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**Correspondence** and requests for materials should be addressed to A.N.L. (anloh@fgcu.edu).

## Impact of climate change on marine pelagic phenology and trophic mismatch

Martin Edwards & Anthony J. Richardson

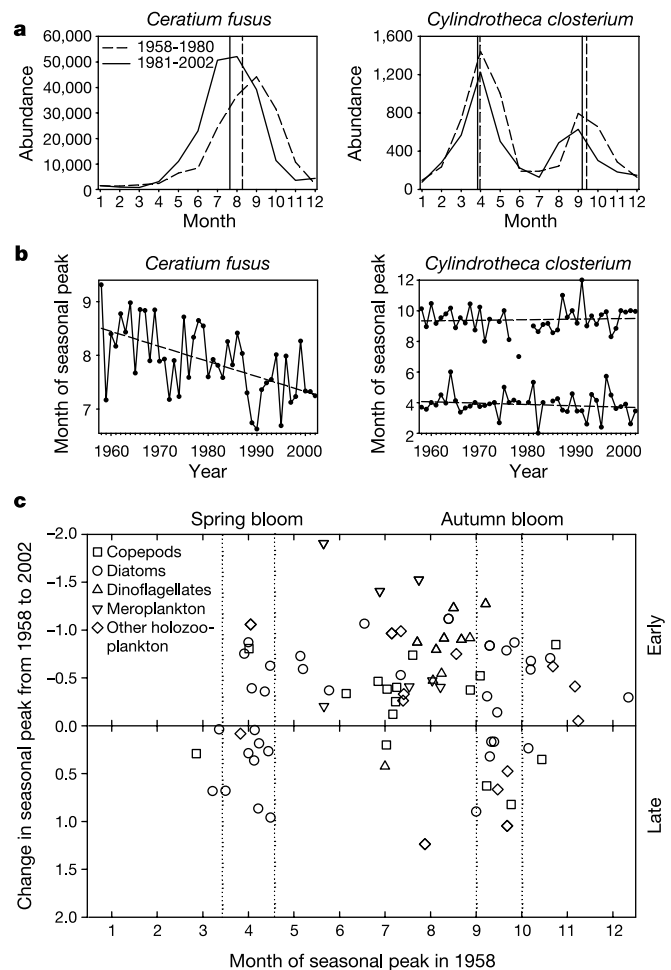
Sir Alister Hardy Foundation for Ocean Science, The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK

Phenology, the study of annually recurring life cycle events such as the timing of migrations and flowering, can provide particularly sensitive indicators of climate change<sup>1</sup>. Changes in phenology may be important to ecosystem function because the level of response to climate change may vary across functional groups and multiple trophic levels. The decoupling of phenological relationships will have important ramifications for trophic interactions, altering food-web structures and leading to eventual ecosystem-level changes. Temperate marine environments may be particularly vulnerable to these changes because the recruitment success of higher trophic levels is highly dependent on synchronization with pulsed planktonic production<sup>2,3</sup>. Using long-term data of 66 plankton taxa during the period from 1958 to 2002, we investigated whether climate warming signals<sup>4</sup> are emergent across all trophic levels and functional groups within an ecological community. Here we show that not only is the marine pelagic community responding to climate changes, but also that the level of response differs throughout the community and the seasonal cycle, leading to a mismatch between trophic levels and functional groups.

The vast majority of documented phenology studies relating seasonal shifts in biology to climate have come from terrestrial and limnological sources (see refs 5, 6). Furthermore, most studies have solely reported phenological changes for a single species and have not explored trophic and ecological interactions<sup>7</sup>. In this study we investigated changes in marine pelagic phenology in the North Sea across three trophic levels using five functional groups. The major functional groups included diatoms and dinoflagellates separately (primary producers); copepods (secondary producers); non-copepod holozooplankton (secondary and tertiary producers) and meroplankton including fish larvae (secondary and tertiary producers). Inter-annual changes in a measure of the timing of the seasonal peak throughout the whole pelagic production season

(the central tendency; see Methods and Fig. 1a, b) were calculated using data from the Continuous Plankton Recorder (CPR)<sup>8</sup>, one of the longest and most spatially extensive marine biological data sets in the world.

