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Growth of *Crassostrea gigas* spat and juveniles under differing environmental conditions at two sites in Wales

Jonathan W. King[1,a], Shelagh K. Malham[1], Martin W. Skov[1], Elizabeth Cotter[2], John W. Latchford[1], Sarah C. Culloty[2] and Andrew R. Beaumont[1]

[1] Centre for Applied Marine Sciences, Marine Science Laboratories, School of Ocean Sciences, University of Wales Bangor, Menai Bridge, Anglesey, LL59 5AB, UK
[2] Department of Zoology, Ecology & Plant Science, Aquaculture and Fisheries Development Centre, University College Cork, Lee Maltings, Prospect Row, Cork, Ireland

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Abstract – Two sites in North Wales, UK, were compared for growth, survival, immunocompetence and gonad development of *Crassostrea gigas*. Juvenile *C. gigas* spat were purchased as a cohort and placed in bags on trestles at the Menai Strait (MS, two heights on the shore) and the Inland Sea (IS) in the summer of 2003. Performance was examined as a function of several environmental parameters; temperature, coloured dissolved organic material absorption coefficient (cDOM), organic and inorganic nutrient levels, chlorophyll *a* and phytoplankton community composition. Both sites produced variations in growth, as assessed by dry weight indices and condition index, which were found to be lower at IS, where the animals were permanently immersed, than at MS, where they were emersed for at least 1 h/tide (at the low shore height) and 4 h/tide (at the high shore height) during spring tides. Condition index was also significantly higher at MS high shore than at MS low shore. Mortality was low at all sites, though an increase at IS at the end of the trial led to a significantly greater final percentage mortality of 15.6%. Gonad development was limited, particularly at IS where most animals remained undifferentiated. Gonad development was significantly higher at MS high shore than at MS low shore. cDOM, organic and inorganic nutrients were similar at the two areas. At IS the temperature and chlorophyll *a* levels were higher than MS. The phytoplankton communities were significantly different, with a bloom of *Prorocentrum micans* dominating at the Inland Sea for much of the experimental period. Differences in the hydrodynamics, as well as Phytoplankton community appeared to be the only parameters that might cause a lower growth rate in the Inland Sea. The possibilities of *P. micans* having a sub-lethal effect on *C. gigas* are discussed.

Key words: Oyster culture / *Crassostrea gigas* / water quality / Immunology / *Prorocentrum micans* / Site selection

Résumé – Croissance du naissain et de jeunes huîtres, *Crassostrea gigas*, sous diverses conditions environnementales de deux sites du Pays de Galles. Deux sites du Nord du Pays de Galles (G.B.) sont comparés au niveau de la croissance, de la survie, de l'immuno-compétence et du développement gonadique de *Crassostrea gigas*. Du naissain de *C. gigas* a été acheté, considéré comme cohorte, et durant l'été 2003, placé en sacs sur des tables (tréteaux) à Menai Strait (MS, à deux différents niveaux de la zone intertidale) et dans la mer intérieure « Inland Sea » (IS). Les résultats sont examinés en fonction de plusieurs paramètres environnementaux, température, matière organique dissoute colorée (cDOM), niveaux de substances nutritives organiques et minérales, chlorophylle *a* et composition phytoplanctonique. Des variations sont observées dans les deux sites, au niveau de la croissance, établies par des indices de poids secs et de condition qui sont plus faibles à IS, où les huîtres sont immergées de façon permanente, alors que celles de MS restent émergées au moins 1 h par marée (en bas de la zone intertidale) et 4 h par marée (en haut) durant les grandes marées. L'indice de condition est significativement plus fort en haut qu'en bas de la zone intertidale à MS. La mortalité est faible quel que soit le site, bien qu'elle augmente à IS en fin d'expérimentation, ce qui conduit à 15,6 % de mortalité à IS. Le développement gonadique est limité, et particulièrement à IS où la plupart des huîtres restent indifférenciées. Le développement des gonades est plus important en haut de la zone intertidale qu'en bas. Les substances organiques et minérales, et DOM sont similaires dans les deux sites. La température et les niveaux de

[a] Corresponding author: j.w.king@bangor.ac.uk
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http://www.ifremer.fr/icsr05/

chlorophylle *a* sont plus élevés à IS qu'à MS. Les communautés phytoplanctoniques sont différentes significativement, avec un bloom de *Prorocentrum micans* dominant à Inland Sea, durant la majorité de la période expérimentale. Des différences, au niveau de l'hydrodynamique et de la communauté phytoplanctonique, semblent être les seuls paramètres qui pourraient causer un taux de croissance plus faible à Inland Sea. Les possibilités qu'aurait *P. micans* de provoquer un effet sub-léthal sur *C. gigas* sont discutées.

