Subtle effect of the Water Accommodated Fraction of oil spills on natural phytoplankton assemblages enclosed in mesocosms

J. González[1*], E. Fernández[1], F.G. Figueiras[2], M. Varela[3]

[1]Departamento Ecoloxía e Bioloxía Animal, Universidade de Vigo, Campus Lagoas-Marcosende 36310 Vigo, Spain.

[2]CSIC, Insituto de Investigacións Mariñas, Eduardo Cabello 6, 36208 Vigo, Spain

[3]Instituto Español de Oceanografía, Paseo Marítimo Alcalde Francisco Vázquez, 10, 15001, A Coruña, Spain

*corresponding author: josegonzalez@uvigo.es

Tel.: +34 986 812641

Fax: +34 986 813792

Abstract.

Four mesocosm experiments were conducted to evaluate the effect of episodic oil spills on coastal marine phytoplankton assemblages. The experimental design was selected to simulate the *Prestige* oil spill, which occurred in Galician coastal waters (NW Iberia) in November 2002. The empirical results indicate that significant direct effects of the water soluble fraction of oil (20-60 µg l^{-1} of chrysene equivalents) on phytoplankton biomass and production were not observed immediately after oil additions. Nevertheless, subtle negative effects on primary production were detected using a modelling approach, being the impact lower in phytoplankton communities dominated by diatoms. Consistent with the reduced direct effect of oil additions on phytoplankton biomass and photosynthesis-related variables, indirect trophic cascading effects, previously reported in microcosm experiments, were not detected. These observations indicate that the effect of punctual inputs of the water accommodated fraction of oil on natural phytoplankton communities was very subtle, undetectable on some occasions, and of much lower magnitude than the effects recorded in microcosm experiments. Consequently, our results suggest that the assessment of the impact of oil spills on phytoplankton communities should not be a priority of the environmental monitoring efforts to be undertaken immediately after oil tanker accidents.

Keywords: Oil spills, Phytoplankton natural assemblages, primary production, mesocosms, Galicia, Prestige.

1. Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are common pollutants in marine ecosystems (NRC 2003). They enter the marine environment through diverse ways, such as spills from oil refineries or effluents from the terrestrial environment, among others. Nevertheless, spills due to tanker accidents are of relevance, because they cause an input of high amounts of oil over short periods of time. After an oil spill, crude oil acts as a reservoir releasing PAHs into the water column, remaining dissolved in seawater even after removing the insoluble fraction (González et al. 2006). Consequently, to characterize the response of planktonic marine primary producers to episodic inputs of PAHs into the sea is thought to be of ecological relevance.

The effect of oil on natural assemblages of phytoplankton remains still largely unknown and the published results seem to be somewhat contradictory, probably due to the different types of oil assessed and experimental approaches used. Investigations conducted over the last decades have focused on the effect of crude oil (Kusk 1978; Ostgaard et al. 1984a, 1984b) and diesel oil (Chan and Chiu 1985) on monospecific cultures, or have tested the effect of individual PAHs on monospecific cultures (Dunstan et al. 1975; Pérez et al. 2010a) and natural assemblages (Thomas et al. 1980; Kelly et al. 1999; Marwood et al. 1999; Hjorth et al. 2007). The effect of the water-accommodated fraction of oil on natural assemblages of phytoplankton has also been assessed (Kelly et al. 1999; Sargian et al. 2005; González et al. 2009; Pérez et al. 2010b), although most of this research was conducted with low water volumes (microcosms) and under laboratory conditions that tried to simulate the natural environment.

Some of these investigations reported negative effects of oil on phytoplankton biomass and photosynthesis related variables (Ostgaard et al. 1984a; Sargian et al.

2005), while others found a positive effect on phytoplankton biomass (Oviatt et al. 1982; Vargo et al. 1982). Differences in sensitivity to PAHs both among species and taxonomic groups have also been found. Thus, greater sensitivity of diatoms to oil additions were reported in some studies (Harrison et al. 1986; Siron et al. 1996; Peterson et al. 2003), whereas other experiments reported different patterns depending on the experimental approach adopted (Dunstan et al. 1975; Vargo et al. 1982; Kelly et al. 1999). Thus, for instance, the diatom *Skeletonema costatum* (Greville) Cleve, which showed a high sensitivity to oil in microcosm experiments, both in terms of biomass and photosynthesis related variables (Ostgaard et al. 1984b), was recognized as one of the most oil tolerant species in experiments conducted in mesocosms (Dunstan et al. 1975; Vargo et al. 1982).

In November 2002 the oil spill caused by the accident of the tanker 'Prestige' affected the NW coast of the Iberian Peninsula. On 13 November, the 'Prestige' started leaking oil 30 miles off the Galician coast (Álvarez-Salgado et al. 2006, González et al. 2006; Ruiz-Villarreal et al. 2006). Six days later, after an erratic course, the tanker broke into two and sank 150 miles offshore, releasing more than 60,000 metric tons of fuel into the water. The effect of this spill on natural phytoplankton assemblages has already been assessed using microcosms (González et al. 2009), revealing two different effects of oil on primary producers. First, it was observed a direct physiological effect that caused a decrease in photosynthetic efficiency following the addition of the water-accommodated fraction of oil. Then, an indirect effect causing changes in the trophic interactions within the microbial plankton community took place. This indirect trophic effect, attributed to a decrease in the abundance and/or activity of consumers, occurred after ~ 3 days of the oil addition and was reflected as increases in phytoplankton biomass and primary production that were not paralleled by enhanced photosynthetic

efficiency. In contrast, *in situ* research conducted in the area to evaluate the effect of this oil spill on phytoplankton, did not reveal significant changes in primary production rates and community composition (Varela et al. 2006). Nevertheless, the temporal scale of this type of *in situ* research, usually conducted several weeks or even months after the spill, does not allow detecting any possible effect of PAHs on phytoplankton, because the volatilization of these compounds occurs over hourly or daily time scales (Ostgaard et al. 1984a; Yamada et al. 2003).

In this investigation, we tried to partially overcome these limitations associated with the microcosm (use of low water volumes) and sea-truth approaches (long time elapsed between the spill and sampling) by conducting oil addition experiments using mesocosms. This experimental approach involves the use of large seawater volumes, thus partially simulating the physical conditions of the natural environment. We hypothesize that the contrasting responses of phytoplankton communities to oil spills previously reported in the literature, are related to the different initial phytoplankton assemblages enclosed and the different experimental approaches adopted. We tested the effect of the water-soluble fraction of oil on phytoplankton communities enclosed in mesocosms on four occasions throughout the seasonal cycle. The results obtained in this investigation contrasted with those previously obtained with microcosm experiments.

2. Material and methods.

2.1. Experimental setup.

With the aim of characterizing the responses of phytoplankton to oil inputs, a series of mesocosm experiments were conducted during the main characteristic periods of the seasonal cycle in the Galician Rías (NW Iberian Peninsula): spring phytoplankton

bloom (March 2005), stratification (June 2005), upwelling (September 2005) and winter mixing (January 2006). Experiments were carried out using a series of 4-6 UV-stabilized reinforced polyethylene bags (1.5 m in diameter and 2 m depth), filled with seawater at a central station in the Ría de Vigo (42º 14.9'N, 8º 47.18'W). The bags were filled from their bottom by inverse filtration through a 200 μm mesh to exclude mesozooplankton. Once filled, the bags were closed at the bottom and moved to a marina where they were attached to the dock. The mesocosms were kept half-submerged in seawater, so irradiance and temperature conditions experienced by the enclosed plankton communities were similar to those in their natural environment.

In each experiment, the bags were randomly positioned in the frame (Fig. 1) to ensure that the results were not affected by their location. Two (in spring and stratification periods) and three (in upwelling and winter experiments) of the mesocosms were kept as controls while the water-accommodated fraction of oil was added to the other bags. Oil addition was made to obtain initial concentrations ranging from 20 to 30 $\mu g\ l^{-1}$ of chrysene equivalents, except in summer, when a higher amount of oil was added to obtain a concentration of ~60 $\mu g\ l^{-1}$ chrysene eq. This decision was adopted as a response to the expected higher volatilization (Siron et al. 1996; Albaigés and Bayona 2003) and biodegradation (Yamada et al. 2003) of PAHs during the stratification period, due to higher seawater temperature.