The x axis of Fig. 1c shows the timing of the seasonal peaks in 1958 of all 66 plankton taxa used in the analysis; this represents the classical view of succession in the temperate marine pelagic ecosystem. Using the linear slope of the time series of the timing of the seasonal peak, we calculated the change in timing of the seasonal cycle (in months) from 1958 to 2002 for each taxon (Fig. 1c; y axis). Substantial temporal modifications in seasonal successional peaks have occurred over the past few decades. In particular, seasonal peaks of meroplankton have moved significantly ( $P < 0.0001$ ) forward (for example, the phylum Echinodermata has moved by 47 days (d)). By contrast, diatom peaks in spring and autumn have collectively remained relatively static, albeit with considerable



**Figure 1** Changes in phenology throughout the pelagic season. **a**, Examples of seasonal cycles for two of the 66 taxa—the dinoflagellate *Ceratium fusus* and the diatom *Cylindrotheca closterium*—used in the analysis for the periods 1958–1980 and 1981–2002. The timing of the seasonal peaks, using the indicator of central tendency, is also shown. **b**, Inter-annual variability of the seasonal peak for the above two species from 1958 to 2002. **c**, The change in the timing of the seasonal peaks (in months) for the 66 taxa over the 45-yr period from 1958 to 2002 plotted against the timing of their seasonal peak in 1958. For each taxon, the linear regression in **b** was used to estimate the difference between the seasonal peak in 1958 and 2002. A negative difference between 1958 and 2002 indicates seasonal cycles are becoming earlier. Standard linear regression was considered appropriate because there was minimal autocorrelation (determined by the Durbin–Watson statistic) in the phenology time series.

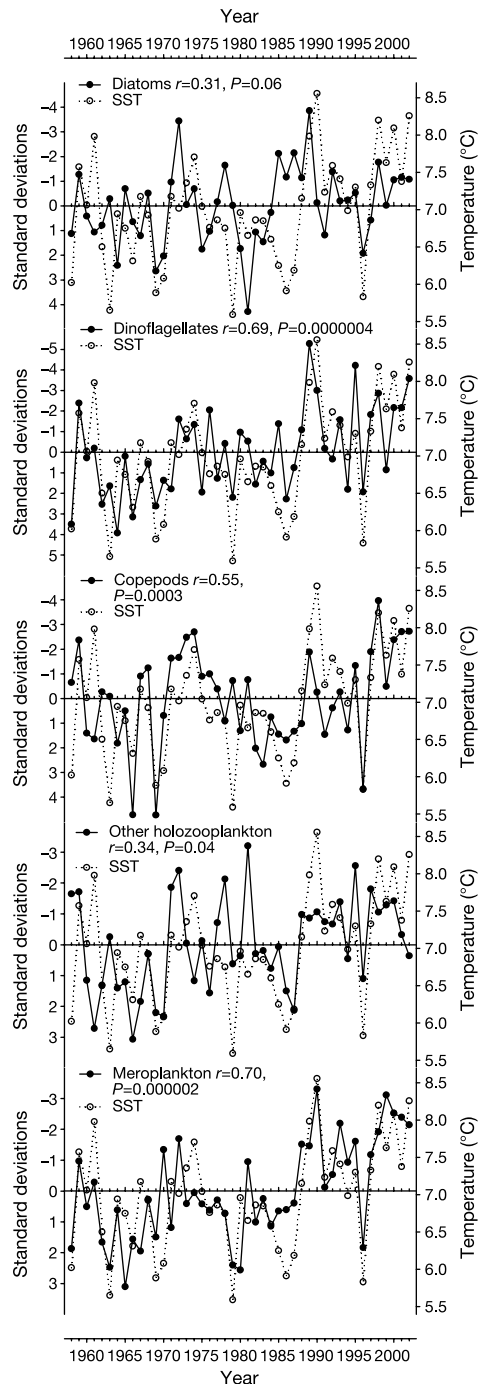
inter-taxon variation. Other holozooplankton taxa (mainly of the class Malacostraca) show a wide variety of responses, although the seasonal cycles of the classes Branchiopoda (genus *Evadne*, 30 d) and Appendicularia (30 d), as well as hyperiids (order Amphipoda, class Malacostraca, 32 d) occur substantially earlier. Cycles of the majority of dinoflagellates are also earlier (principally the genera *Ceratium*, 27 d, *Protoperidinium*, 26 d and *Dinophysis*, 24 d), having

important implications for the monitoring and study of harmful algal blooms.