1 Introduction

When locating sites for expansion of the pacific oyster industry in the UK, it is necessary to identify whether a potential location is suitable for oyster growth, both in terms of normal conditions and irregular events. Oyster growth and survival can be influenced by many natural factors. Many of the requirements for a suitable site are known and have been described in detail, for example in Spencer (1990), which includes specific information on the two study sites in this paper; the Menai Strait (MS) and the Inland Sea (IS), both on Anglesey, North Wales. However, as Werstink et al. (2005) point out new sites are often selected using ill-defined criteria and methods. A number of studies examine the effect of environmental parameters (e.g. Brown and Hartwick 1988; Grant et al. 1998; Dégremont et al. 2005). Volety et al. (2005) use a mathematical modelling approach to identify habitat suitability for *Crassostrea virginica* in Florida. Before such an approach is tried for *C. gigas* in the UK, further data for ground-truthing is required and investigations made into what components of the ecosystem need to be monitored for inclusion in site assessments (and for how long; Mazurié et al. 2005). The long term aim of such an approach should be to provide a standard protocol for identifying suitable sites for expansion of bivalve farming, within a coastal zone management framework. Increasing understanding of the factors that influence growth performance and how they interact is an ongoing requirement due to the complexity of the interactions of *C. gigas* with the ecosystem where they are placed.

In this study the performance of *C. gigas* (growth, mortality, gonad development and immunocompetence) was studied in the context of several environmental parameters; temperature, chlorophyll *a*, phytoplankton community, colour dissolved organic matter (cDOM, a measure of terrestrially-derived material in the water) inorganic and organic nutrients. The effect of emersion time was also studied at MS as Spencer et al. (1978), working within 1 km of the MS study site, found that growth was inversely proportional to emersion time.

2 Materials and methods

2.1 Areas

Two sites were used in this study, one on the North shore of the western Menai Strait and the other in a bay on the East shore of the Inland Sea (Fig. 1). The Menai Strait has strong currents, net transport of water to the South West (25−30 million tonnes/tidal cycle), a flushing time of 2−3 days and up to 7.5 m tidal range. Two heights were studied in the Strait, "high shore" with 4 h exposure on spring tides, and "low shore", exposed for 1 h on spring tides. The Inland Sea was man made in the 19th century, and has limited tidal flushing through small culverts under road bridges. Due to the limited tidal flows though the culverts a complex tidal situation arises in which the tide is not diurnal, as it is outside the influences of the bridges, and tidal range is about 1 m with the highest tidal range during neap tides. Because of this and the topography at the IS study site, only one height was possible, with the oysters immersed at all times.

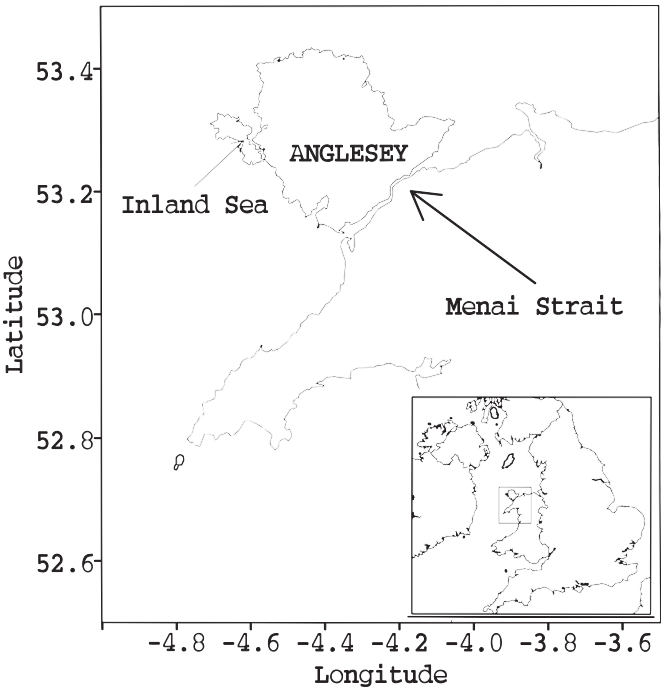

Fig. 1. The location of the Inland Sea and the Menai Strait, North-West Wales, UK.

2.2 Experimental design

A single cohort of *C. gigas* seed oysters (shell length 10−12 mm) was purchased from Guernsey Sea Farms, and placed on three sites in two areas in June 2003 randomly distributed into 0.5 m^2, 4 mm mesh bags on trestles (1000 oysters per bag, $n = 5$ bags per site). The average weight of oysters per bag was 292 g; this start weight and number is within the typical range for newly introduced spat on farms in the Irish Sea. An additional 3 bags at each site were left undisturbed as a control for mortality counts. Water quality samples (nutrients, chlorophyll *a*, cDOM, phytoplankton samples) from each area and oyster samples for laboratory analysis (growth, condition index, gonad development and immunological parameters) were taken fortnightly from each site on spring tides. Temperature probes (Guernsey Sea Farms Ltd.)

were placed in two bags in IS and in one bag at each height on the shore in MS. Temperature readings were recorded every 30 min throughout the summer and pooled for each area. An additional single surface seawater temperature was taken during each visit using a thermometer.