Twelve hours after filling the bags an initial sampling was performed (day 0). Immediately after this sampling, the water-accommodated fraction of oil was added and samples were collected to determine the initial concentrations of PAHs. The accommodated fraction of oil was obtained by adding 15 kg of oil, similar to the one spilled by the "Prestige" (provided by the "Oficina Técnica de Coordinación del Programa de Intervención Científica en la Catástrofe del Prestige"), in 300 litres of

seawater prefiltered by 0.2 μm. The mixture was shaken during 4 hours to extract the soluble fraction of oil in seawater. The final concentration obtained by this method was ~700 μg PAHs l^{-1}. The soluble fraction was then separated from the insoluble fraction of oil and added to the mesocosms to obtain the desired initial concentrations.

Samples were taken 1, 2, 3, 4, 6 and 8 days after the first sampling. The mesocosms were sampled with 2 m long methacrylate tubes used as pipettes to obtain homogeneous samples, integrate the vertical variability of the water column and avoid resuspension of sedimented matter during sampling.

2.2. PAHs.

PAHs concentrations in the mesocosms were determined as described in the MARPOLMON protocol (UNESCO 1984) and referred to a chrysene standard. Measurements of chrysene standards against "Prestige" oil extracted according to this protocol yield a factor of 5.5 ± 0.4 μg "Prestige" oil μg $chrysene^{-1}$. Naphtalene and alkylated derivates (methylnaphtalenes, dimethylnaphtalenes and trimethylnaphtalenes) dominated (89% of the total PAHs concentration) in the water-accommodated fraction of the "Prestige" fuel (González et al. 2006). Therefore, our experiments basically assessed the effects induced by these compounds.

PAHs concentrations tested in these experiments (19-58 μg l^{-1} chrysene eq.) simulate those which probably occurred in the sea just after the "Prestige" oil spill (González et al. 2009), because studies conducted in the region 20 days after the disaster (González et al. 2006) reported lower values (0.09-4.84 μg l^{-1} chrysene eq.), which were in the range of PAHs concentrations observed after similar episodes (Table 1).

2.3. Nutrients.

Samples to determine dissolved inorganic nutrients concentrations (ammonium, nitrate, phosphate and silicate) were prefiltered through 0.45 µm polycarbonate filters and kept frozen until analysis by the standard segmented flow technique using spectrophotometric methods (Álvarez-Salgado 1993).

2.4. Chlorophyll *a*.

Water samples of 250 ml were taken from each bag and filtered sequentially through 20 and 0.2 µm pore size polycarbonate filters, which were kept frozen (-20 ºC) until pigment extraction in 90% acetone over 24 h in the dark at 4 ºC. A Turner TD-700 fluorometer calibrated with pure chlorophyll *a* (Sigma) was used to determine chlorophyll *a* concentrations.

2.5. Phytoplankton composition.

The study of phytoplankton taxonomic composition was determined using three different methods depending on cell size.

Organisms with a cell size >20 µm were determined in samples of 100 ml preserved in Lugol's iodine solution (2% final concentration). Samples were allowed to settle in composite sedimentation chambers and diatoms and pigmented dinoflagellates were counted and identified to the species level when possible with an inverted microscope following the Utermöhl's technique. Two transects were scanned at x400 and x250 to enumerate small-sized species, whereas the whole slide of the chamber was scanned at x100 magnification to count larger, often less abundant, species. Pigmented dinoflagellates were differentiated following Lessard and Swift (1986) and using epifluorescence microscopy. Biovolumes calculated according to Hillebrand et al.

(1999) were used to estimate cell carbon biomass of diatoms and dinoflagellates following Strathmann (1967).

Nanophytoplankton (2-20 μm) was determined by epifluorescence microscopy in 10 ml samples fixed with buffered 0.2 μm filtered formaldehyde 2% final concentration. Then, samples were filtered through 0.2 μm black Millipore-Isopore filters, which were immersed in low fluorescence immersion oil and examined at x1000. Pigmented organisms were enumerated under blue light excitation. Dimensions were measured to calculate cell biovolumes assuming a spherical shape. Biovolumes were converted to cell carbon following Verity et al. (1992).

Samples of 1.8 ml were taken to determine picophytoplankton, particularly *Synechococcus* and picoeukaryotes, since *Prochlorococcus* is present in very low abundance in these coastal waters (Rodríguez et al. 2003). Samples preserved in 1% paraformaldehyde + 0.05% glutaraldehyde were frozen at –80ºC before analysis with a FACSCalibur flow cytometer, as described in Calvo-Díaz and Morán (2006). Carbon biomass was estimated assuming a spherical shape and using the volume-to-carbon factors of 230 fg C μm^{-3} for *Synechococcus* and 237 fg C μm^{-3} for picoeukaryotes (Worden et al. 2004).

2.6. Primary production.

Primary production rates were measured by the radiocarbon method. Three 100 ml light and one dark acid-cleaned pyrex bottles were filled with seawater from each bag. Each bottle was inoculated with 370 kBq (10 μCi) $NaH^{14}CO_3$ and incubated for 2-3 hours simulating natural conditions. To this aim, incubators were refrigerated with running seawater from the laboratory's continuous supply under natural irradiance conditions. Samples were then sequentially filtered through 20 and 0.2 μm pore size

polycarbonate filters, which were exposed to concentrated HCl fumes for 24 h to eliminate unincorporated ^{14}C. Radioactivity of the samples was measured with a liquid scintillation counter using the external standard and the channel ratio methods for quenching correction.

2.7. Photosynthetic efficiency.

The photosynthetic efficiency of Photosystem II, expressed as Fv/Fm, was measured using a Fast Repetition Rate Fluorometer (FRRF), as described in Pérez et al. (2006). Three 40 ml water samples were taken from each mesocosm and kept in darkness for 30 minutes before measurements to allow relaxation of non-photochemical quenching. Blank values were obtained by measuring Fv/Fm on water samples collected from the mesocosms previously filtered through a GF/F glass fiber filter.

2.8. Primary production model.

Primary production was modeled according to Anderson and Williams (1998). The model is based on the equations of Fasham et al. (1990) with primary production rates (dP/dt) estimated as: $dP/dt = (1-\gamma_1)\, J\, Q\, \mu_{pmax}\, P$.

In this equation, the term γ_1 corresponds to the fraction of produced organic matter that is excreted during photosynthesis (dissolved organic matter production), so the expression $(1-\gamma_1)$, represents the production of particulate organic matter incorporated into phytoplankton biomass. The term Q represents nutrient limitation (Anderson and Williams 1998), while J describes light limitation (Evans and Parslow 1985), both terms ranging from 0 to 1. The phytoplankton maximum growth rate, i.e. phytoplankton growth rate under not-limited conditions of irradiance (J = 1) and nutrients (Q = 1), is denoted by μ_{Pmax} and P represents phytoplankton biomass. Two

different values of μ_{Pmax} were used along each experiment, due to the changes recorded in the composition of the phytoplankton community (see results). As the model runs in nitrogen units, phytoplankton biomass (P) and primary production (dP/dt) were transformed to nitrogen using the Redfield's ratio.

Model outcomes were compared to empirical values by means of linear regression. A t-test (two tailed, alpha = 0.05) was used to determine if the slopes differed significantly from unity.

3. RESULTS

3.1. PAHs concentrations.

Average initial PAHs concentration tested in all the experiments was 19.04 ± 3.40 $\mu g\ l^{-1}$ chrysene equivalents (Fig. 2), except for the summer experiment, when it was approximately three times higher (58.02 ± 8.53 $\mu g\ l^{-1}$ chrysene eq.). PAHs concentrations decreased exponentially at a rate of approximately -0.35 d^{-1} in all the experiments except during summer stratification, when this rate doubled (-0.7 d^{-1}). After the first day, PAHs concentrations in all the experiments, even in summer, ranged between 5 and 12 $\mu g\ l^{-1}$ of chrysene eq.. PAHs concentrations were also measured in the control bags to discard any contamination in the water collected, obtaining values lower than 0.5 $\mu g\ l^{-1}$ of chrysene eq. in all samples.

