Marine Management Organisation Project FES 228 ‘Does phytoplankton abundance control shellfish success in Poole Harbour?’

An investigation into the annual cycle of phytoplankton abundance in Poole Harbour and its relationship with Manila clam nutrition

Phytoplankton (diatom) diversity in Poole Harbour (15/11/2011) and individual Manila clams (*Ruditapes philippinarum*) from three size classes collected in Poole Harbour.

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Contents

1. Summary

In 1988 government scientists and local shellfish farmers introduced the non-native Manila clam (*Ruditapes philippinarum*) to Poole Harbour in the belief that this high-value species would provide good possibilities for commercial cultivation (Humphreys, 2010). Although the clam subsequently naturalised (Jensen et al. 2004) recurrent bouts of natural mortality (Humphreys et al. 2007) have reduced the viability and attractiveness of the species to local shellfish farmers. It is unclear what causes the observed non-fishing mortality but disease and food supply are two possibilities. In order to better understand the role of food supply and physical factors on clam biology, we measured a suite of biological and physical parameters in Poole Harbour using *in situ* monitoring whilst simultaneously assessing clam condition over an annual cycle (June 2011 to August 2012). We recorded a period of consistently low autumn and winter phytoplankton abundance between October and March. In contrast, phytoplankton abundance showed a highly variable pattern in spring and summer. This variability was driven by both prolific summer diatom blooms and a more gradual seasonal change in the abundance of the smaller phytoplankton, i.e. the picoeukaryotes. The phytoplankton abundances observed in this study represent some of the highest levels ever recorded in Poole Harbour but, and in contrast with some previous observations, are consistent with the expected seasonal cycle. The condition of the Manila clam population closest to our monitoring site was at its lowest in March, approximately coinciding with the annual minimum in phytoplankton abundance. There were significant differences in clam condition at different sites within the Harbour although it was difficult to relate these differences to food supply. We conclude that changes in the composition and abundance of phytoplankton in Poole Harbour correlates closely with clam physiological status, as indicated by condition index measurements, and that the low winter food availability likely increases clam vulnerability to disease and other external factors. Together, these environmental factors contribute to a variable clam survivorship between years.

2. Introduction

2.1 Economic importance of the Manila clam

The Manila clam (*Ruditapes philippinarum*) is one of the world's major aquaculture species. Global production of the Manila clam has undergone a rapid increase in recent years. According to FAO statistics, the total global Manila clam production in 2010 was 3,644,311 tonnes. Of this total, 99% came from aquaculture with a value of $3.3 billion. Compared to 1988, when the clam was first introduced to Poole Harbour, there has been an approximately 20-fold increase in the global aquaculture production of the Manila clam. Over the same period there has been a decline in the global capture fishery (FAO, 2012). At its peak in 2004 reported Manila clam landings from Poole Harbour reached over 500 tonnes with a value of around £1.5 million. However by 2009 total reported landings had dropped dramatically to less than 10% of these figures. Although these figures do not include unofficial landings and therefore only represent a proportion of the total catch from the Harbour, they do indicate a significant decline in the commercial value of the Poole Harbour population over recent years. One reason for this is late winter/early spring mortality which at present is poorly explained (Humphreys et al. 2007).

2.2 Shellfish aquaculture and U.K. government policy

The recently published U.K. Marine Policy Statement (HM Government, 2010) highlights the urgent need to develop efficient, competitive and sustainable aquaculture industries as a national priority linked to food security. There is some evidence that the U.K. sector is expanding; in 2008 the estimated value of farmed shellfish in the U.K. was £33 million from 38,600 tonnes, an increase of 40% over the previous year (HM Government, 2011). Developing our understanding of the biology of important aquaculture species such as the Manila clam in marginal or unusual habitats is therefore essential in contributing to current U.K. policy objectives.

2.3 Poole Harbour as an important site for U.K. shellfish aquaculture

Poole Harbour is a complex estuary ecosystem with lagoonal characteristics. On the north side of the Harbour the town of Poole forms a continuous belt of urban development whereas on the south and western sides of the Harbour land use is mostly agricultural with some nature reserves (Figure 1). Much of the harbour is designated as a Special Protection Area (SPA) under the EU Birds Directive, and is also a Site of Special Scientific Interest and a Ramsar Site. Shorebirds, such as the oystercatcher, have benefited from the introduction of the Manila clam to Poole Harbour (Caldow et al. 2007). Poole Harbour is micro-tidal with a mean tidal range of 1.8 m at springs and 0.5 m at neaps with a 'double high water' pattern. During spring tides around 45% of the total water volume leaves on the ebb with the equivalent figure being about 22% on neap tides (Humphreys, 2005). The Harbour is generally very shallow and this feature, along with the tidal pattern and the geological composition of the sea bed, aids shellfish farming operations which are a successful local industry. Consequently Poole Harbour contains one of the most significant

Pacific oyster farms in mainland Britain. The main freshwater inputs to the Harbour are the Frome and Piddle rivers both of which have extensive agricultural catchments and flow into the Wareham channel. Several sewage treatment works discharge into the Harbour, the main input being from the Poole works which discharges into Hole's bay, a subsidiary basin of the Harbour (see Figure 1). The main Poole Harbour shellfish cultivation areas ('lease-beds') are 'class B' aquaculture production sites (CEFAS, 2012) meaning that some depuration is required. 'Class C' and 'Prohibited' areas also exist within the Harbour for some species (CEFAS, 2012). Poole Harbour is classified as a 'sensitive area (eutrophic)' and 'polluted water (eutrophic)' under the urban waste water treatment and waste water directives (Environment Agency 1997, 2001).

2.4 Scientific characterisation of Poole Harbour

The project team recognised that Poole Harbour, despite being an ecosystem of international significance, has very limited data on the organisms at the base of the food web, the phytoplankton. This gap in our knowledge is important because phytoplankton (primary) production will drive a substantial proportion of secondary production, including benthic invertebrates such as clams and oysters, upon which the Poole Harbour fisheries are based. With the support of the Fisheries Challenge Fund and the Marine Management Organisation we investigated the relationship between phytoplankton abundance and Manila clam condition over a seasonal cycle. The collection and analysis of this type of high resolution dataset is a first in Poole Harbour which aids in our understanding of the Harbour ecosystem and its dependent fisheries.

2.5 Clam nutrition and phytoplankton abundance

Laboratory investigations of Manila clam nutrition have measured the ability of individual clams to clear (i.e. filter) different types of phytoplankton from the surrounding seawater and how clearance efficiency varies as a function of clam size and phytoplankton type (e.g. Nakamura 2001). Such studies indicate that Manila clams are able to filter phytoplankton between 2 and 200 μm with a similar efficiency and in addition, some authors conclude that *Ruditapes philippinarum* is able to exploit food resources between 2 and 8 μm more effectively than the closely related *R. descussatus* (Nakamura 2001). Phytoplankton between a size of 2 and 10 μm would typically be single-celled diatoms or dinoflagellates, and at about 200 μm would likely be large chain-forming diatoms such as *Skeletonema* or *Chaetoceros*. 'Bloom' events, where the abundance of phytoplankton increases rapidly at certain times of the year, are dominated by chain-forming diatoms in a nearby ecosystem (the Solent; Iriarte & Purdie 2004) and Environment agency reports (1997, 2001) have previously highlighted the occurrence of high diatom abundances in Poole Harbour. In general food availability can drive rapid changes in the condition index of suspension feeding bivalves (Norkko et al. 2005) with gametogenesis and spawning also playing a major role.