2.3 Analysis of oyster condition

Oysters were analysed from the MS (high), MS (low) and IS having been divided into three groups, the first to measure growth parameters ($n = 20$), the second for histological examination of gonads ($n = 30$) and the third for immunological assessment ($n = 60$). Each experimental oyster bag was checked for mortality (%, $n = 200$) in situ with empty shells being returned to the bag afterwards. A control at each site was sampled for mortality only at the end of the experiment. The Kruskal-Wallis test was used to compare sites.

Growth (g) was measured as tissue and shell dry weight (g). To obtain dry weights, samples were oven dried at 60 °C for 48 h. An ANOVA was used to test for significant differences in growth with Tukey's pairwise comparisons to determine how the sites differed from each other. A $\log_{10}$ transformation log was needed to give a normal distribution.

Condition index was calculated as:

$$\text{CI} = (\text{tissue dry weight / shell dry weight}) \times 100.$$

A two factor ANOVA, following $\log_{10}$ transformation, and Tukey's pairwise comparisons were used to test for significant differences.

For histological examination oysters were fixed in Davidson's solution (Shaw and Battle 1957) at 4 °C for 48 h, embedded in paraffin wax, sectioned at 7 μm, and stained in Harris' haematoxylin and counterstained in eosin (Humanson 1979). The prepared microscope slides were examined under a light microscope to determine sex and stage of gametogenesis. The oysters were classified into distinct phases of gonad maturity according to the classification of Steele and Mulcahy (1999); χ^2 tests were used to compare gonad development.

To assess immunocompetence, animals were returned to the laboratory and placed in aerated through-flow tanks and left undisturbed for 2 days before processing.

Thirty oysters were taken and half subjected to an applied stress ("stimulated") as described in Lacoste et al. (2002) while the remaining half were sampled immediately ("unstimulated") This was to gain an indication of whether the animals were able to respond well to stress or not.

Haemolymph was collected as described in Lacoste et al. (2002) with modifications. Haemolymph was pooled from 10 animals and 2 μl of haemolymph was added to 10 μl of Alsevers solution to fix the cells and to obtain more accurate cell counts. The remaining cells were adjusted to 10^6 cells ml^{-1} with MHBSS (HBSS adjusted to a salinity of 31 ppm and containing 0.03 μg ml^{-1} EGTA) and used immediately in the phagocytosis assays. This was repeated three times.

For blood counts, cells fixed in Alsevers were counted and the total number of haemocytes.ml^{-1} calculated. The number of haemocytes ml^{-1} from un-stressed animals was then subtracted from those which had undergone an applied stress to give an overall value for the site and density. Results were plotted as a time series.

For the phagocytosis assay, the bacterium *Vibrio anguillarum* was grown, fixed and labelled (fluorescein 5-isothiocyanate, Isomer 1, (FITC) (Sigma)) according to Lacoste et al. (2002), Mortensen and Glette (1996). The phagocytosis assay was performed as described in Lactose et al. (2002) with modification due to the small volume of blood obtainable from 10−12 mm shell length oysters. Briefly, 20 μl haemolymph was used with twenty μl of FITC labelled bacteria and the slides incubated in the dark for 30 min before rinsing (MHBSS) and addition of 20 μl of ethidium bromide (50 μg ml^{-1} in PBS) (Sigma) to counter stain unphagocytosed bacteria. The number of phagocytosing haemocytes were counted using a 488 nm emission filter on a Zeiss microscope. Three counts of 200 cells were made from each of the three replicate slides. The percentage of phagocytic cells for each slide was then calculated and the mean and standard error recorded for each time point. Finally the percentage phagocytosis for each site and stocking density was calculated by taking the un-stressed value from the results obtained from the oysters which underwent an applied stress. Results were analysed with a two factor ANOVA.

2.4 Environmental parameters

Environmental parameters were recorded at the two sites, MS and IS. The two heights on the shore were sufficiently close not to take separate measurements. On each sampling occasion 200 ml of water was filtered (25 mm Whatman GF/F filter, nominal pore size 0.7 μm) in triplicate for chlorophyll *a* analysis. Six litres of seawater were also taken for cDOM and nutrient analyses; 2 L was sub-sampled for phytoplankton analysis and stored at 4 °C after the addition of Lugol's iodine.

Chlorophyll *a* was extracted overnight in 90% acetone and fluorescence read using a Turner 10 AU fluorometer calibrated for total chlorophyll *a*. The concentration of total chlorophyll *a* in the original sample was then calculated.

Nitrite and nitrate were analysed using the method of Grasshoff (1976), modified for flow injection analysis by Anderson (1979), and further by Johnson and Petty (1983).