3.2. Nutrients.

Differences in nutrient concentrations between control and oiled bags were not statistically significant (ANOVA, $p > 0.05$) at any time.

In spring, the concentration of dissolved inorganic nitrogen and phosphorous decreased throughout the experiment. This decrease was faster during the first two days. Dissolved silicate concentration also decreased at the beginning of the experiment, but recovered after Julian Day (JD) 64 (Fig. 3).

In the stratification period, nutrient concentrations were lower than in spring. Values remained rather constant, although a slight increase was observed in the last two-three days, probably attributable to accidental water inputs into the bags.

The temporal distribution of nutrient concentration observed in the upwelling experiment was similar to that described for Spring. Levels of dissolved inorganic nitrogen and phosphorous showed a decreasing trend, especially at the beginning of the experiment. Silicate concentration also decreased during the first 48 hours, increasing thereafter until the end of the experiment.

The highest dissolved nitrogen, phosphorous and silicate concentrations were measured in Winter, showing very low variability throughout the experiment.

3.3. Chlorophyll *a*.

In general, no significant differences in chlorophyll *a* concentrations were found between treatments and controls (Fig. 4). Although punctual differences with respect to the control bags were observed in some of the experiments, a clear response pattern of chlorophyll *a* to oil input was not detected. The higher chlorophyll *a* concentrations recorded in oiled bags on days 187 and 189 during the stratification experiment were probably due to the accidental water input commented before. Chlorophyll *a* concentration showed a similar pattern in the two size fractions tested (0.2-20 μm and >20 μm) (data not shown).

3.4. Phytoplankton community.

Differences in community composition among control and oiled bags were not found. Nevertheless, the initial phytoplankton community composition and its evolution differed among experiments (Fig. 5). The spring experiment was conducted during a phytoplankton bloom dominated by diatoms. In this upwelling region, stratification periods typically succeed bloom periods during summer. Therefore, the phytoplankton community enclosed in the stratification period was dominated by senescent diatoms, probably due to nutrient depletion (Fig. 3). The phytoplankton community enclosed during the autumn experiment was characteristic of an upwelling event when diatoms dominated. Finally, the winter community corresponded to that typical of a mixing period, with flagellates forming the bulk of the phytoplankton biomass.

The picophytoplankton contribution to total phytoplankton biomass was low (~ 5 %) in all the experiments except in winter, when this size fraction represented up to a 30 % of total phytoplankton biomass. Picoeukaryotes were always more important than the cyanobacteria *Synechococcus*, which accounted for <10% of the total picophytoplankton biomass.

Nanophytoplankton biomass did not exceed 10 % of total phytoplankton biomass in spring and summer experiments, but accounted for 20 % of total phytoplankton biomass during the final phase of the upwelling experiment. Nanophytoplankton attained higher importance in winter, when their contribution to total phytoplankton biomass was 40 %.

In spring, the phytoplankton community was largely dominated by diatoms, mainly *Chaetoceros socialis* Lauder and *Lauderia annulata* Cleve, with the two species showing a different temporal evolution. Phytoplankton biomass increased during the two initial days at a rate of 9.50 ± 3.23 mmol C m^{-3} d^{-1} (r^2 = 0.90), due to the active

growth of these two species. However, between days 63 and 64 (JD), a decrease in biomass was observed, after which values remained rather constant until the end of the experiment. This pattern was due to a decrease in the abundance of *L. annulata* from day 63 onwards, whereas *C. socialis* kept its abundance constant along the rest of the experiment. The decrease in the abundance of *L. annulata* was due to sinking, which probably relates to the increase in silica concentration measured in the mesocosms (Fig. 3) as a result of triggered silica redissolution. The biomass of dinoflagellates, was low, but showed a progressive increase from the beginning of the experiment.

During summer stratification, the phytoplankton community was also dominated by diatoms, with *Leptocylindrus danicus* Cleve being more abundant during the initial days and *C. socialis* achieving a higher contribution to phytoplankton biomass at the end of the experiment. Phytoplankton biomass increased during the two initial days at a rate of 4.59 ± 2.18 mmol C m^{-3} d^{-1} ($r^2 = 0.82$). Although a decrease in biomass was observed during the next day (186), total biomass increased afterwards (9.47 ± 0.33 mmol C m^{-3} d^{-1}; $r^2 = 0.99$) coinciding with the change in community composition.

A diatom bloom occurred at the beginning of the upwelling experiment when phytoplankton biomass increased 15 mmol C m^{-3} during the first day. However, the bloom quickly collapsed showing a continuous decrease in biomass until the end of the experiment. *Chaetoceros debilis* Cleve, *Chaetoceros affinis* Lauder and *Leptocylindrus minimus* Gran dominated the phytoplankton outburst, all of them following a similar temporal trend. The decrease in diatom biomass marked the beginning of the recovery in silicate concentration (Fig. 3). Although diatoms accounted for more than 75 % of the total phytoplankton biomass during the three initial days, dinoflagellates progressively acquired a higher contribution in terms of biomass until they accounted for more than 60 % of the total phytoplankton biomass at the end of the experiment.

Diatoms were virtually absent in the winter experiment, when small flagellates, small naked dinoflagellates and picopytoplankton dominated the phytoplankton community. Few changes occurred during this experiment, although biomass showed a slight increase (0.79 ± 0.12 mmol C m^{-3} d^{-1}; $r^2 = 0.96$) during the three initial days. Then, values remained fairly constant until the end of the experiment.

3.5. Primary production and photosynthetic efficiency of PSII.

Primary production rates in the oiled treatments, although differed in magnitude among experiments, showed a similar pattern as compared to primary production values measured in the controls in all the studied periods (Fig. 6). Except in the experiment conducted in spring, when primary production was significantly lower at oiled bags than in the control ($p < 0.01$) on the second day, no significant differences were observed during the three initial days. After that, enhanced phytoplankton production rates with respect to the control were not observed, except at some particular days in the experiments conducted during stratification and winter periods. Differences in the summer experiment might be related to an accidental water input, as explained above. The temporal evolution of primary production rates in the two size fractions tested (0.2-20 μm and >20 μm) was similar in each experiment (data not shown).

The photosynthetic efficiency of PSII, expressed as the ratio Fv/Fm, did not show a clear response to oil addition in any of the experiments. In all the experiments the measured values of this ratio ranged between 0.35 and 0.45 and were quite constant along each period, with no statistically significant differences among the control and the oiled bags (data not shown).

3.6. Primary production model.

Due to the absence of a clear response of phytoplankton to the water soluble fraction of oil in the empirical measurements, a mathematical model was used to reproduce primary production rates from measured variables and thus, to determine the existence of possible subtle, not detectable, effects due to the sensitivity of the empirical approach.

With the aim of reproducing the temporal evolution of primary production in the control bags during each experiment, the model was tuned by varying their key parameters. Measured primary production rates were not adequately reproduced by manipulating the parameters defining nutrient uptake and light absorption in the model equations (Q and J). By contrast, reasonably accurate estimates of these rates were obtained when varying μ_{Pmax}. As μ_{Pmax} largely depends on the species composition of the phytoplankton community, the tuning procedure was made by assigning different μ_{Pmax} at each of the two phases detected in the phytoplankton composition during each experiment (Fig. 5, see also the results describing the temporal evolution of the phytoplankton community). These changes in community composition were especially evident during the two bloom experiments carried out in spring and upwelling periods, and were less marked, although still detectable, in the stratification and winter experiments.