2.6 Objectives of MMO-funded project FES 228

1. To generate an annual cycle of biological and physical data for Poole Harbour using *in situ* (sonde) monitoring over a summer to summer period.
2. To compare a variety of phytoplankton assessment techniques, and to understand the seasonal pattern of change in phytoplankton abundance in Poole Harbour.
3. To relate findings on phytoplankton abundance to observed changes in Manila clam condition over the year.
4. To report on the sustainability of the Manila clam fishery and on further research objectives arising from this project

3. Material and methods

3.1 Study and sampling sites

A multiparameter water-quality sonde (YSI 6600v2-4) was attached to a fixed structure at 1 m depth on the Othniel Shellfish Ltd platform in Poole Harbour (N 50°41’52.57” W 1°58'31.23"; Figure 1). Water samples were collected by dipping a 5 l bottle to 0.5 m depth at the same location. The Othniel platform is permanently moored in the Wych channel just North of Brownsea Island (Figure 1). At this site the water depth is approximately 3-5 m and the platform is exposed to a good tidal exchange between the shellfish lease-beds and the upper reaches of the Harbour to the West, and the Harbour mouth to the East. Manila clams were harvested from this area using a pump-scoop dredge and analysed (see below) on a monthly basis. Clams were also sampled (by hand dredge) from off Holton Mere in the Wareham channel (Figure 1) on a monthly basis. In addition, Manila clams collected from the vicinity of the platform (Figure 1), were held within a plastic mesh bag suspended from the Othniel platform at approximately 1 m depth as additional experimental subjects for condition analysis.

3.2 In situ *(sonde) monitoring*

The sonde measured chlorophyll fluorescence (relative fluorescence units; RFU, or µg/l via an on-board calibration), phycoerythrin fluorescence (RFU or cells/ml via an on-board calibration), dissolved oxygen (mg/l or % saturation), turbidity (NTU), pH, temperature (°C) and conductivity/salinity (presented here as parts per thousand; ppt). The sonde measured all parameters at 12 minute intervals. The sonde was deployed on 14/06/11 and recovered on 03/08/12. We visited the sonde on the following dates for routine maintenance (cleaning, data download and reprogramming, battery changes): 29/06/11, 18/07/11, 25/07/11 (sonde repositioning only), 29/07/11, 15/08/11, 08/09/11, 20/09/11, 11/10/11, 01/11/11, 04/01/12, 24/01/12, 10/02/12, 06/03/12, 04/05/12, 06/06/12, 25/06/12, 02/07/12, and 13/07/12 (sonde cleaning only). These maintenance visits introduced approximately 2 h gaps in the dataset. Asides from these gaps there are two more extended gaps in the dataset: 27/11/11 – 05/12/11 (sonde recalibration at supplier’s workshop) and 05/04/12 – 12/04/12 (cable failure).

3.3 Water sampling

At the conclusion of each sonde maintenance visit water was sampled as described above. This water was kept cool and in darkness during transport back to the laboratory where it was processed approximately 3 h later. One hundred ml subsamples were fixed with lugol’s iodine and later quantified using settling chambers and inverted microscopy. Three 50 ml subsamples were syringe-filtered through a 25 mm diameter GF/F glass fibre filter (0.7 µm pore size) using a swinnex unit. Filters were stored at -20°C and later analysed for chlorophyll *a* via fluorometry. The GF/F filtrate was stored at -20°C (nitrate and phosphate samples), or room temperature (silicate samples), and later analysed using standard methods. 0.5 ml

subsamples were analysed by flow cytometry (accuri C6) for quantification of major algal groups (picoeukaryotes, *Synechococcus*, nanoeukaryotes and cryptophytes) using standard methods (PML, 2012). The entire 0.5 ml volume was passed through the cytometer at 100 µl/min using forward scatter (size) and red (chlorophyll) fluorescence as dual triggers.

3.4 Manila clam analysis

Three groups of Manila clams were analysed as part of this study. For the lease-bed clams between 18-30 individuals were collected, and in the Wareham channel between 10-20, each month. The condition index of each clam was determined as a proxy for the physiological condition of the overall population. Clams were frozen immediately upon collection to prevent any change in condition and then processed in the laboratory later; clams were thawed and the flesh was removed from the shell by dissection and washed in tap water to remove any traces of salt. Once the clam had been dissected, both the flesh and the shell were dried for 24 hours at 105°C until they reached a constant weight (Sahin et al. 2006). The dry flesh and shell were then weighed and the condition index was calculated using the following equation (Sahin et al. 2006): Condition index = meat dry weight (g) / shell dry weight (g) x 100.

3.5 Data analysis

We tested the difference between the condition index of the Wareham channel and lease-bed clams using Mann-Whitney rank sum tests. Due to logistical constraints we were not always able to sample the two populations on exactly the same date, so only compared the two populations on those dates for which we were able to sample both populations. This yielded 5 comparisons spread over the year (31 Aug, 30 Sept, 24 Nov, 04 Jan, 06 Jun).

The condition index (CI) of the lease-bed clams was correlated (Spearman) with phytoplankton abundance, temperature and salinity. As the sampling date of the clam sampling was not always equal to the water sampling date (which yielded the phytoplankton abundance data), the closest sampling date was used in some of the analysis. For temperature and salinity we used the mean value from the sonde dataset for the month (30 days) preceding the condition index measurement (Norkko et al. 2005). All tests were conducted using SigmaStat 3.11.

4. Results

4.1 Physical characteristics of Poole Harbour water: temperature, salinity, dissolved oxygen and pH

The sonde recorded a temperature range of 1.8 to 21.8°C over the study period with the minimum in February 2012 and the maximum in August 2011 (Figure 2). Very cold air temperatures resulted in ice formation at the edges of the Harbour in February and this coincided with the minimum water temperature recorded. Salinity varied between 18.2 and 35.1 ppt with the minimum occurring in May 2012. In most months of the study salinity varied between approximately 28 and 35 ppt with the magnitude of daily and weekly fluctuations in salinity showing a clear relationship with the tidal cycle. Heavy rainfall in May, June and July 2012 (nationally, Met office data indicate that June 2012 was one of the wettest on record) resulted in lower monthly median salinities at the study site and much lower outliers than at the same time the previous year (Figure 2). Dissolved oxygen ranged between 4.4 mg/l (September) and 14.4 mg/l (May) over the year (Figure 3). The low point in dissolved oxygen occurred at high water (2.16 m) on the 1st of September at 23:43 BST. The maximum monthly range in dissolved oxygen occurred in August 2011 (8.1 mg/l) with daily variations in the summer months strongly linked with the daylight hours (Figure 3). Daily variation in dissolved oxygen was much lower at other times of the year (data not shown). pH ranged from 7.9 to 8.57 over the year with small daily changes attributable to the tidal cycle and photosynthetic activity (data not shown).