Ammonium analysis was based upon the method of Holmes et al. (1999). Phosphate was determined using a method based upon the methods of Grasshoff (1976) and Johnson and Petty (1982). For silicate the method used was based upon the technique established by Grasshoff (1976), then developed by Parsons et al. (1984). Dissolved organic carbon (DOC) was determined using an MQ Scientific TOC analyser (MQ Scientific, Inc., PO Box 2435, Pullman, WA 99165-2435, USA), following the high temperature combustion oxidation (HTCO) methods of Qian and Mopper (1996). Dissolved organic nitrogen (DON), total dissolved phosphorus (TDP), Dissolved organic phosphorus (DOP) and total dissolved nitrogen (TDN) concentrations were determined using the method of Kattner and Becker (1991).

Phytoplankton samples were analysed for community composition using standard methodologies (Sournia 1978): a 120 ml sub-sample (1/10th of original) was extracted for each

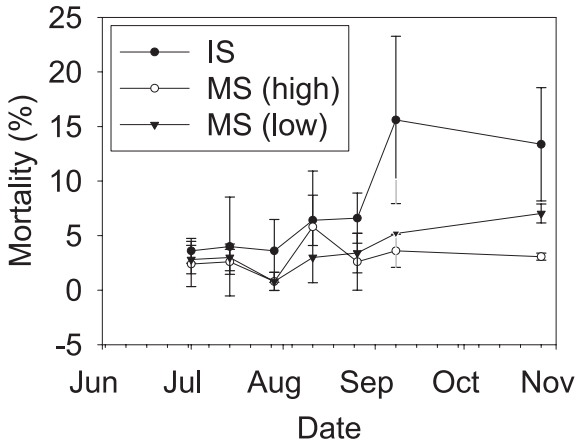

Fig. 2. Mortality of oysters was low, though the Inland Sea sample increased at the end of the trial, to become significantly higher (Kruskal-Wallis, $p = 0.01$). IS: Inland Sea, MS (high): Menai Strait high shore (4 h emersion on spring tides), MS (low): Menai Strait low shore (1 h emersion on spring tides).

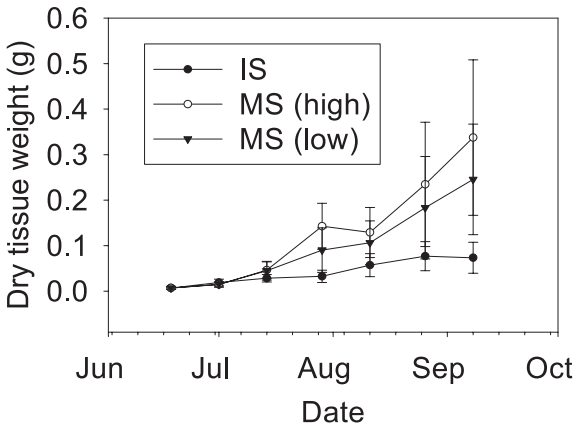

Fig. 3. Growth of oysters over experimental period (g dry tissue). There was a significant difference between sites (ANOVA, $p = 0.001$) with Tukey's pairwise comparisons showing that IS oysters grew significantly less over the trial period. There was no significant difference between MS (high) and MS (low). IS: Inland Sea, MS (high): Menai Strait high shore (4 h emersion on spring tides), MS (low): Menai Strait low shore (1 h emersion on spring tides).

Table 1. Mortality, growth and condition index (mean + SD) of oysters at the end of the trial for both sites Inland Sea and Menai Strait (High and Low: two heights on the shore).

Mean + SD	Inland Sea	Menai Strait	
		High	Low
Mortality[1] (%)	13.4 ± 0.6^{a}	3.1 ± 0.3^{b}	7.0 ± 0.9^{ab}
Growth[2] (g dw)	0.07 ± 0.03^{a}	0.34 ± 0.17^{b}	0.25 ± 0.12^{b}
Condition Index[3]	4.59 ± 0.46^{b}	6.19 ± 1.47^{a}	5.03 ± 0.91^{b}

Significant tests: (1) Kruskal-test, $p = 0.001$, (2) ANOVA, $F = 49.55$, $p < 0.001$, (3) ANOVA, $F = 10.09$, $p < 0.001$. Values with different superscripts are significantly different and those with the same superscript are not significantly different.

water sample, inserted in sedimentation chambers over night and viewed in an upside-down light microscope (Nikon Diaphot). Plankton were quantified at ×20 magnification, using ×40 and ×100 magnification (oil immersion) for identification when required. The majority of plankton >10 μm were identified to genus level or more. Identification was based on Ricard (1987), Hartley et al. (1996) and Tomas et al. (1996) for diatoms, and Sournia (1986) and Chrétiennot-Dinet (1990) for other microphytoplankton.

3 Results

3.1 Condition of oysters

Mortality (Fig. 2) was low at all sites although there was an increase in IS towards the end of the trials, which led to mortality on the last sampling date being significantly higher in IS than for MS high-shore site (Mean final values: see Table 1). No other significant differences were observed. The control bags had a similar mortality rate to the last of the fortnightly counts, suggesting that repeated sampling did not significantly increase the mortality of the remaining oysters.

There was a significant difference in growth between sites (Mean final dry tissue weights: see Table 1). Tukey's pairwise comparisons revealed that oysters grew significantly larger in MS than in the IS, and that in MS there was no significant difference in growth between the shore heights (Fig. 3).