The good agreement found between measured and estimated primary production rates at the control bags (Fig. 7), allows us to infer μ_{Pmax} values in the control treatments from this modelling exercise, both for the initial and the decay phases. Thus, in the initial phase of the spring experiment (days 61-64 in Fig. 5), μ_{Pmax} (2.9 d^{-1}) was higher than during the decay phase (1.6 d^{-1}). The stratification experiment (Fig. 5) also showed two well-differentiated phases but with higher μ_{Pmax} in the second (2.8 d^{-1}) that during

the initial phase (1.9 d^{-1}). In the upwelling period two well defined phases in growth rates were again identified (Fig. 5), with higher values during the initial phase (3.2 d^{-1}) than in the post bloom phase (1.4 d^{-1}). In the winter experiment (Fig. 5) the initial μ_{Pmax} (0.9 d^{-1}) remained relatively constant during most of the experiment (from day 26 to day 32). However, μ_{Pmax} increased to 3.0 d^{-1} from day 32 onwards.

When μ_{Pmax} values estimated for the control bags were used to model the behaviour of the oiled bags, primary production rates were overestimated in three out of the four experiments (Fig. 8). The exception to this pattern was found in the case of the upwelling experiment, in which primary production in the oiled mesocosms could be reliably estimated using the μ_{Pmax} values estimated for the corresponding control bags.

We then introduced a toxicity factor (T) into the model equation, $dP/dt = (1-\gamma_1)\ J\ Q\ \mu_{pmax}\ P\ T$. This toxicity factor refers to processes of unknown nature but probably not related to photochemical processes, as suggested by the lack of effect of oil additions on variable fluorescence (Fv/Fm). The value of T (1 in the control bags) was tuned in the treated bags to reproduce primary production rates measured in the oiled bags.

Once the primary production rates measured in oiled bags were reasonably well reproduced by the model (Fig. 8), the value of T could be used to infer the impact of PAHs on primary production. Thus, primary production in the oiled mesocosms of the spring experiment showed a 16% decrease during the initial phase of the bloom. The impact was slightly lower (13%) during the decay phase. There was no toxicity effect of PAHs during the initial phase of the summer experiment, which was, however, significant in the final phase, causing a 20% decrease in primary production. Toxicity effects were not detected in the upwelling experiment. Finally, in winter, as in summer, negative impacts were not observed during the initial phase of the experiment, although the final phase showed the highest impact (57%). Nonetheless, this high impact of oil in

winter should be taken with caution because of the weak relationship ($r^2 = 0.122$, Fig. 8) between estimated and measured primary production.

4. Discussion.

The experimental results obtained in this mesocosm study showed that phytoplankton was not significantly affected by PAHs, thus clearly in contrast previous studies using a microcosm approach (González et al. 2009). Oil addition to mesocosms did not cause immediate and persistent negative effects on photosynthetic efficiency or primary production, like those occurring in microcosms, since a significant decrease in primary production was only observed on the third day of the spring experiment (Fig. 6). Moreover, the increase in phytoplankton biomass and primary production recorded in microcosm experiments after the first 3 days, which was attributed to indirect trophic effects, was not so evident in mesocosm experiments. In the particular case of the stratification experiment (Fig. 4), where these increases were observed, their magnitude was not as high as in the microcosm experiments. Consequently, comparison of the results emerging from the two experimental approaches allows us to conclude that, although the toxic effects of oil spills on phytoplankton appear to be detectable using either a micro or mesoscosms experimental approach, the magnitude of effects measured in mesocosms were considerably more subtle than those recorded in microcosms. The reason for these different responses of phytoplankton to oil additions was likely related to the contrasting evolution of PAHs concentrations in the two experimental approaches. PAHs concentrations in the oiled mesocosms decayed throughout the experiments at much higher rates (-0.7 d^{-1} in summer and -0.35 d^{-1} in the other three experiments) than in microcosms (-0.11 d^{-1}) (González et al. 2009),

indicating that the higher air-sea interface of the mesocosms imposed a major volatilization of the water accommodated fraction (Dunstan et al. 1975; Ostgaard et al. 1984a; Yamada et al. 2003). Thus, one day after oil addition, PAHs concentration in the mesocosms was lower than the values registered in microcosms after five days. High volatilization rates should also occur at sea, as deduced from sea-truth PAHs concentrations 20 days after the Prestige oil spill (González et al. 2006), which were five times lower than the concentrations initially added to the mesocosms.

However, the results obtained in this investigation differed from those obtained in other mesocosm studies. In this study, we tried to simulate typical PAHs concentrations expected at sea just after the Prestige disaster. However, our initial PAHs concentrations were considerably lower than those usually tested in this type of experiments. Typically, the initial concentrations used were higher (Siron et al. 1996; Nayar et al. 2005), or oil is repeatedly added to maintain a high concentration throughout the experiment (e.g. Vargo et al. 1982). On other occasions, an oil slick was left at the surface to act as PAHs reservoir (Ostgaard et al. 1984a). This difference in the quantity and mode of oil addition might explain why the results obtained in the above mentioned mesocosm experiments were similar to the results observed in our previous microcosm experiments (González et al. 2009). Nevertheless, the use of a constant hydrocarbon concentration (Vargo et al. 1982; Siron et al. 1996) would prevent understanding the real effect of episodic spills on marine phytoplankton, as would be the case for an oil spill derived from a tanker accident.

Although persistent and statistically significant effects of oil were not detected in the experimentally determined variables, the modelling approach used here allows us to find out negative effects of PAHs on primary production in three of the experiments conducted in this study. The reason for this apparent mismatch between empirical and

modeled results plausibly stem from the small magnitude of the toxic effect which probably lies within or close to the experimental error associated to the determination of primary production rates. In this regard, it is worth to mention that the corresponding variation coefficients of primary production rates empirically determined in this study (11.2 %, 16.17 % and 15.49 % for the experiments of spring, stratification and winter, respectively) were close to the estimates of the magnitude of the effects on primary production rates inferred from the value of the T factor of the model. Nevertheless, in spite of the small magnitude of the detected effect, its trend was consistent in the three experiments, being always negative.

The observed non-existing (upwelling) or low (spring) impact of oil additions on phytoplankton communities dominated by diatoms and the higher impact on nano- and picoplankton (winter) agree with the results obtained in microcosm experiments (González et al. 2009), which revealed a higher impact on phytoplankton communities dominated by smaller cells. In addition, these results indicate that although a direct effect of the water accommodated fraction of oil on the natural phytoplankton community existed, this was extremely subtle, and of a much lower magnitude that the effects registered under laboratory conditions of microcosms experiments.

Consequent with the reduced direct effect of oil additions on phytoplankton production, the indirect trophic cascading effects previously observed in microcosms should not be expected to occur in mesocosms. The significant higher chlorophyll *a* concentrations detected on some occasions in the oiled bags during this study (Fig. 4) were of low magnitude, and were not paralleled by clear effects of oil on the rest of variables measured. This allows us to infer that indirect phytoplankton recovery processes associated with trophic interactions within the microbial plankton community, if existing at sea, are probably of reduced magnitude and, therefore, of relatively low

ecological significance. Consequently, our results suggest that the assessment of the impact of oil spills on phytoplankton communities should not be a priority of the environmental monitoring efforts to be undertaken immediately after oil tanker accidents.

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Figure Legends:

Figure 1. Distribution of the mesocosms along the external dock of a marina in the Ría de Vigo during one of the experiments. The distribution of the bags along the dock was randomly established for each experiment.

Figure 2. Temporal evolution of PAHs concentration (expressed in $\mu g\ l^{-1}$ of chrysene equivalents) in the four experiments conducted along the seasonal cycle. Error bars represent the standard error of the measurements.

Figure 3. Temporal evolution in the control bags of nutrient concentrations ± standard error in the four experiments conducted. Not statistically significant differences were found among control and oiled mesocosmos (ANOVA, $p > 0.05$) Ammonium and nitrate concentrations are represented on the left panel. Phosphate and silicate concentrations are on the right panel. All concentrations are in mmol m^{-3}.

Figure 4. Evolution of total chlorophyll *a* concentration in the four experiments conducted ± standard error. Asterisks show statistically significant differences (ANOVA) with respect to the control bags (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

Figure 5. Temporal evolution in the control bags of the biomass (mmol C m^{-3}) of diatoms, pigmented dinoflagellates, pigmented nanoflagellates and picophytoplankton in the four experiments conducted. Pigmented picoplagellates and *Synechococcus* are included in picophytoplankton. Oiled mesocosmos did not show statistically significant differences respect to the control (ANOVA, $p > 0.05$). Standard error is represented by error bars.