4.2 Inorganic nutrients

Nitrate ranged from 1.86 to 48.4 μM L^{-1}, phosphate between 0.08 and 0.83 μM L^{-1} and silicate between 1 and 46.91 μM L^{-1} over the 14 month monitoring period (Table 1). Nitrate concentration was generally elevated in the winter i.e. between November and March relative to the preceding June to November period. Nitrate concentration between April and August 2012 was generally higher than in 2011 with the peak occurring in July after heavy rain (see below). Phosphate showed little obvious trend over the year. Silicate concentration showed a similar pattern to nitrate concentration. The concentration range we measured showed broad agreement with that measured previously in the Solent (e.g. Iriarte & Purdie 2004).

4.3 Phytoplankton and chlorophyll a *abundance*

Phytoplankton abundance was assessed in 4 separate ways. Firstly, water sampled at the sonde was filtered onto glass fibre (GF/F) filters and analysed by fluorometry to give an estimate of the chlorophyll *a* concentration in Harbour water. Secondly, the sonde measured both chlorophyll and phycoerythrin fluorescence at 12 minute intervals, giving a similarly indirect measurement of phytoplankton abundance. Thirdly, water samples were fixed and assessed by microscopy which allowed a direct assessment of the population size of the larger species. Lastly, phytoplankton abundance was measured using flow cytometry. Although flow cytometry yields accurate, high resolution quantification of almost the entire

assemblage (including the picophytoplankton), it lacks information on species composition. We combined the three measurements to give a complete picture of phytoplankton abundance over the annual cycle. The filter data indicated that chlorophyll *a* concentration ranged from a minimum of 0.5 µg/l (06/03/2012) to a maximum of 3.9 µg/l (03/08/2012). In autumn and winter chlorophyll *a* concentrations were consistently low with higher values in the spring and summer (Figure 4).

As expected in a high-resolution dataset, the patterns in chlorophyll fluorescence as detected by the sonde were relatively complex. The most notable feature of the chlorophyll fluorescence dataset were the large spikes that occurred at regular intervals for discrete periods. The position of the sonde in the Wych channel meant that it was well placed to monitor the composition of water exchanging between Poole Harbour and Poole Bay; the influence of the flood and ebb at the study site was clear in the salinity profile (Figure 5). During certain periods the spikes in chlorophyll fluorescence showed a clear phasing with the tidal cycle. For example, during early July 2011 chlorophyll fluorescence showed regular and rapid increases (in some cases to detection saturation) and all of these peaks occurred during the flood tide (e.g. Figure 5). These 'tidal spikes' occurred over the entire monitoring period though they did vary in their magnitude with the largest spikes associated with the periods of elevated phytoplankton abundance detected by the other methods. The sonde dataset will be lodged at the British Oceanographic Data Centre and will undergo further analysis to work out the exact causes and significance of these tidal spikes. At present the dataset are undergoing quality control as three periods 17/11/12 to 27/11/12, 24/05/12 to 06/06/12, and 23/06/12 to 25/06/12 showed a gradual increase which was instantly corrected by the cleaning of a maintenance visit. Thus, the increase most likely indicated the build-up of fouling organisms on the sensor surface and so these data will be omitted from the analysis. Such a build up was never detectable by the naked eye before cleaning commenced however.

A total of 21 samples were analysed by microscopy for phytoplankton abundance. Depending on an initial assessment of the abundance of cells relative to sediment and detritus in the water, between 5 ml and 25 ml of the 100 ml collected was identified and counted. Taxonomic identification was to a mixture of species, genus, and family level and in some cases size categories of types were also recorded; the phytoplankton types recorded are listed in Table 2. A total of 61 phytoplankton types were identified in this way, consisting of 41 types of diatom, 17 dinoflagellates, 1 euglenoid, 1 silicoflagellate and 1 chlorophyte. The two highest abundances occurred in August 2011 (5,005 cells/ml) and August 2012 (4,321 cells/ml). These samples were overwhelmingly dominated by the diatoms *Skeletonema* and *Chaetoceros* which, between them, accounted for over 99% of the total cell abundance. Microscope counts of *Skeletonema* will be an underestimate as for practical reasons the number of cells per chain was not counted but one chain was recorded as one cell. An approximate average number of cells per chain would be 6. For flow cytometric assessment of phytoplankton abundance 0.5 ml of

unfiltered water was analysed on each sampling date. The minimum abundance was found on January 2012 (4,119 cells/ml) and the maximum (101,551 cells/ml) in August 2012. The same water sample was used for both microscope and flow cytometric analysis. There was no significant correlation between phytoplankton abundance estimated with microscopy and flow cytometry. Both methods indicated a similar general pattern however, with a period of relatively low phytoplankton abundance in the autumn and winter with higher values during the spring and summer (Figure 4).

4.4 Manila clam condition

At the beginning of the study, in July 2011, 100 (3-4 year old) clams were collected and suspended in a mesh bag at the Othniel platform. Over the next month these clams underwent a 1-2 point drop in condition index relative to the clams fished nearby and by September 2011 a trend of declining shell size was detected indicating that attrition between individual clams (due to being held captive in the bag) was resulting in physiological stress and therefore non-representative results. Mortality increased and by the end of the study almost all the clams in the bag had died. These data are not presented.

Clams (3-4 year old individuals) fished from the vicinity of the Othniel platform on a monthly basis showed a general decline in condition from July 2011 to March 2012, when the annual minimum in condition index was recorded. From March onwards condition index generally increased until the end of the study period (Figure 6). In contrast, clams collected in the Wareham channel (mostly 1-2 year old individuals) showed consistently lower condition index with no clear seasonal change. In all comparisons (see above) a highly significant difference in condition index was detected between the two populations ($P<0.01$).

4.5 Relationship between phytoplankton abundance, temperature and salinity and Manila clam condition (leasebed clams).

Scatter plots and Spearman rank correlations (Figure 7) between food availability and condition index indicated a modest and significant positive correlation between phytoplankton abundance (estimated via flow cytometry) and condition index ($P<0.05$). Correlations between phytoplankton abundance (estimated via microscopy) and chlorophyll *a* were weak and not significant. A significant positive correlation was found between temperature and condition index ($P<0.05$). There was no correlation between salinity and condition index.

