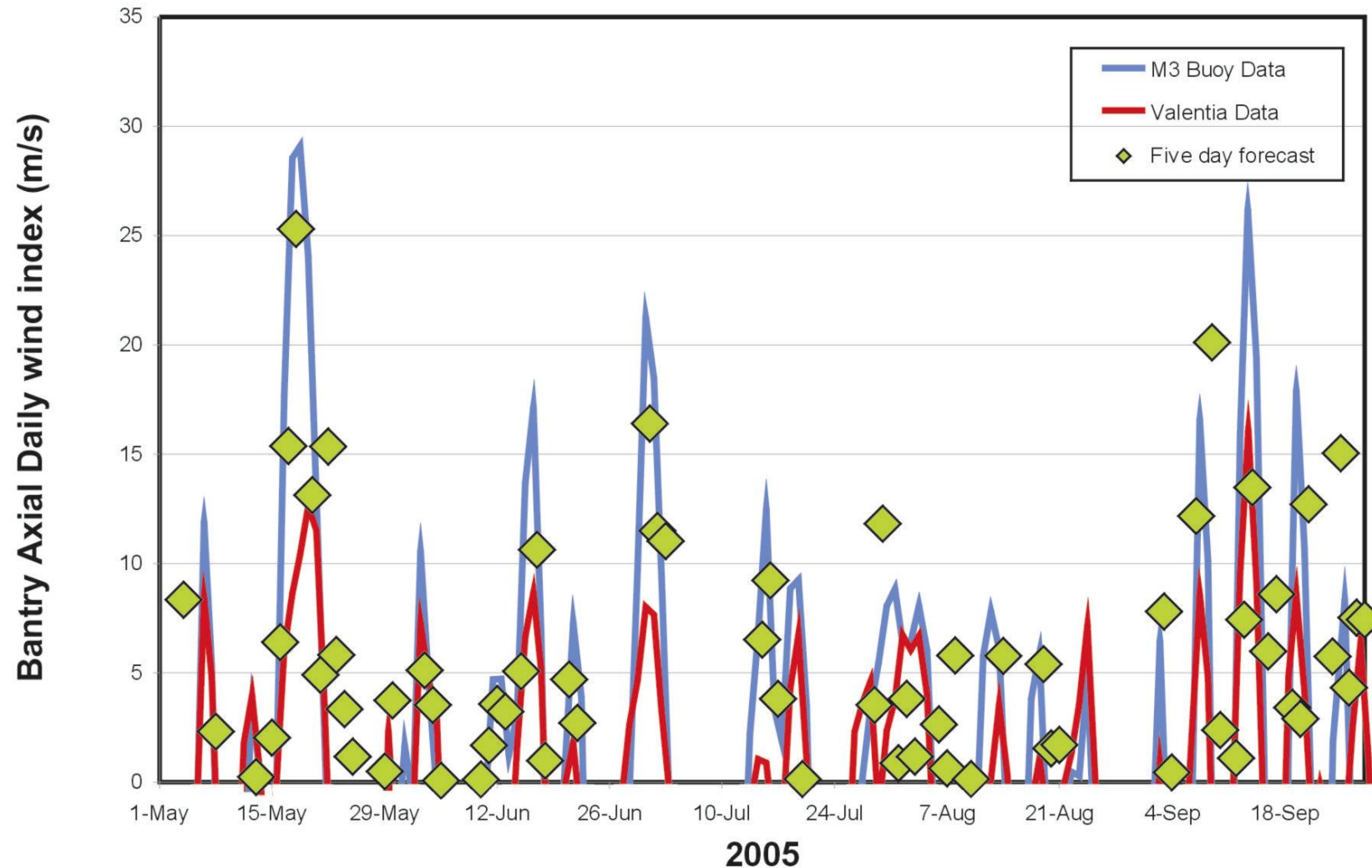


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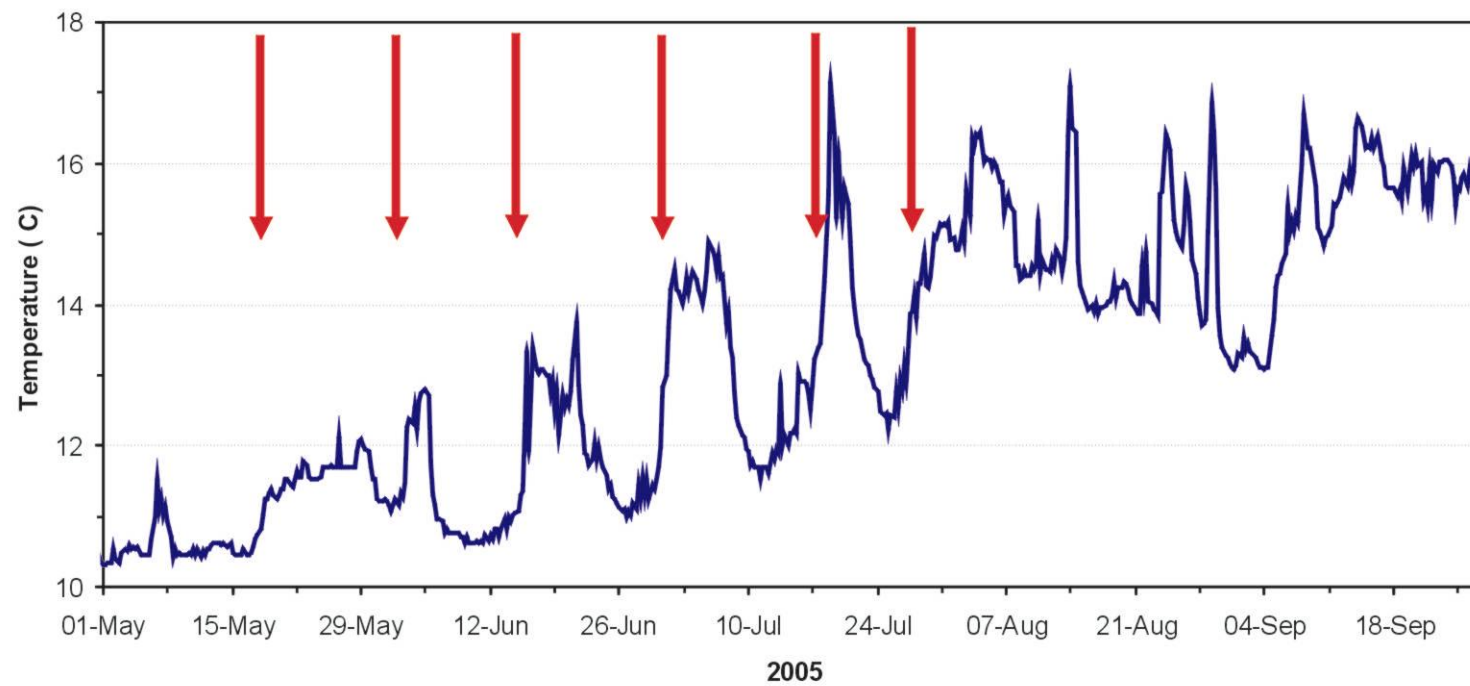


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