Figure 6. Temporal evolution of primary production rates (mg C $m^{-3}\ h^{-1}$) ± standard error in the four experiments conducted. Asterisks show statistically significant

differences (ANOVA) respect to the control bags (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

Figure 7. Relationships between empirically determined (X axis) and modeled (Y axis) primary production rates in the control bags. Filled circles correspond to initial phases and open circles to the final phases of the experiments. See text for additional details.

Figure 8. Relationships between empirically determined (X axis) and modeled (Y axis) primary production rates in the oiled bags. Modeled primary production (white triangles) overestimated ($P < 0.001$, dotted lines and equations on the top left in the panels) measured primary production in three experiments (spring, summer and winter). Solid line and equations on the bottom right correspond to the relationships between measured and modeled primary production rates (black squares) after a toxicity factor (T) due to PAHs was included in the model. See text for additional details.

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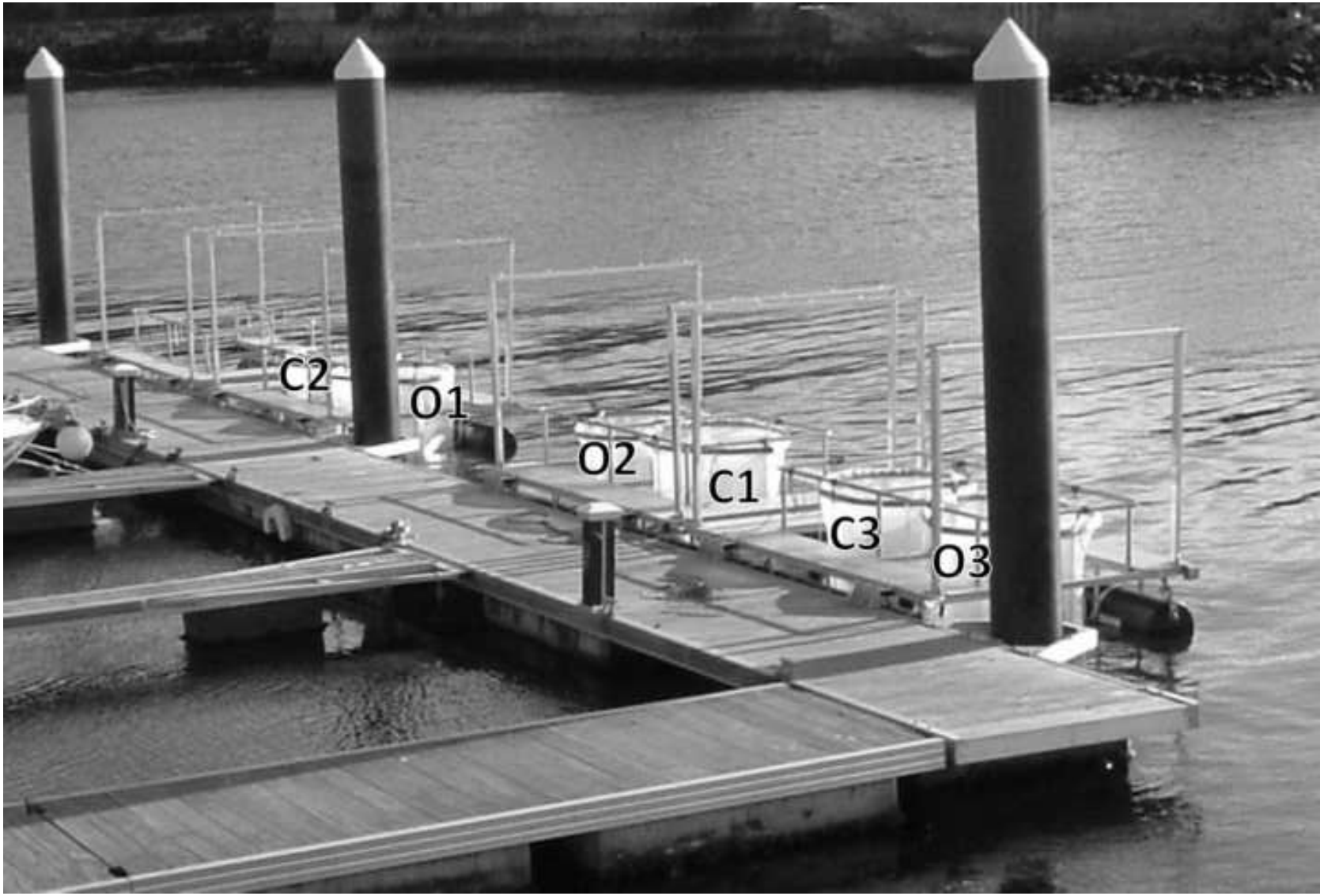

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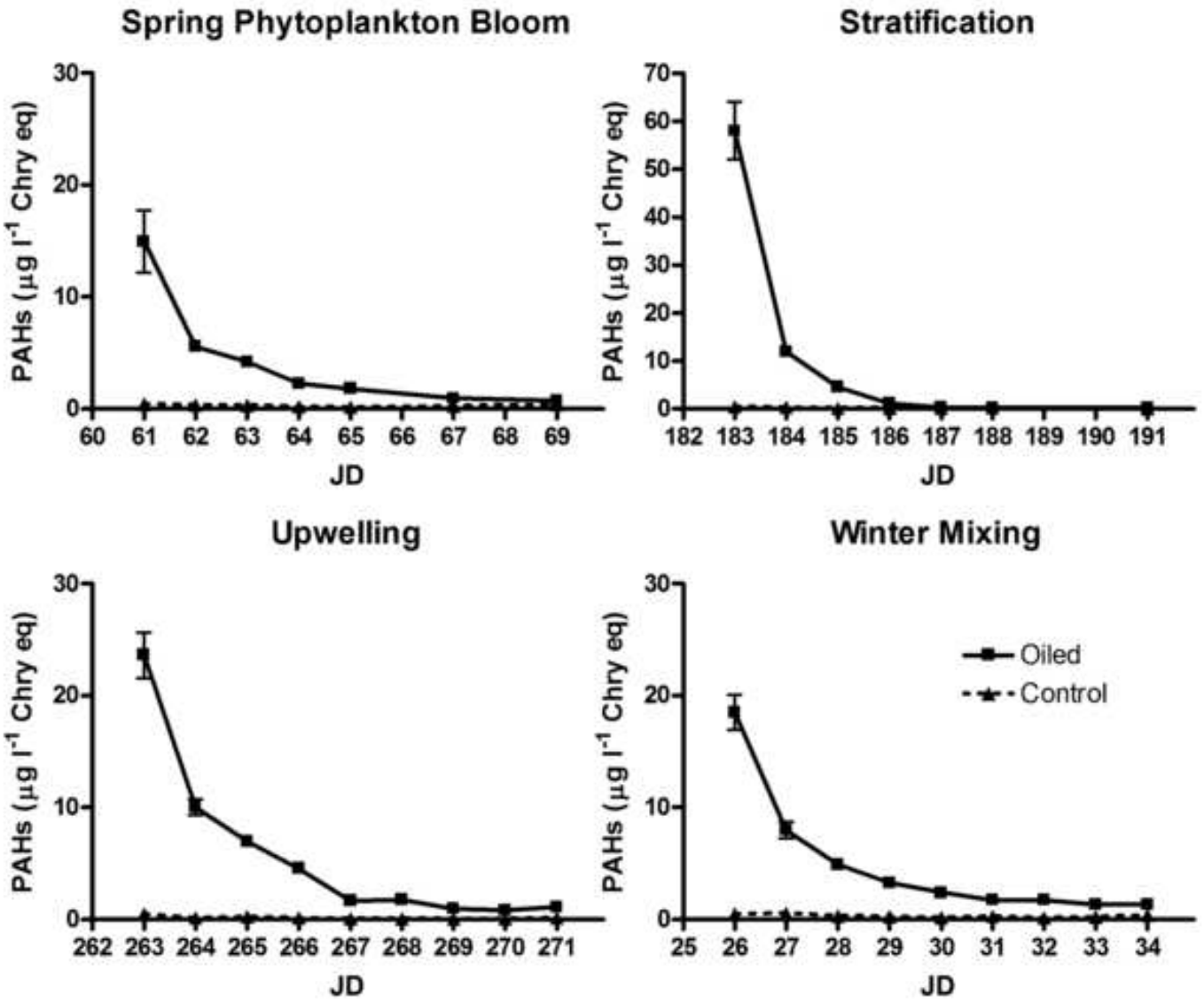