5. Discussion

5.1 Physical data

The first objective of this study was to generate an annual cycle of physical and biological data for Poole Harbour. Previous reports (EA 1997, 2001) have concentrated on the nutrient status of the Harbour, the need to collect basic data to classify the water quality and ecological status of the Harbour, and the likely biological consequences of excessive nutrient deposition within the Harbour. Perhaps the most important parameter in the classification of water quality is dissolved oxygen which has been difficult to measure previously due to the rapid bio-fouling of deployed instruments (e.g. EA, 1997). The dissolved oxygen dataset of this study (measured optically) appears robust and indicates the expected diurnal range in dissolved oxygen as a function of water column productivity during the growing season. At the study monitoring site the levels of dissolved oxygen were within the ranges generally agreed to be satisfactory (> 4 mg/l or > 60 % saturation; e.g. Elliott et al. 2012) except for one brief incursion below these levels in September 2011, which may have been linked to a die-off of the recurrent diatom blooms which were recorded in both August 2011 and August 2012 (see below). At the monitoring site salinity generally showed little variation and simply matched the magnitude of tidal exchange. During the very wet spring of 2012 however very low salinities were recorded (down to 18 ppt) which indicate the extent to which heavy rainfall and river flows into the Harbour can cause substantial inter-annual variation. These low salinities may have had physiological consequences for some bivalves within the Harbour. The range of temperature (1.8-21.8°C) at the monitoring site will be greater than that in Poole bay and all of these parameters, which we measured at 1 m depth, will have a much greater range within the interstitial water which surrounds the Manila clam and other bivalves living within the mud, especially in those individuals which are exposed by the tide or which experience extended periods of very shallow overlying water. The above observations provide a baseline which we hope to build upon in years to come.

5.2 Phytoplankton abundance in Poole Harbour

A meta-analysis of the available nutrient and chlorophyll (mainly Environment Agency) data for Poole Harbour (Moss, 2012) indicated that summer levels of chlorophyll *a* exceeded 10 µg/l at multiple locations within Poole Harbour (including the Harbour mouth) in the years leading up to 2001. Such levels of chlorophyll *a*, along with cell counts in excess of 4,000 cells/ml, were used as part of the evidence base during the classification of Poole Harbour as eutrophic (EA, 1997, 2001). In the present study we recorded a maximum of 3.9 µg/l chlorophyll *a* during the summer blooms and >4,000 cells/ml from our microscope counts. Our microscope count methodology is comparable to that used by the Environment Agency whereas our chlorophyll *a* protocol may have been slightly different. In addition, during the spring and summer diatom blooms our counts are a considerable underestimation of true cell abundance due to the practical difficulties of counting heavy aggregations of

chain-forming diatoms. In this study the use of flow cytometry, which also counts picoeukaryotes such as *Micromonas* and *Ostreococcus* as well as cyanobacteria such as *Synechococcus*, gives more accurate phytoplankton abundance data. Whilst the flow cytometry data are interesting in observing seasonal changes in these less visible species, they are not relevant to water quality thresholds designed for simpler methods. Previous observations (EA, 1997), based on chlorophyll *a*, highlighted the potential of "winter diatom blooms" as a feature of Poole Harbour. Our data represent a comprehensive assessment of the phytoplankton of Poole Harbour and we found no evidence of winter diatom blooms. Through the winter months phytoplankton abundance is at its lowest levels. In this sense the phytoplankton assemblage shows the typical seasonal pattern of a temperate estuary. The diatom blooms in the spring and summer achieve very high biomasses and are dominated by *Skeletonema* and *Chaetoceros*. These species are typical of U.K. coastal waters.

The sonde dataset, which will be subject to further investigation, indicates that tidal scouring may lead to suspension of the microphytobenthos and its transport around the Harbour. Such an effect has also been observed during the *in situ* monitoring of the Bury Inlet (Elliott et al. 2012). A considerable extent of Poole Harbour is intertidal and supports abundant microphytobenthos populations. It is possible that suspension of this algal matter provides a significant component of bivalve nutrition in the summer months. Mucilaginous diatom mats were frequently observed by the Environment Agency in the past (EA, 1997, 2001) supporting the idea that suspension of microphytobenthos is a frequent occurrence. However, at this stage it is difficult to ascribe the source of the chlorophyll signal observed by the sonde. It is also possible that Poole Harbour acts, at times, as a sink for phytoplankton blooms in Poole Bay. The exchange of algal matter between the bay and the Harbour is an ongoing area of investigation which may indicate the relative importance of bay phytoplankton in the nutrition of harbour bivalves.

The abundance data generated by flow cytometry are an order of magnitude higher than that recorded by microscopy. This is not surprising given that the picoeukaryote algae, which numerically dominate coastal phytoplankton assemblages, are missed by microscopy. Despite this they are likely important for bivalve nutrition assuming that they are filtered with a reasonable efficiency (Nakamura, 2001). The higher picoeukarote abundance in spring and early summer 2012 relative to 2011 could be due to an increased input of river and effluent input in 2012. The highest nitrate value in our dataset (48.4 μmol L^{-1}) was found on 13th July 2012 after a period of heavy rain. This sample also contained the highest silicate value (46.9 μmol L^{-1}) and was sampled at the lowest point of the tide (salinity of ~24 ppt recorded by the sonde). Amongst others, the Western Channel Observatory at Plymouth Marine Laboratory has documented the rapid response of phytoplankton assemblages in U.K. coastal waters to the nutrient input caused by heavy rainfall. It seems reasonable to suppose that the atypical rains in spring and summer 2012 caused higher phytoplankton biomasses relative to 2011, especially amongst the picoeukaryotes. Due to their size the picoeukarote populations in Poole Harbour

likely respond quicker than the nanoeukaryotes (such as the larger diatoms) to elevated nutrients.

5.3 Clam condition, phytoplankton abundance, and temperature

In general the condition index of suspension feeding bivalves responds quickly to food availability i.e. < 1 week (Norkko et al. 2005). Linking food abundance and quality to such rapid changes in condition remains challenging. Manila clam modelling studies have struggled with the use of chlorophyll *a* data as a proxy for food resources and have highlighted the general lack of knowledge about Manila clam nutrition (Flye-Sainte-Marie et al. 2007). In our study a modest correlation was found between condition index and phytoplankton abundance estimated by flow cytometry but with no other estimate of food supply. Unsurprisingly, condition index also correlated with temperature since phytoplankton abundance is seasonal. Nevertheless, the correlation does highlight the link between picoeukaryote abundance and clam condition and suggests that this component of the phytoplankton assemblage may be significant in Manila clam nutrition. As a non-native at the edge of its range, the Manila clam could also be subject to physiologically stressful winter temperatures which, combined with low food supplies, may lead to the spring mortality events previously observed. On the other hand, warmer winters may also contribute to the spring mortality since clam reproduction is temperature induced, and after warm winters spent filtering nutritionally poor water (at a considerable metabolic cost) clam physiological reserves may be inadequate to meet the great demands of reproduction (i.e. gametogenesis and spawning). Distinguishing between these possibilities depends upon the extent to which individual clams require a minimum condition state before being triggered by temperature to enter their reproductive cycle. Lipid content has been used as a proxy for the metabolic reserve that individual clams are able to accumulate and measurements of the lipid status of the Poole Harbour populations over the annual cycle would be a useful next step. In addition, aquarium work with defined diets would be useful in order to test the relative importance of picoeukaryote and nanoeukaryote algae in Manila clam nutrition.