The general pattern observed for taxa that peak when the water column is mixed or in a transitional state is to show considerable variability in phenology, whereas taxa associated with low turbulent conditions have virtually all advanced in their seasonality (34 out of 37 taxa between May–August). The benthic larval component of the zooplankton (meroplankton) has also shown larger shifts forward in seasonality compared with the holozooplankton. During summer, meroplankton have moved forward collectively by 27 d, dinoflagellates by 23 d, copepods by 10 d and non-copepod holozooplankton by 10 d over the 45-yr study period. Diatoms as a group showed the largest variations in phenology, with particular taxa occurring both earlier and later during the spring and autumn blooms. Diatoms show a wide variety of life strategies, and, following the Sverdrup model<sup>9</sup>, delays in the spring bloom for some species could be associated with recent increases in wind forcing in the North Sea and trends in the North Atlantic Oscillation index<sup>10</sup>. Collectively, however, the mean movement in the spring bloom was 0 d and the mean movement in the autumn bloom was to 5 d earlier. Many long-term phytoplankton studies have noted that the timing of the spring bloom is in fact fairly constant, occurring approximately the same time each year under highly variable environmental conditions<sup>11,12</sup>. Other studies have also shown that the development of the pycnocline (which shows considerable geographical/temporal variability in European shelf seas) is not an essential prerequisite for the development of the spring bloom<sup>13,14</sup>. Recent research has implicated photoperiod in the control of diatom spore growth and germination<sup>11,12,15</sup>. Thus the temporal stasis in the spring bloom could be a consequence of the diatom community in this study being dominated by taxa that form resting spores.

To explore the relationship between the timing of the seasonal peak and hydro-climatic change we correlated the annual centre of gravity index for the period 1958–2002 for the five functional groups (expressed as principal components) with spring sea surface temperature (SST) (data supplied by Hadley Centre for Climate Research). We adjusted significance levels to account for temporal autocorrelation, and found highly significant correlations between the first principal component (PC1) of the peak in the seasonal cycle of summer plankton and SST (Fig. 2), particularly for dinoflagellates ( $r = 0.69, P < 0.0001$ ; PC1 45.5% of the total variability) and meroplankton ( $r = 0.70, P < 0.0001$ ; PC1 37.2%). Also during summer, diatoms ( $r = 0.31, P > 0.05$ ; PC1 22%), copepods ( $r = 0.55, P < 0.001$ ; PC1 33%) and other holozooplankton ( $r = 0.34, P < 0.05$ ; PC1 24%) showed positive correlations between phenology and SST. By contrast, diatoms, copepods and other holozooplankton that had peaks in spring and autumn (that is, taxa with bimodal seasonal cycles; data not shown) showed no relationship between phenology and SST. For additional information on a species-by-species basis, individual correlations and their confidence limits are given in the Supplementary Information.

The relationship between SST and the seasonal development of some plankton taxa, particularly the meroplankton and holozooplankton, can be explained by the species-specific effects of temperature on many aspects of plankton physiology, such as adult mortality, reproduction, respiration, embryonic and gonad development<sup>16–20</sup>. Dinoflagellates may not only be responding physiologically to temperature, but may also respond to temperature indirectly if climate warming enhances stratified conditions and/or if these conditions appeared earlier in the season. Stratified conditions are predicted to intensify with patterns of climate change in the North Sea<sup>21</sup>. The magnitude of changes in phenology reported in this study are greater than those from previous studies on terrestrial communities<sup>4</sup> and indicate that marine pelagic communities are particularly sensitive to climate change. These large phenological shifts have occurred with an increase in SST of 0.90 °C (estimated from regression) during the study period (1958–2002).