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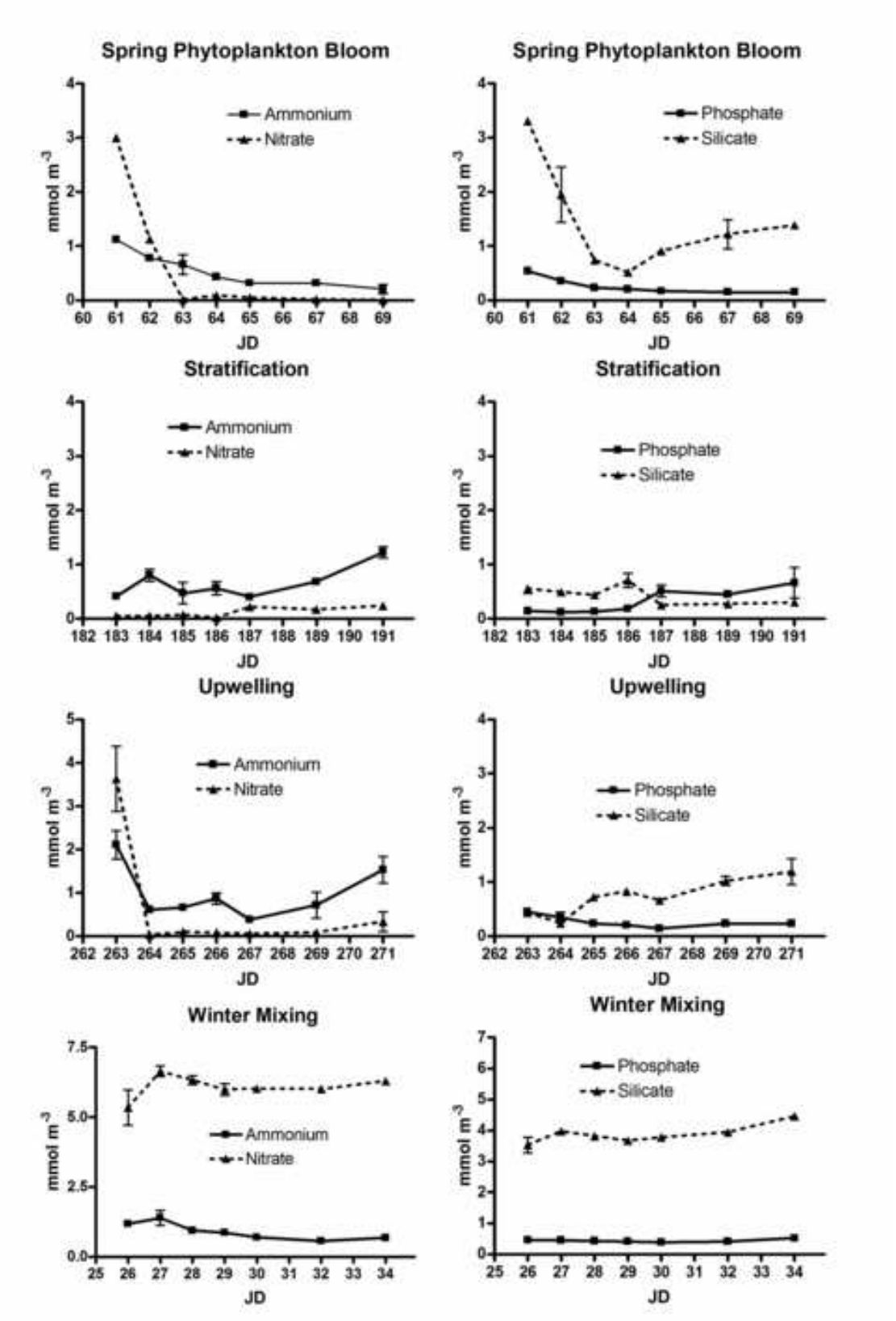

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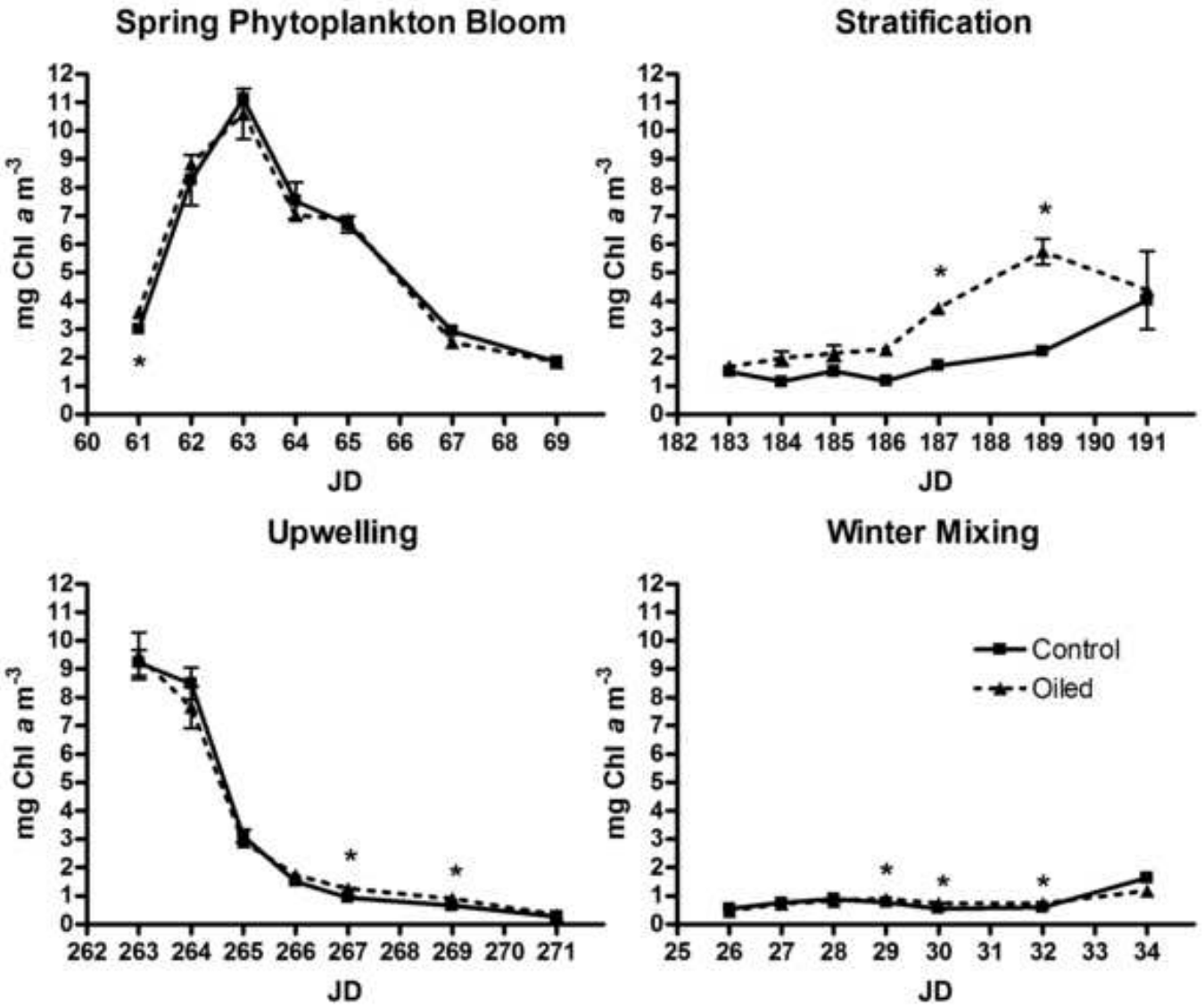