5.4 Future research and outputs

During the design of this project we recognised that, as an enclosed estuary subject to considerable human and freshwater inputs, non-algal particulate organic matter might occur at significant concentrations in Poole Harbour. The effects of such matter could potentially be both positive and negative; there is the possibility that it could provide nutrition to the clams but also, if non-nutritious, a negative effect as it could increase the metabolic cost of filtration and compete with (i.e. displace) algal cells during the feeding process (Nakamura 2001). Estimating the seasonal change in non-algal particulate organic matter remains challenging. In other parts of the world, e.g. Japan, Manila clam nutrition has been positively linked with sedimentary organic matter using stable isotope analysis (Watanabe et al. 2009). Therefore, a complete picture of clam nutrition likely requires an estimation of more

than phytoplankton, microphytobenthos and their detritus (Watanabe et al. 2009) but also an estimation of the alternative sources of particulate organic matter which are potentially significant. This is an area for which we are now gathering preliminary observations in Poole Harbour.

The reported exploitation of the Manila clam in Poole Harbour has suffered from disease as well as the spring mortalities described in Humphreys et al. (2007). Brown ring disease (caused by the bacterium *Vibrio tapetis*) is probably the dominant pathogen of the Manila clam and *Vibrio* was recently highlighted as an 'emerging threat' for U.K. aquaculture (Callaway et al. 2012). Viral infections, e.g. herpes, are also likely to be significant. In practise disease threats have been well understood by the responsible shellfish producers of Poole Harbour who have adapted their operations accordingly. Perhaps the most significant threat to the sustainability of the Poole Harbour Manila clam fishery is currently illegal fishing activity. The potential for a return to active cultivation of the Manila clam in Poole Harbour remains uncertain. Future research detailing more exactly the nutritional requirements of the Manila clam, and the interaction between clam physiological condition and pathogens seem to be two areas of priority in establishing the potential for the successful exploitation of the Manila clam in Poole harbour.

The abundant data and samples obtained through this project provide a unique and invaluable record of the water characteristics of Poole Harbour over a full annual cycle which we will continue to analyse. Further work will include cohort analysis of the two clam populations over the year of study and the examination of further correlations between clam growth rates and the measured physico-chemical features of the water column, both individually and in interaction. We intend in due course to disseminate our findings through conference and journal papers, acknowledging MMO support in such cases.

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7. Figures and tables

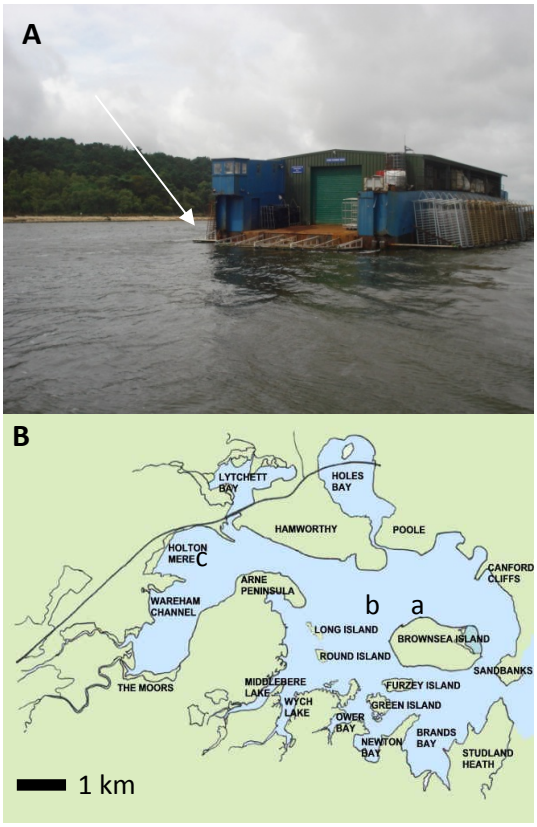

Figure 1. A) Sampling and monitoring site at the Othniel platform North of Brownsea Island within Poole Harbour. The sonde was attached to a fixed structure on the Eastern end of the platform (arrow). B) Poole Harbour showing the location of the platform (a) and the main aquaculture lease-beds (b). Manila clams were fished at (b) via pump-scoop dredge and also at (c) by hand dredge (see text). The Harbour mouth, opening to Poole bay, is between Sandbanks and Studland. Map courtesy of the Poole Harbour Study Group.

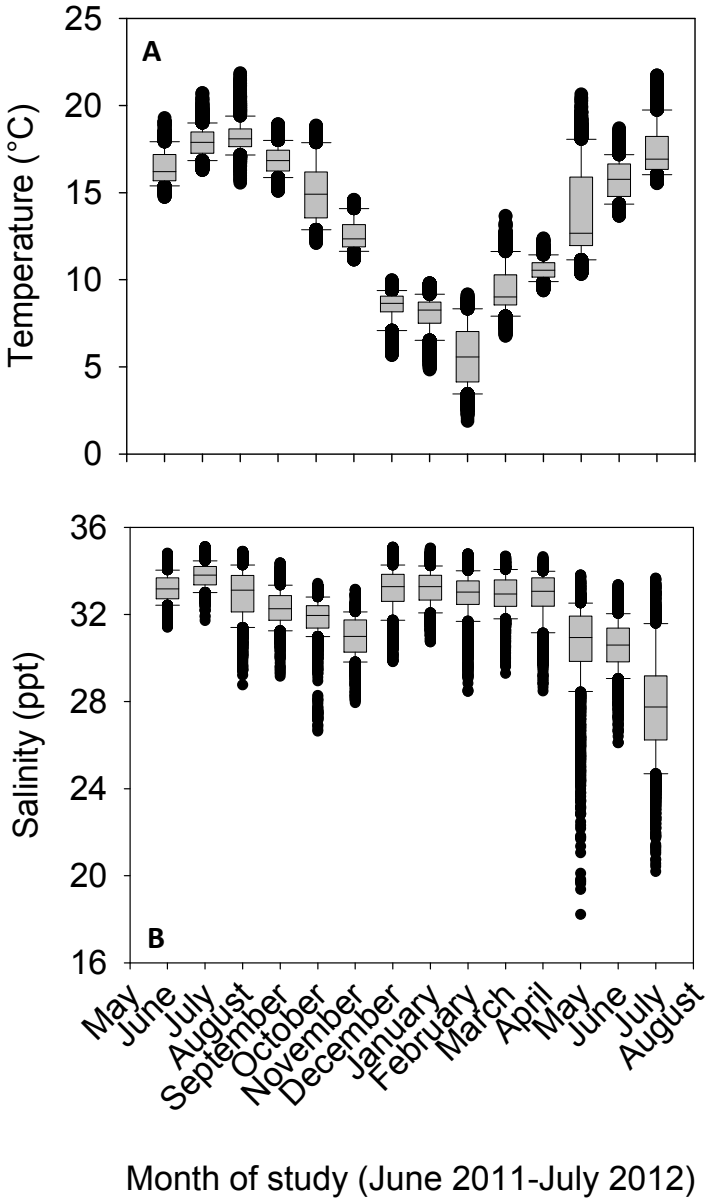

Figure 2. A) Box plot of temperature measured every 12 minutes at 1 m depth in the Wych channel (Poole Harbour) from June 2011 to June 2012. The ends of the boxes represent the 25th and 75th percentiles, the median is represented by the line within the box, and the error bars represent the 10th and 90th percentiles. Outliers are also plotted. B) Box plot of salinity measured at the same location with the same resolution.