**Figure 2** The relationship between the interannual variation in the timing of the seasonal cycle for various functional groups during the summer stratified period and SST. Note the high correlations for dinoflagellates and meroplankton. The time series of the timing of the seasonal cycle for each functional group was represented by principal component analysis of all constituent taxa. Negative standard deviations represent earlier seasonal cycles.

Although we have observed considerable inter-annual variability in plankton phenology, significant underlying patterns over the past few decades have emerged. Diatom blooms in spring, and hence the beginning of the pelagic seasonal cycle, have remained relatively fixed in time, and are presumably dependent on day length or light intensity<sup>11,15</sup> rather than on temperature-mediated physiological responses in their life strategies. Conversely, organisms that are dependent on temperature to stimulate physiological developments and larval release have significantly moved forward in their seasonal cycle in response to temperature, a trend that has continued over the last decade (with the exception of 1996, a negative North Atlantic Oscillation index year). Although many pelagic organisms are responding to climate warming, it is the intensity of the response that varies considerably amongst the pelagic assemblage. The different extent to which functional groups are moving forward in time in response to warming has led to mismatch between successive trophic levels and a change in the synchrony of timing between primary, secondary and tertiary production. Because efficient transfer of marine pelagic production to higher trophic levels, such as commercially important fish species, is largely dependent on the temporal synchrony between successive trophic production peaks<sup>2,3</sup>, our study suggests that marine trophodynamics may have already been radically altered (notwithstanding some species adaptations), and will continue to do so in the coming decades if the climate continues to warm at its present rate. In addition to the effects of overfishing, the decline in abundance of key planktonic prey, and shifts in their seasonality, have recently been implicated in exacerbating the decline in North Sea cod stocks<sup>22</sup>. Planktonic phenological shifts of the magnitude reported in this study, coupled with large-scale shifts in plankton biogeography<sup>23</sup>, will undoubtedly have a considerable effect on ecosystem function in the North Sea and may help to explain the recently reported dramatic ecological changes observed in the North Sea<sup>24</sup>. □

Methods

Plankton data

The CPR survey is the longest running, large-scale marine biological survey in the world. The CPR is a near-surface (10 m) plankton sampler voluntarily towed each month behind merchant ships on their normal routes of passage. Methods of analysis for ~400 phyto- and zooplankton taxa have remained almost unchanged since 1958<sup>8</sup>. In this study we use data from the most consistently sampled region in the entire survey, the central North Sea (55–58° N). From 1958 to 2002 all months have been sampled in this region. Species found in more than 1% of the samples were included in the analysis, as variations each year in the seasonal cycle of rarer species are not adequately estimated. To aid interpretation, species were assigned to functional groups, namely diatoms, dinoflagellates, copepods, non-copepod holozooplankton and meroplankton.

Sea surface temperature

SST was used as an indicator of climate change in the ocean because organisms respond physiologically to temperature and it has been found to be important in many terrestrial phenological studies<sup>1</sup>. Monthly mean gridded (1° × 1°) SSTs from a blend of satellite AVHRR (advanced very high resolution radiometer) and *in situ* observations were obtained from the Hadley Centre of the UK Met Office (HadISST). We calculated mean spring SST in the central North Sea, because this time of year is important for the seasonal cycles of many pelagic organisms. Similar results, however, were obtained for mean annual SST (data not shown).

Data analysis

Most terrestrial phenological studies have used the first arrival (for example, of migratory species such as swallows) or leaf emergence date (for example, oaks) of a particular species to describe phenological changes<sup>25</sup>. In the present study, we estimated the timing of the seasonal peak throughout the entire growing season (the central tendency, *T*) using the month co-ordinate of the centre of gravity of the area below graphs of monthly means<sup>26,27</sup>:

$$T = \frac{\sum_{i=1}^{12} M_i x_m}{\sum_{i=1}^{12} x_m}$$

where *x<sub>m</sub>* is the mean abundance in month *M* (January = 1, ..., December = 12).