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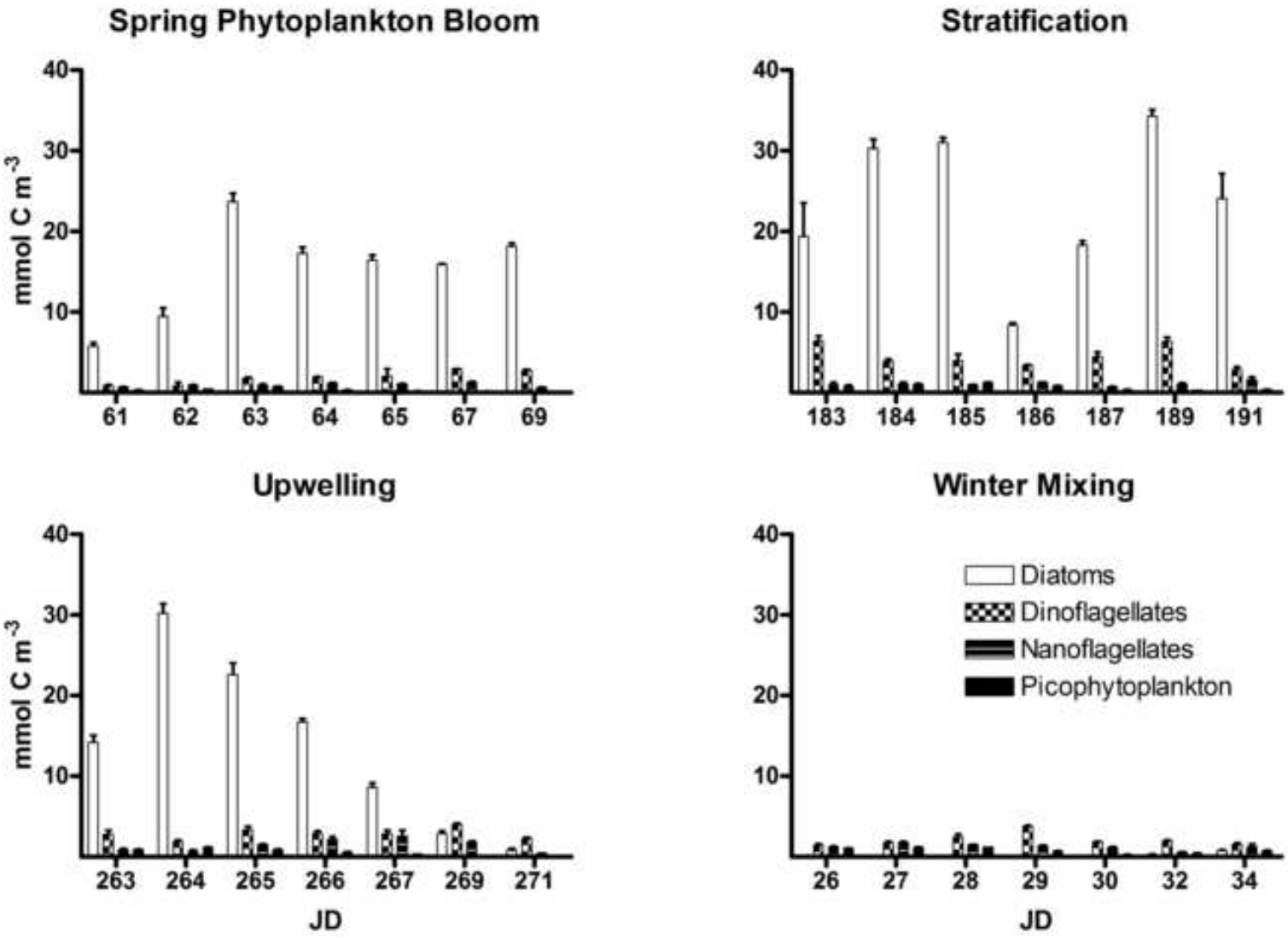

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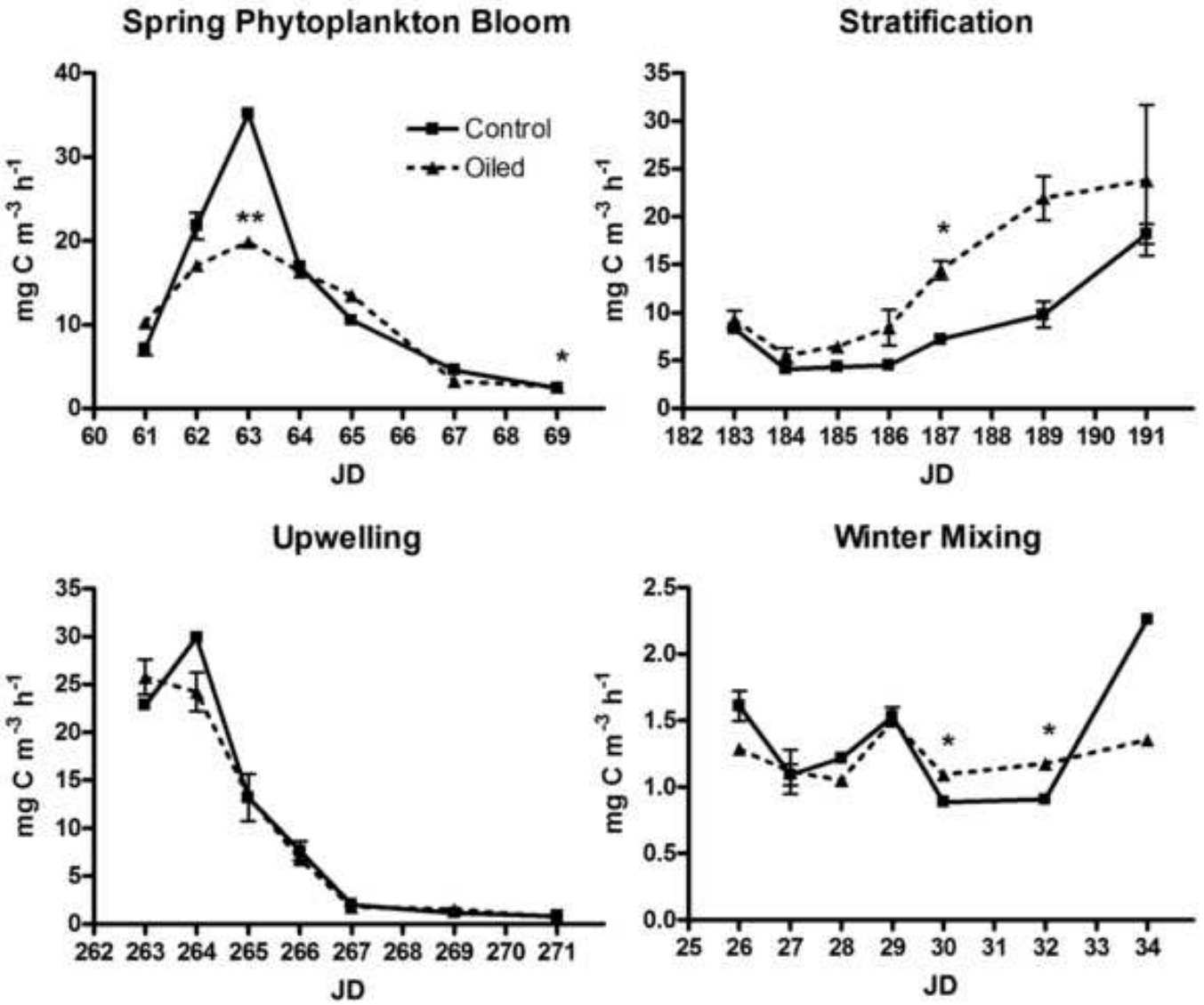