Condition index was also significantly different (Mean final values: see Table 1). Tukey's pairwise comparisons showed that the MS high-shore site had better condition than the other two sites, which were not significantly different from each other.

Gonad development did not reach the ripe/spawning stage, with a few exceptions in MS high shore (3.4%), and resorption appeared to be occurring in half of these. The high shore site had significantly more sexually mature animals and fewer undifferentiated than expected (as few as 50%). The converse was true for the lower site ($\chi^2 = 7.192$, $p = 0.027$). The IS specimens had significantly less gonad development than expected with never less than 86% of oysters being undifferentiated at each sampling period ($\chi^2 = 64.19$, $p < 0.001$).

IS oysters were immunosuppressed compared with those in MS sites. In the former, both blood counts and phagocytosis counts (ANOVA, $F = 242.88$, $p < 0.001$) were not raised in the stressed animals compared to their unstressed counterparts (Figs. 4a,b), although no site showed a reaction for the first two weeks of the experiment. Results obtained at the beginning of September were not valid as there was an oil leak in the aeration system when the animals were in the laboratory tanks prior to analysis.

3.2 Environmental parameters

Inorganic nutrient levels were low and usually similar in the two sites, although there were occasional peaks in ammonia and silicate levels were higher in the IS than the MS in the mid-period of the trial (Fig. 5). Organic nutrients (Fig. 6) were similar, although there was a large peak in TDP and DOP in early September at IS and a drop in DON in July at MS. DOC and cDOM (Fig. 7) were generally higher at IS.

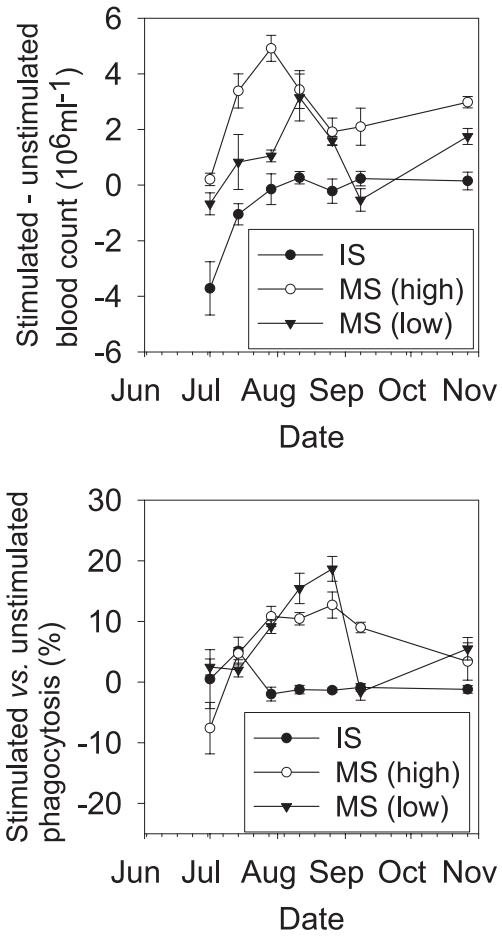

Fig. 4. (a) Stimulated – unstimulated blood counts of oysters at different sites (10^6 cell ml^{-1}), showing that IS samples are unable to respond to stress, unlike samples from MS (ANOVA, $p < 0.001$). IS = Inland Sea, MS (high) = Menai Strait high shore (4 h emersion on spring tides), MS (low) = Menai Strait low shore (1 h emersion on spring tides). (b) Stimulated – unstimulated phagocytosis at different sites (%). Oysters at IS show an average of around 0, being immunosupressed, whilst those at MS respond to stress. (ANOVA, $p < 0.001$).