This index is sensitive to changes in the timing of the seasonal cycle (Fig. 1; see also refs 26, 27). The average seasonal cycle over the 45-yr period for each taxon was used to determine whether taxa were unimodal or bimodal (spring and autumn). A fundamental difference between terrestrial and many pelagic ecosystems is that there is just one seasonal primary production peak in terrestrial ecosystems but usually two in temperate marine

environments: one in spring and the other in autumn. During autumn, the pelagic environment reverts back to spring-like conditions, accompanied by another burst in phytoplankton production, albeit not as intensive as the spring bloom. As a result, many pelagic organisms, in particular the floral community (predominantly the class Bacillariophyceae, the diatoms, that are nutrient limited during the summer period), display bimodal seasonality. For unimodal taxa the timing of the seasonal peak was calculated throughout the entire year, whereas for bimodal taxa the timing of the seasonal peak was calculated separately for the first six months and the last six months of the year.

To summarize phenology time series of the different species within each functional group, standardized principal components analysis (PCA) based on a correlation matrix was used<sup>28</sup>. These PCAs based on phenology were correlated with mean spring SST and were adjusted for temporal autocorrelation using the modified Chelton method<sup>29</sup>.

Potential biases

A possible source of bias with the central tendency index is associated with changes in the time each month that sampling takes place. Six routes are generally towed within the central North Sea, which helps to minimize bias, although mean sampling time each month has still moved forward by an average of 2.4 d over the entire period from 1958 to 2002. This is relatively small compared with the large shifts earlier of dinoflagellates (23 d) and meroplankton (27 d) over the 45-yr study (Fig. 1). In addition, the slightly earlier sampling each month, over more recent times, would actually tend to delay and not bring forward the timing of peak abundance as calculated by *T*, because the seasonal cycle when calculated on a monthly basis would shift to the right. To minimize the likelihood of committing type I errors when identifying changes in the timing of the seasonal cycle for each taxon over the 45-yr study period, we used a conservative significance level of 1%. For some taxa that are not speciated (for example, fish larvae) it is difficult to separate observed trends in phenology from changes in community composition caused by climate-induced biogeographical shifts. However, on the basis of the strong and consistent relationships observed between these taxa and SST, coupled with similar patterns in the majority of speciated taxa, we conclude that many components of the pelagic assemblage are responding rapidly to temperature change through changes in the timing of their seasonal cycles.

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**Correspondence** and requests for materials should be addressed to M.E. ([maed@sahfos.ac.uk](mailto:maed@sahfos.ac.uk)).

## Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating

Susan Kalisz<sup>1</sup>, Donna W. Vogler<sup>1,2</sup> & Kristen M. Hanley<sup>1</sup>

<sup>1</sup>University of Pittsburgh, Department of Biological Sciences, Pittsburgh, Pennsylvania 15260, USA

<sup>2</sup>State University of New York, College at Oneonta, Biology Department, Oneonta, New York 13820, USA

The evolution of self-fertilization in hermaphrodites is opposed by costs that decrease the value of self progeny relative to that of outcross progeny<sup>1–3</sup>. However, self-fertilization is common in plants<sup>4</sup>; 20% are highly selfing and 33% are intermediate between selfing and outcrossing<sup>5</sup>. Darwin<sup>6</sup> proposed an adaptive benefit of self-pollination in providing reproductive assurance when outcrossing is impossible<sup>6–9</sup>. Moreover, if outcross pollen receipt is inconsistent within or between years, these conditions likewise favour self-pollination<sup>10</sup>, and this can result in a mixture of self and outcross seed production (mixed mating). Despite wide acceptance, the reproductive assurance hypothesis has lacked the support of complete empirical evidence to show that variable pollination can create both the ecological and genetic conditions favouring self-pollination. We recently showed in *Collinsia verna* that during periods of infrequent pollinator visits, autonomous self-pollination boosted seed output per flower<sup>11</sup>, the key ecological condition. Here we show low inbreeding depression and marker-based estimates of selfing, demonstrating that when the pollination environment in wild populations necessitates reproductive assurance, selfing rates increase. We provide a complete demonstration of reproductive assurance under variable pollination environments and mechanistically link reproductive assurance to intermediate selfing rates through mixed mating.