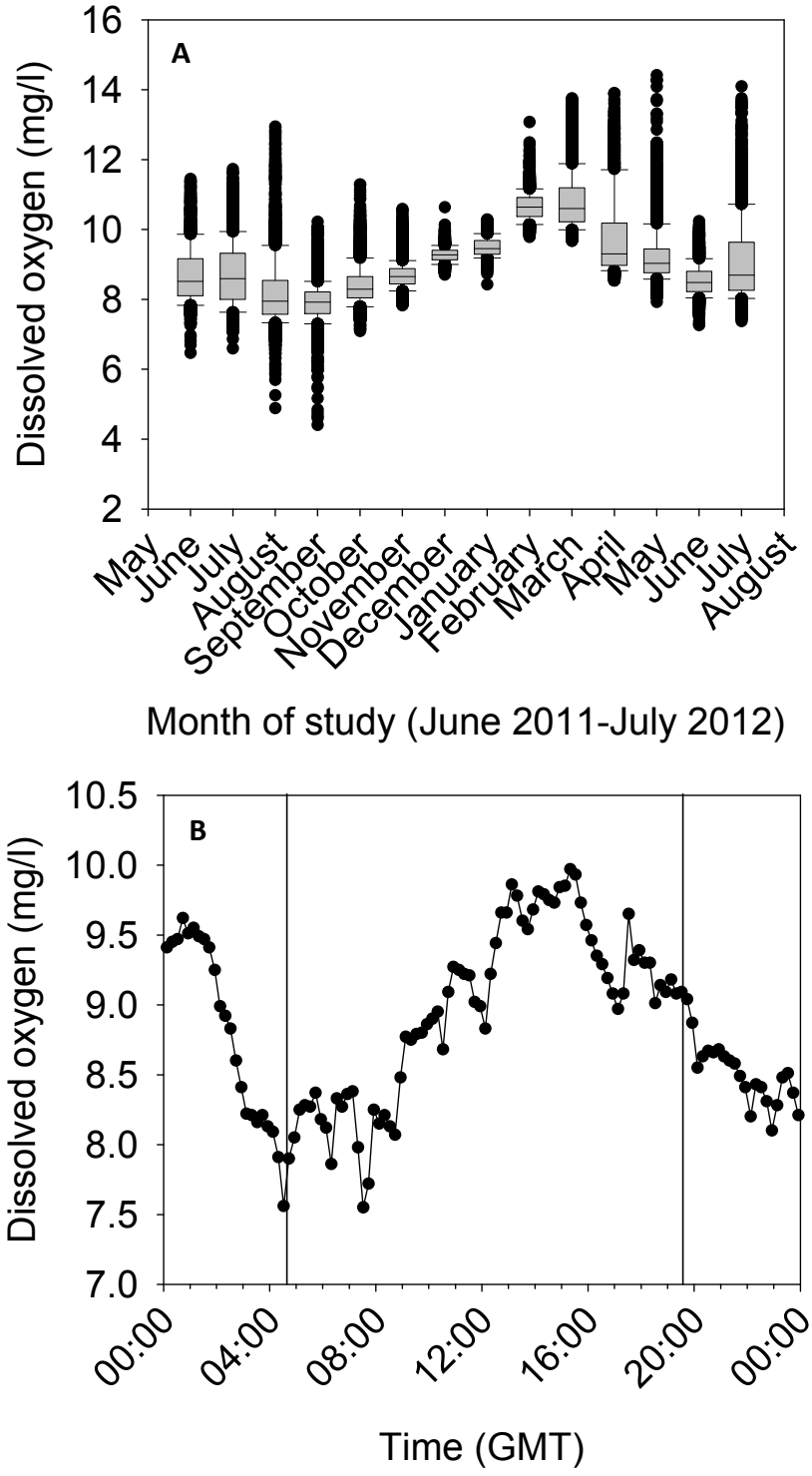

Figure 3. A) Box plot of dissolved oxygen measured every 12 minutes at 1 m depth in the Wych channel (Poole Harbour) from June 2011 to July 2012. See Figure 2 legend for details. B) Daily variation in dissolved oxygen on the 10th of August 2011. The vertical lines indicate sunrise and sunset.

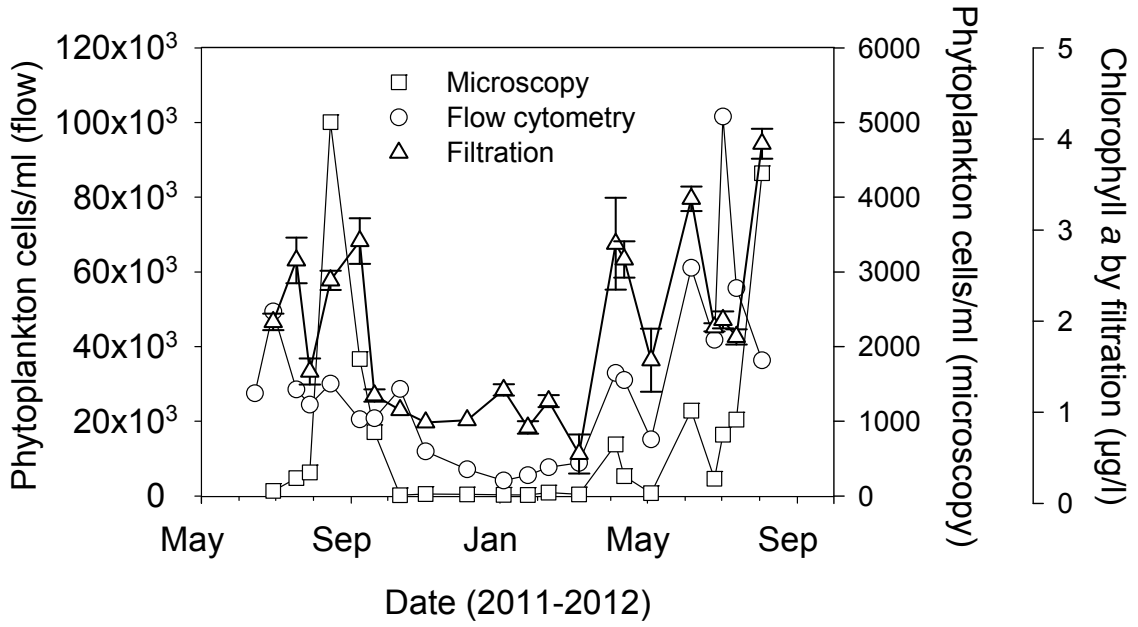

Figure 4. The annual cycle in phytoplankton abundance in Poole Harbour estimated by microscopy, flow cytometry and through quantification of chlorophyll *a* (see text).