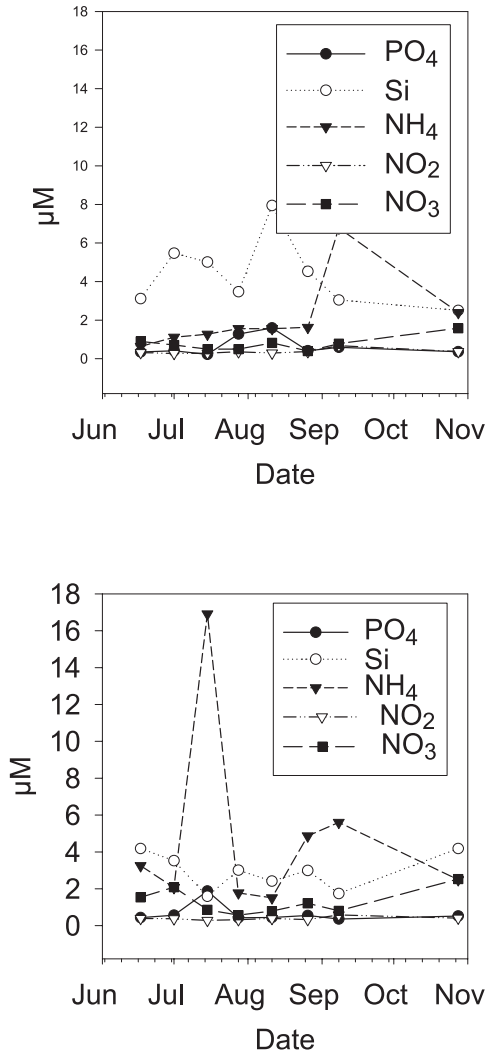

Fig. 5. Time series plot of inorganic nutrient levels, (a) Inland Sea (b) Menai Strait. Levels are generally low, apart from a large peak in NH_4 in the Menai Strait in July. Levels were similar at the two sites, though silicates were higher in the Inland Sea for the mid part of the trial.

Temperature was higher on average at IS than at MS (Fig. 8) though not excessively high for *C. gigas*. Point temperatures indicated that the values given by the continuous recorders were not greatly affected by the twice daily emersion during spring tide periods.

Pairwise comparisons showed that chlorophyll *a* levels were either equal to or significantly higher in the Inland Sea than in the Menai Strait (ANOVA $F = 36.1$, $p < 0.001$ for location). Averaged over the whole period levels were 75% higher in the Inland Sea (Fig. 9).

A principle components analysis (PCA) of the phytoplankton communities at each site (Fig. 10) showed a clear difference between the two areas. A large bloom of the heterotrophic thecate dinoflagellate *Prorocentrum micans* occurred in IS for much of the experimental period, this bloom peaked at 10^6 cells per litre and on occasion represented 95% of the phytoplankton cells present. The numbers of this organism were so large that a correlation matrix was used to standardise the variables prior to PCA. This approach results in 22% of the total variability being explained in the first two principal components. Samples from the two sites were very well separated, with no single species or small group of species having a huge influence on the location of a sample on either axis, indicating that overall there were a large number of species whose abundance was different between the two locations. However numbers of each were generally small; the only other taxonomic group that had significant numbers of cells present in the IS were the Chlorophycae group, which was regularly recorded at 10^4 peaking at 10^5 at the end of August.

4 Discussion

Oysters grew significantly larger in the Menai Strait compared to the Inland Sea and had better condition. Samples from

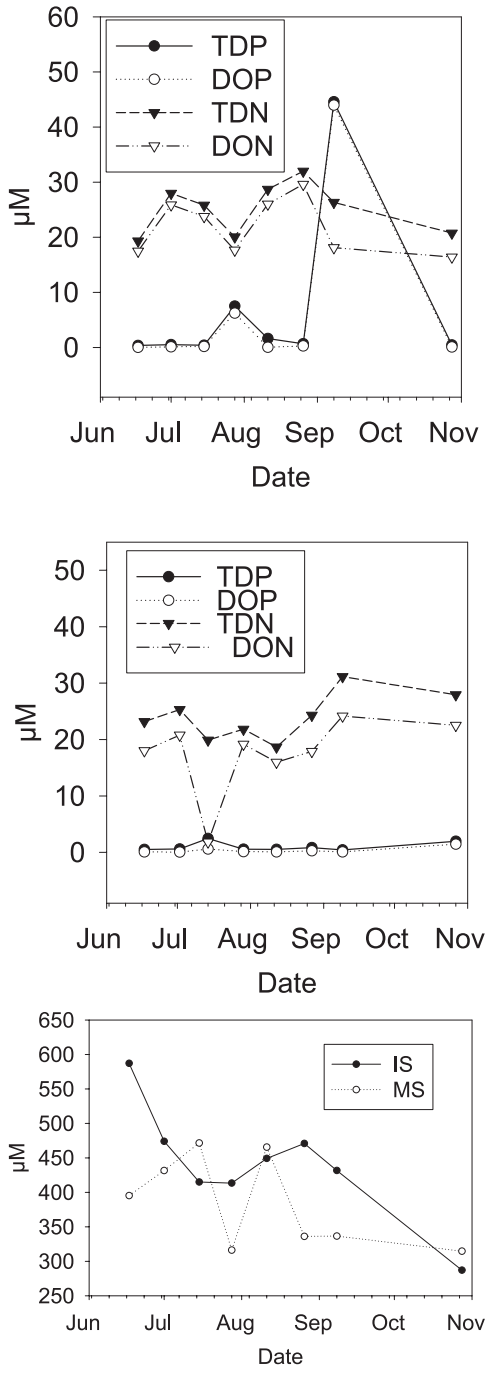

Fig. 6. Time series plot of organic nutrients, (a) TDP, DOP, TDN and DON, Inland Sea (b) TDP, DOP, TDN and DON, Menai Strait (c) DOC, Menai Strait (MS) and Inland Sea (IS). The two sites had similar values, though there was a large peak in TDP and DOP in early September at the Inland Sea, and in July DON had a lower value in the Menai Strait.