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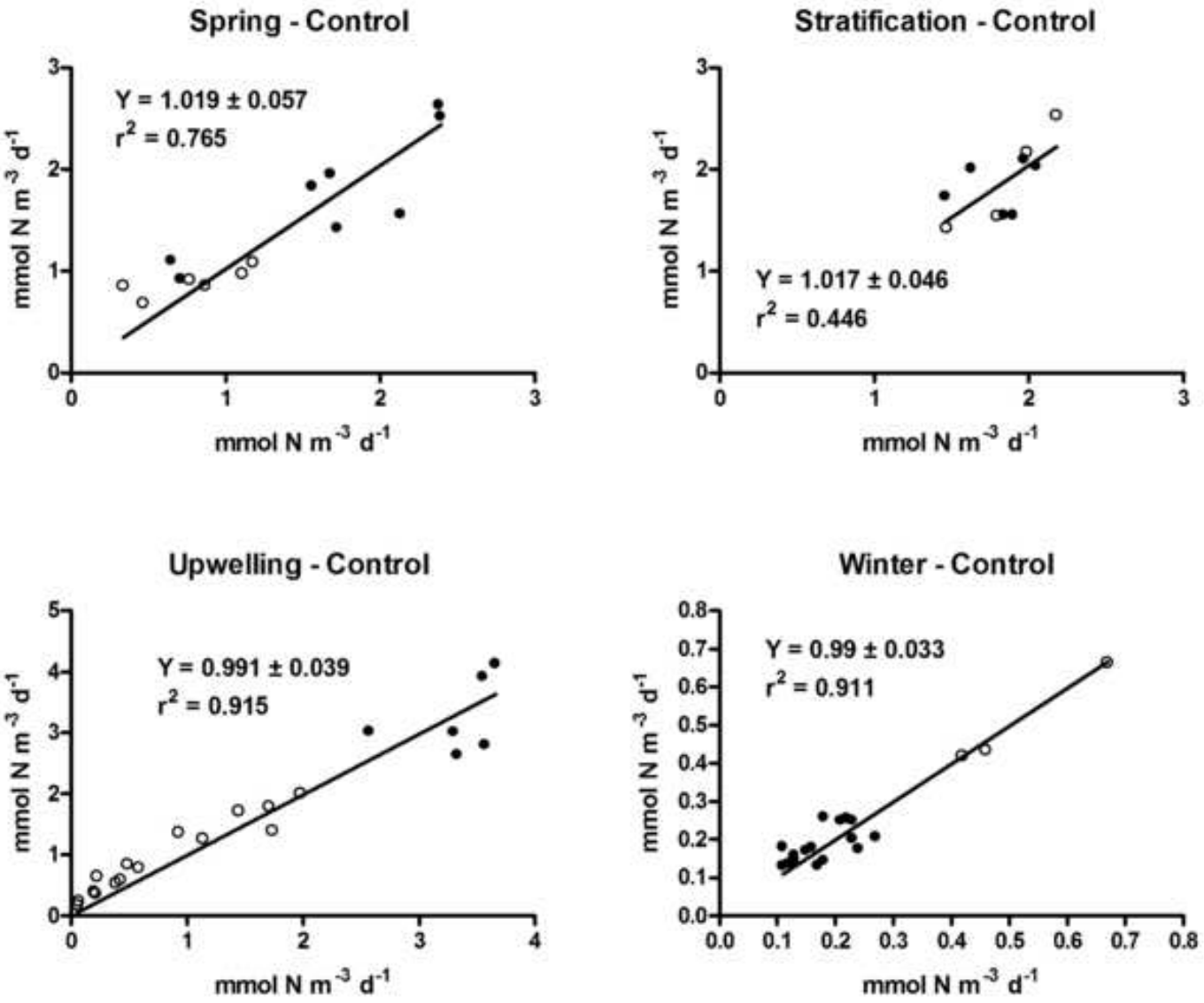

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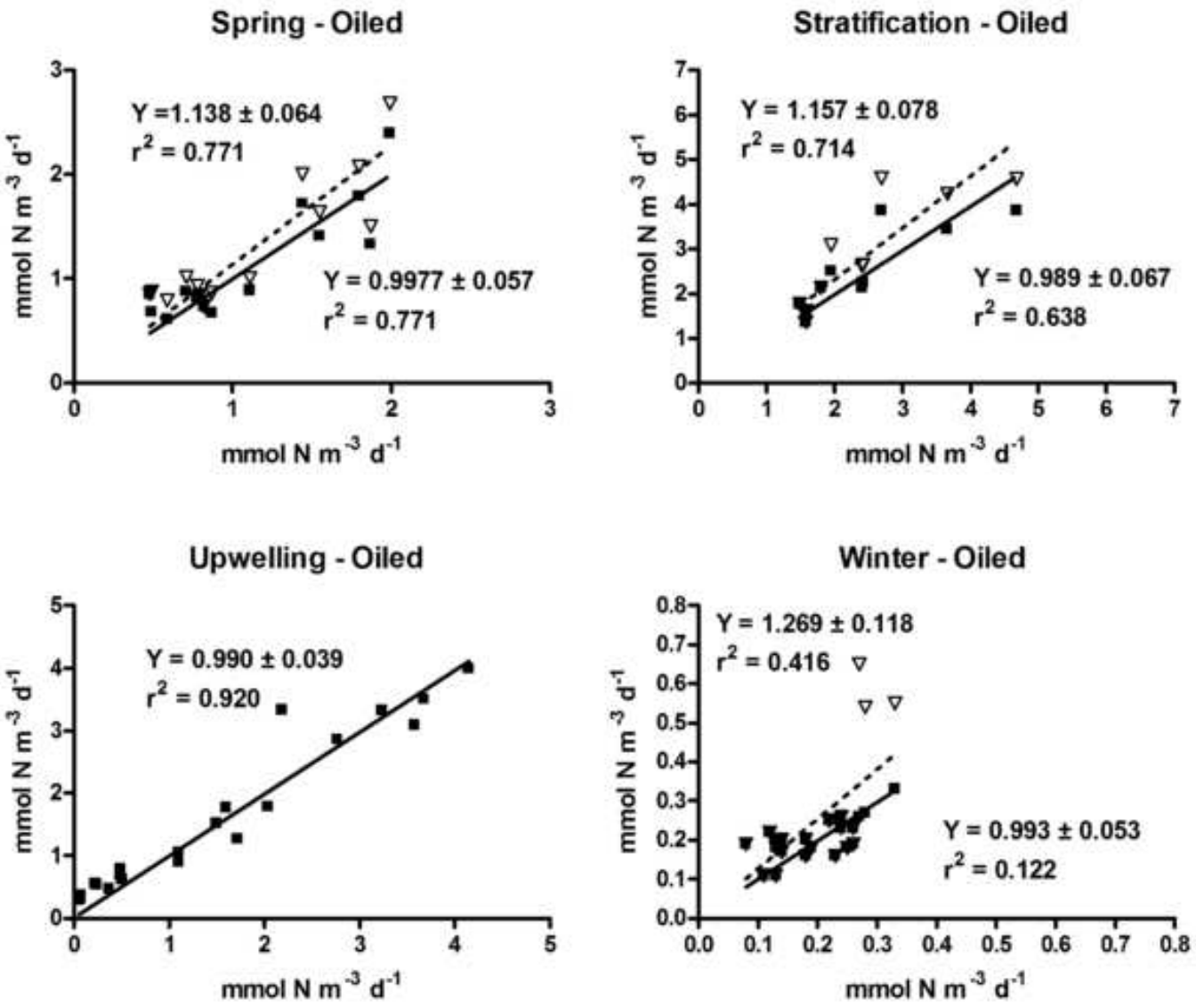

Table 1

PAHs concentrations measured in coastal waters after some of the most important oil spills investigated over the past two decades.

Oil Spill	PAHs Concentration	References
"Prestige", 2002	0.1-4.8 µg/L, after two weeks	González et al. (2006)
"Erika", 1999	23.5- 54.9 ng/L, after three months	Tronczynski et al. (2004)
"North Cape", 1996	13.7-49.7 µg/L, after four days 0.2-115 µg/L, after one week	Redy & Quinn (2001)
"Exxon Valdez", 1989	10-29.3 µg/L	Neff & Stubblefield (1995)
"Bahia Paraíso", 1989	50-100 µg/L	Kennicutt et al. (1991)