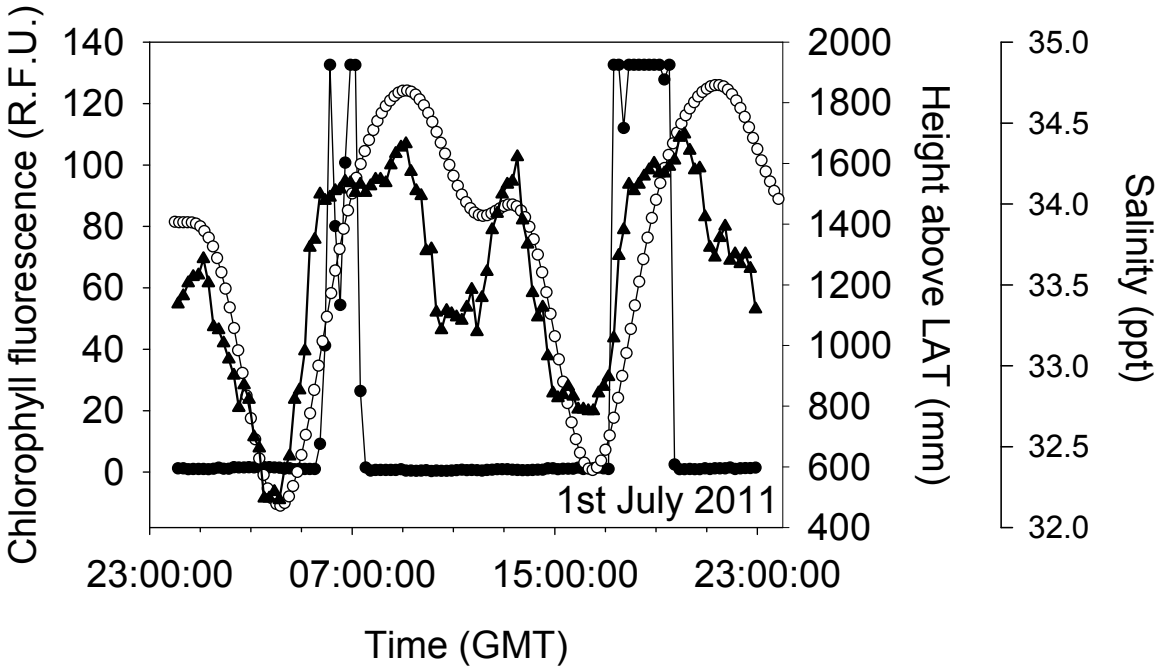

Figure 5. An example of the timing of chlorophyll fluorescence (●) spikes recorded at the study site with respect to tidal height (○) and salinity (▲). Tidal data (courtesy of Tim Smith, U.K. Hydrographic Office) are predictions for height above chart datum (approximately equivalent to lowest astronomical tide; LAT) at pottery pier (Brownsea Island). Pottery pier is <1 km from the Othniel platform.

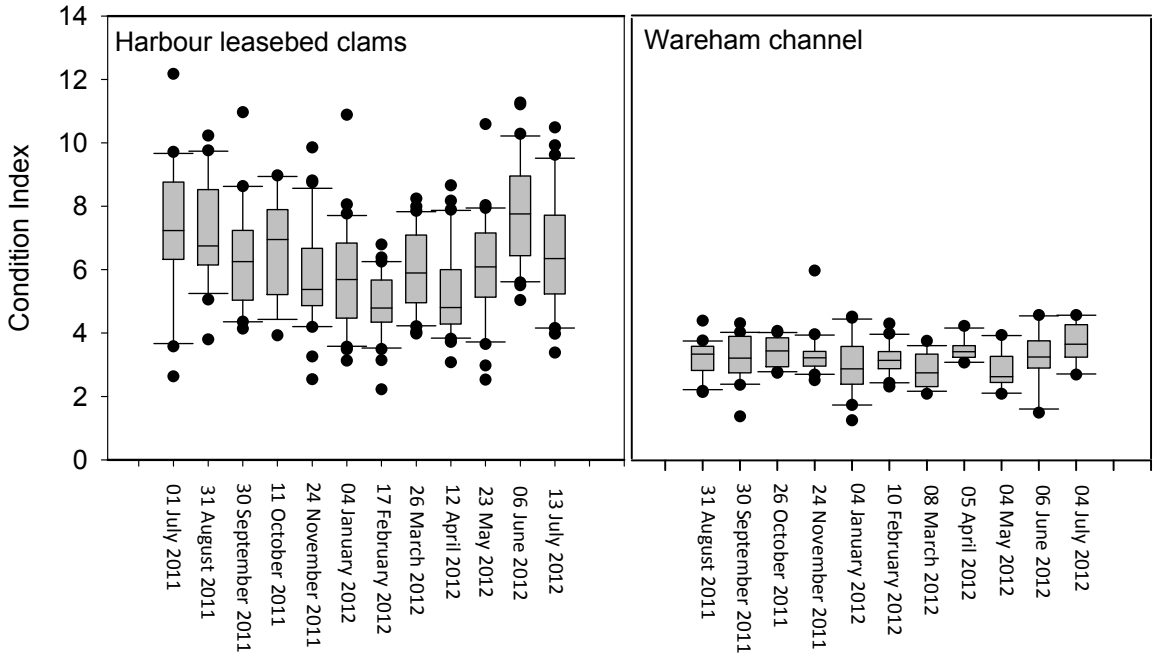

Figure 6. Seasonal change in the condition of Manila clams over an annual cycle in Poole Harbour at two different locations. The ends of the boxes represent the 25th and 75th percentiles, the median is represented by the line within the box, and the error bars represent the 10th and 90th percentiles. Outliers are also plotted.