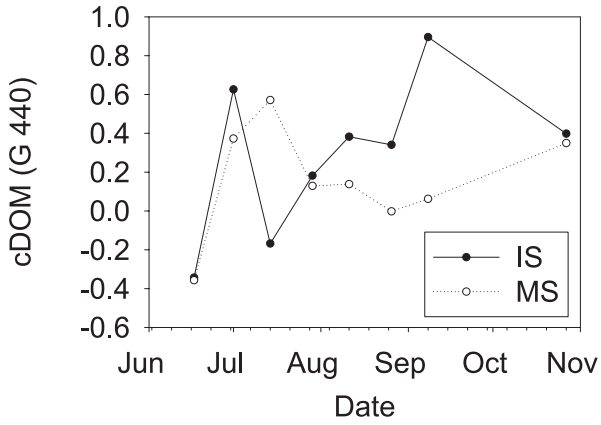

Fig. 7. Time series plot of cDOM for the Menai Strait (MS) and Inland Sea (IS). Levels were generally low, indicating that run-off from land is not a major influence.

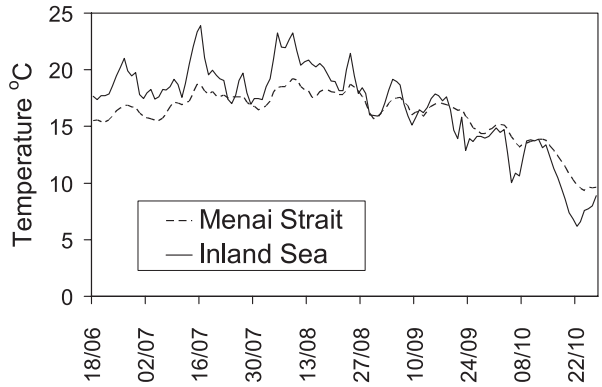

Fig. 8. Daily temperatures (°C) during the experimental period. The Inland Sea (IS) was generally warmer until the end of August, and the Menai Strait (MS) was warmer from mid-September onwards.

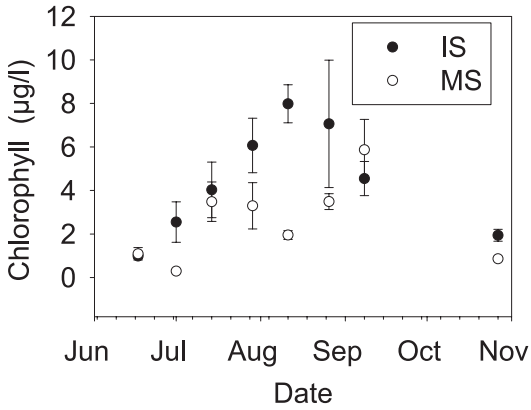

Fig. 9. Time series plot of chlorophyll *a* levels. Over the whole period levels in the Inland Sea (IS) were either significantly higher or similar than the Menai Strait (MS), ANOVA, $p < 0.001$. On average IS levels were 75% higher than at MS.

the Menai Strait had greater gonad development, though maturity/spawning was a rare occurrence. As well as poorer growth performance, oysters in the Inland Sea appeared immunosuppressed, being unable to respond to an applied stress with increased in blood cell counts or % phagocytosis. This suggests that some factor of the site results in the oysters being in poor condition and potentially susceptible to infection.

There was little difference in growth between the two sites in the Menai Strait, which contradicts the findings of Spencer et al. (1978) who found that growth was inversely proportional to emersion time. One possible explanation for this is that in the Menai Strait an infestation of the polychaete *Polydora* sp. occurred from the beginning of September onwards, with infection rates higher in the animals lowest on the shore as in

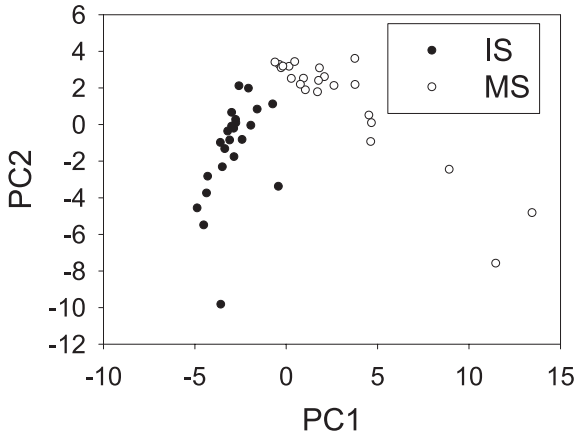

Fig. 10. Principle components analysis of the phytoplankton communities of the Inland Sea (IS) and Menai Strait (MS). There is no overlap between samples from the two sites, indicating that they are quite different.

the findings, for example, of Caceres-Martinez et al. (1998), who also noted a correlation between infestation rate and mortality and an inverse correlation between infestation rate and condition index. It maybe therefore that the greater infestation with *Polydora* sp. in oysters on the low shore site counteracted the effect of emersion on growth rate. The bags situated on the low shore site were also considerably more fouled than those higher up, this is likely to have reduced food availability to these animals and have therefore led to a reduced performance in the lower shore site, countering any expected higher growth rate due to greater emersion. In spite of these factors, Menai Strait samples consistently performed well.