Populations of flowering plants that lack mates or pollinators, such as those at the edge of a species' range or colonizing species, rapidly evolve autonomous self-fertilization<sup>12,13</sup> (within-flowers selfing without a pollen vector)<sup>8</sup>, and this is thought to occur because selfing provides reproductive assurance<sup>6–9</sup>. Other ecological factors, such as unpredictable outcross pollen receipt within or

among years<sup>8,10,12,14</sup> may also, in theory, produce conditions that favour autonomous selfing through reproductive assurance<sup>10,15,16</sup>. For autonomous selfing to evolve, its benefits must be balanced against the potential costs. Alleles promoting self-fertilization ability increase in frequency because plants that carry them can serve as pollen parents in two ways: both by fertilizing ovules on other plants (that is, outcrossing) and by fertilizing their own ovules (that is, selfing)<sup>17</sup>. Thus, individuals with alleles that cause more selfing have an advantage in transmission over individuals with alleles for outcrossing<sup>18</sup>. In contrast, selfing is disfavoured when there is inbreeding depression ( $\delta$ , low vigour of self progeny)<sup>1,2</sup> and/or when the production of selfed progeny pre-empts the production of outcrossed progeny (pollen or seed discounting)<sup>8,16</sup>. Previous investigations have failed to show that when outcross pollen receipt is inconsistent, selfing is favoured and outweighs these costs<sup>19</sup>. We are currently unable to predict when autonomous self-fertilization will provide reproductive assurance. An unequivocal demonstration of reproductive assurance under unreliable pollinators requires several types of data<sup>4,14,19</sup>. Plants must fail to receive outcross pollen, but this failure need not occur every season. During periods of low or no outcross pollen receipt, autonomous selfing must boost seed production. Finally, the combined costs (seed discounting, pollen discounting and inbreeding depression) must not completely negate the fitness gain of selfing.

Costs incurred by autonomous selfing vary depending both upon the timing of self-pollination relative to outcross pollen receipt<sup>8</sup> and the availability of pollinators. When pollinators are present, autonomous self-pollination that occurs after all opportunities for outcross pollen receipt have passed (delayed selfing) incurs no pollen or seed discounting costs<sup>8,10</sup>. Additionally, even if inbreeding depression ( $\delta$ ) is high, the survival of any progeny produced by delayed selfing always provides reproductive assurance<sup>10</sup>. In contrast, if autonomous self-pollination coincides with outcross pollen receipt (competing selfing), then pollen discounting, seed discounting and inbreeding depression can disfavour selfing<sup>8</sup>. In theory, if the fitness of self progeny produced by competing selfing is less than or equal to roughly half the fitness of outcrossed progeny (that is  $\delta > 0.5$ ), then the fitness gain due to the transmission advantage is lost<sup>1,2</sup>. Finally, when pollinators are absent, there are no seed and pollen discounting costs of autonomous selfing<sup>3,8,10,20</sup>. In a field experiment, we previously investigated autonomous self-pollination in the winter annual wildflower, *C. verna* (Plantaginaceae). We showed that this species autonomously self-pollinates<sup>21</sup> in a field experiment that compared fruit set of emasculated versus control flowers (Tables 1 and 2 in ref. 11). Further, we quantified the timing of autonomous selfing by comparing both the timing of pollen deposition and the number of pollen grains on the stigmas of flowers in open-pollinated conditions relative to flowers in pollinator-excluded treatments. Selfing in *C. verna* is autonomous and predominantly delayed, with the potential for some competing selfing (Fig. 2 in ref. 11).

Because competing selfing can occur in *C. verna*, it is important to estimate the magnitude of inbreeding depression. Here we report results from three wild populations (BT, EF and TMC; see Methods) located in southwestern Pennsylvania, USA. We produced both selfed and outcrossed progeny on plants from each population and compared their lifetime performance. Mean trait values of self versus outcross progeny for each population were compared, and indicate that only one of the 15 comparisons was statistically significant (seed weight; BT population,  $P < 0.001$ ). All three populations show markedly low average levels of inbreeding depression (Fig. 1;  $\delta < 0.15$  for all traits measured, in all populations), lower than the  $\sim 0.5$  value that opposes competing selfing. Additionally, previous field estimates of early inbreeding depression in the three study populations revealed no significant difference (for all six comparisons  $P > 0.2$ ) in the fruiting success of selfed versus