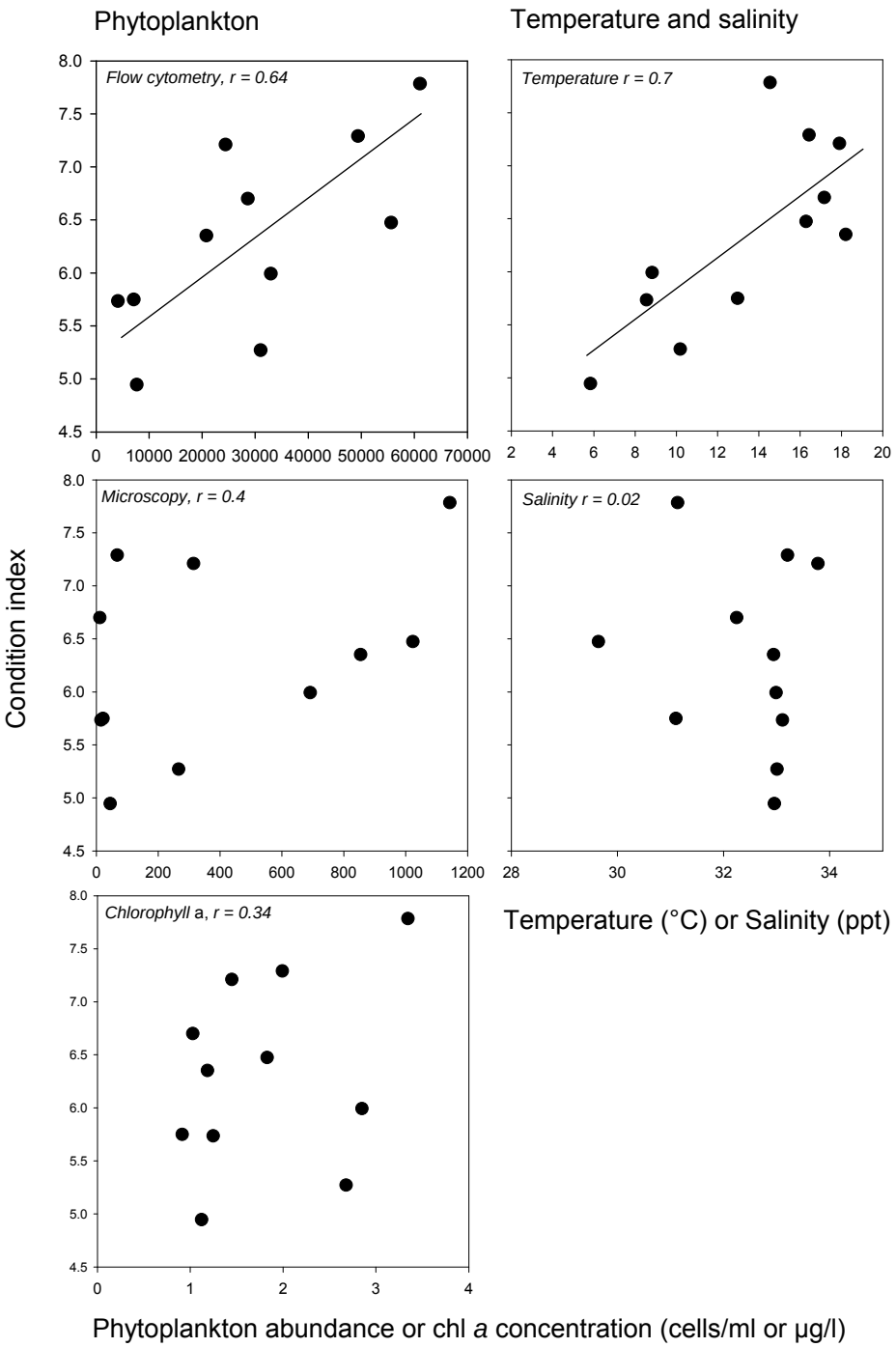

Figure 7. Scatterplots of Manila clam condition index vs. phytoplankton abundance, temperature and salinity in Poole Harbour over an annual cycle. The correlation (Spearman) coefficient is given and a line included where the correlation between the two variables is significant (P<0.05).

Date	Nitrate	Phosphate	Silicate
29/06/2011	3.6	0.3	8.5
18/07/2011	1.9	0.61	7.8
29/07/2011	2.1	0.43	7.1
15/08/2011	2.1	0.41	6.9
08/09/2011	7.7	0.39	14.5
20/09/2011	5.7	0.42	5.1
11/10/2011	5.4	0.53	14.7
01/11/2011	10.4	0.57	9.8
05/12/2011	20.6	0.58	23.2
04/01/2012	41.2	0.62	24.0
24/01/2012	23.7	0.58	12.9
10/02/2012	34.9	0.6	16.0
06/03/2012	30.2	0.49	9.1
05/04/2012	7.6	0.12	2.0
04/05/2012	37.1	0.61	25.2
06/06/2012	12.0	0.32	4.5
25/06/2012	29.9	0.44	14.5
02/07/2012	12.4	0.21	6.7
13/07/2012	48.4	0.83	46.9
03/08/2012	6.0	0.08	1.0

Table 1. Inorganic nutrient concentrations (µmol L^{-1}) in Poole Harbour over a 14 month period sampled from 0.5 m depth in the Wych channel (see text).

Phytoplankton type				
Diatoms	**Dinoflagellates**	**Silicoflagellates**	**Euglenoids**	**Chlorophytes**
Actinoptychus	*Alexandrium*	*Dictyocha fibula*	Unidentified euglenoid	*Scenedesmus*
Araphiated diatom <20	Armoured dinoflagellate <20			
Araphiated diatom 20-50	Armoured dinoflagellate 20-50			
Araphiated diatom >50	Naked dinoflagellate >50			
Asterionella formosa	*Gonyaulax*			
Asterionellopsis glacialis	*Heterocapsa*			
Biddulphia alternans	*Heterocapsa triquetra*			
Centric diatom <20	*Polykrikos*			
Centric diatom 20-50	*Prorocentrum micans*			
Centric diatom >50	*Prorocentrum* <20			
Cerataulina pelagica	*Prorocentrum* 20 -50			
Chaetoceros sp. (Hyalochaete)	*Protoperidinium bipes*			
Chaetoceros sp. (Phaeoceros)	*Protoperidinium* <20			
Cylindrotheca/Nitszchia	*Protoperidinium* 20-50			
Dactyliosolen fragilissimus	*Scripsiella*			
Ditylum brightwelli				
Eucampia zodiacus				
Guinardia delicatula				
Guinardia striata				
Gyrosigma/Pleurosigma				
Heliotheca tamesis				
Lauderia annulata				
Leptocylindrus danicus				
Leptocylindrus minimus				
Licmophora				
Melosira				
Odontella				
Paralia sulcata				
Pseudo-nitzschia <5				
Pseudo-nitzschia >5				
Pseudo-nitzschia (unmeasured)				
Raphiated diatom <20				
Raphiated diatom 20-50				
Raphiated diatom >50				
Rhizoselenia <10				
Rhizoselenia 10-20				
Rhizoselenia >20				
Skeletonema				
Striatella unipunctata				
Thalassiosira <10				
Thalassiosira 10-50				

Table 2. Phytoplankton types recorded in Poole Harbour over a 14 month period sampled from 0.5 m depth in the Wych channel (see text).