The higher temperatures and Chlorophyll *a* levels alone in the Inland Sea would suggest that this site was in fact a better site for oyster production. The lack of major differences in cDOM, organic and inorganic nutrients would not contradict this. Hydrodynamically the sites were very different, with a good deal more tidal flushing and current in the Menai Strait than in the Inland Sea. Although there are no precise values for this, flow rate through the bags is likely to be less in the latter site. However water movement caused by the rise and fall of the tide and gentle wave action has been specifically identified as providing adequate water exchange over the oysters in the Inland Sea (Spencer 1990). Furthermore fouling was considerably less at this site than at MS, increasing the rate of through-flow in bags at IS.

The only remaining variable that was recorded was the phytoplankton communities.

These were strikingly different in the Inland Sea and Menai Strait, with the Inland Sea strongly dominated by flagellates, microflagellates and cyanobacteria, and only a reduced number of diatoms (in some periods virtually devoid of these). A single species dominated; the semi-benthic *Prorocentrum micans,* with up to 10^6 cells L^{-1} making up to 95% of the phytoplankton cells in the samples. The Chlorophycae made up the second most abundant group in the Inland Sea, frequently having 10^4 cells L^{-1} and peaking at 10^5 cells L^{-1} at the end of August.

P. micans has been reported as a non-toxic harmful algal bloom (HAB) and opinion that it is harmless is sufficiently strong for it to sometimes be used as a control in experiments on toxic effects of algae on e.g. blue mussels, as a comparison with *P. lima.* (Anon. 1995a), trials with *Artemia fransiscana* (Anon. 1995b) and as a control for *Alexandrium minutum* (Guidande et al. 2002). Furthermore it was found not to be discriminated against in ingestion trials on larval crabs (Hinz et al. 2001), not toxic to abalone larvae or spat (Botes et al. 2000) and to "contribute nutritionally" to the diet of 1st stage larvae of *Cancer magister.* (Sulkin et al. 1998).

Whilst no studies have directly indicated that *P. micans* has a sub-lethal effect on *C. gigas* a number of studies provide circumstantial evidence. *P. micans* has been associated with 7-epi-PTX-2SA in mussels (a derivative of PTX-2), but only as one of the dominant species present at the site (Pavela-Vrančič et al. 2002). It is not clear whether *P. micans* was responsible for the toxin, whether it stimulated another species to produce it or whether its presence was purely coincidence. Kim et al. (1999) found that *P. micans* produced low levels of H_2O_2 and Ianora et al. (1999) found that it reduces male copepod fertilization capacity. As with all non toxic HABs *P. micans* has led to shellfish kills at very high concentrations (Wang et al. 1998) and at the collapse of the bloom (Matthews and Pitcher 1996). Burkholder (1998) included *P. micans* in a list of toxic estuarine and marine dinoflagellates and also used it as an example of a benthic dinoflagellate that produces ciguatoxins or ciguateratoxins.

Several other *Prorocentrum* spp. are harmful to shellfish. (e.g. Luckenbach et al. 1993; Wikfors and Smolowitz 1995) and it has been suggested that *P. minimum* expresses toxin transiently (Wikfors 2005) with effects ranging from no symptoms to mortality and including tissue pathologies that are sometimes transient. The successful use of *P. micans* as a control in relatively short-term experiments such as those cited above does not therefore rule out a possible harmful effect. Neither would short term trials necessarily reveal sub-lethal effects that only result in symptoms over a long period.

P. micans, during some periods of the trial, provided almost the entire diet of the oysters in the Inland Sea. Rey et al. (2001) found *P. micans* to be nutritionally valuable for *Calanus helgolandicus*, with similar C and N levels to *Rhodomonas sp.* but there was low growth efficiency due to the indigestible cellulose theca.

Our histology samples did indicate that the digestive glands of *C. gigas* at IS were full of *P. micans*, or an organism of similar shape and size, and the digestive gland appeared to be red on most sampling occasions (Cotter, unpubl. data). This suggests that *C. gigas* were able to feed in the Inland Sea, in spite of its relatively low tidal flushing.

Before the establishment of the present farm a successful 100 t long-line farm operated in the Southern end of the Inland Sea that achieved good growth rates for this species (John Coppock, pers comm.). That site has now been partly covered by the construction of a new bridge. Further studies would therefore be required to ascertain whether the *P. micans* bloom was caused by changes in the Inland Sea due to this new construction, or whether it is limited to a small part of the Inland Sea where the current farm is situated.

Until further studies can be carried out on *P. micans* and possible sub-lethal effects on *C. gigas*, we would recommend that areas where *P. micans* occurs in any kind of numbers should be treated with caution. Models or protocols that assess the suitability of sites for oyster farming should not use chlorophyll *a* data alone to assess quality of food supply.

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