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Plankton response to nutrient enrichment is maximized at intermediate distances from fish farms

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ABSTRACT: Plankton community composition changes in response to nutrient enrichment were examined at 2 sites in the eastern Mediterranean. Samples from bacteria up to mesozooplankton were taken upstream and downstream of finfish farms in the north Aegean (Lesvos, Greece) and on the southern coast of Cyprus. The pattern of change appeared to be similar, albeit of different magnitude, in both areas. In Cyprus, results showed an increase in bacteria and a decrease in diatom abundance close to the cages, response to effluents was limited, and consequent growth was controlled by microzooplankton. In Lesvos, results showed increased abundance of bacteria, nanoflagellates and mesozooplankton, a decrease in diatoms and an increase in larger dinoflagellates and ciliates downstream of the farm. A shift towards a more diverse microplankton community was observed, consistent with the intermediate disturbance hypothesis. The community consisted of larger-sized microzooplankton, and changes persisted throughout the downstream stations. Results showed that the plankton response to nutrient enrichment is more pronounced at intermediate distances from the farm. The community composition changes observed in Lesvos indicate that even in exposed sites with high current velocities there is an influence of farm cages. Changes manifest in terms of size for some groups and abundance for others. Although fish farms have been associated with degradation of the environment in which they are located, this was not apparent in the present study. Indeed, the shifts induced by farm effluents can help us identify drivers of change and assess community responses to perturbations in a dynamic environment.

KEY WORDS: Fish farm · Impact · Oligotrophic conditions · Picoplankton · Microplankton · Mesozooplankton · Intermediate disturbance hypothesis · IDH · Mediterranean Sea

INTRODUCTION

The plankton community structure of the eastern Mediterranean plays an integral part in forming the unique characteristics of this sea. Azov (1991) characterized the area as a marine desert owing to low surface chlorophyll concentrations, which were mostly associated with picophytoplankton. An acute phosphate limitation which follows an increasing gradient moving eastwards is also of note and was described extensively by Krom et al. (2010) as oligotrophic and dominated, as far as phytoplankton populations go, by small-sized species. In fact, the Levantine Basin is considered ultra-oligotrophic (Bar-Zeev et al. 2011). This low biomass by no means limits diversity, which is thought to be high compared to the sea's surface and volume (Bianchi & Morri 2000). The low primary productivity of the eastern part co-occurs with a shift towards a community where most nutrients are recycled within the microbial loop (Turley 1999), which is also thought to account for the low fisheries production of the area. Even though overall productivity remains low, areas of higher productivity are spread throughout the region and include locations where

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river inputs (e.g. Northern Adriatic, Turley 1999) and flows from other water bodies (Northern Aegean, Siokou-Frangou et al. 2002) provide nutrients to the starved system. These areas are potentially more susceptible to effects of eutrophication, and the microbial loop is often replaced with a more linear food chain (phytoplankton–zooplankton–fish (Siokou-Frangou et al. 2010).

An additional localized potential source of nutrients in the area is the operation of fin-fish farms. This sector has thrived in the past decades and is expected to continue to do so, taking into consideration the continuing dependence of markets on fish-based protein (FAO 2012a) Greece is the largest producer of gilthead seabream *Sparus aurata* and European seabass *Dicentrarchus labrax* in the region, followed closely by Turkey, Egypt, Italy and Spain (FAO 2012b). Production of these species in Greece for 2010 was in the vicinity of 100 000 t.

Although the amount of nutrients released by fish farms is high (Islam 2005, Karakassis et al. 2005), detecting them in the field has proved very elusive (Pitta et al. 2005, Yucel-Gier et al. 2008). This has been attributed to environmental factors such as fast dispersion of nutrients (Gowen & Bradbury 1987). More recently, Pitta et al. (2009) showed that nutrients released from farms are rapidly assimilated into the food web, resulting in increased grazing pressure on phytoplankton and a subsequent quick transfer of nutrients to higher trophic levels.

The transfer up the food web to higher trophic levels has also been shown through increased biomass of wild fish near fish farming zones (Machias et al. 2004), increase of local fisheries landings in the wider vicinity of fish farms (Machias et al. 2006, Arechavala-Lopez et al. 2011), use of areas proximate to cages by juveniles as a settlement site (Fernandez-Jover et al. 2009) and even increase of high level predators such as dolphins (Piroddi et al. 2011). Despite these findings, changes in plankton community structure continue to be difficult to detect, with existing studies finding seasonal as opposed to farm-related changes (Yucel-Gier et al. 2008, Skejic et al. 2011). Pearson & Black (2001) pointed out that it has only rarely been possible to demonstrate any linkage between the nutrients produced from farming and a biological response. It is also not typically feasible to attribute wider-scale effects to nutrients from fish farms due to the failure to connect nutrient enrichment from the cages to wider-scale effects. A review by Sarà (2007b) noted that effects of fish farming on the water column are often neglected, even though the changes of patterns in nutrient flows can trigger deviations in ecological processes on a much wider scale.

The aim of this paper is to provide a comprehensive study of changes in community structure in the vicinity of fish cages encompassing a wide range of size classes and trophic functions from bacteria to mesozooplankton in response to nutrient enrichment. The range of organisms studied provides valuable insights into how communities, fine-tuned to deal with oligotrophic conditions, respond to nutrient addition in exposed coastal areas of the Eastern Mediterranean.

MATERIALS AND METHODS

Study areas

The study areas in Lesvos and Cyprus are located in the eastern Mediterranean. They were both sampled in July 2008. In total, 7 stations were sampled in Lesvos and 10 in Cyprus (Fig. 1).

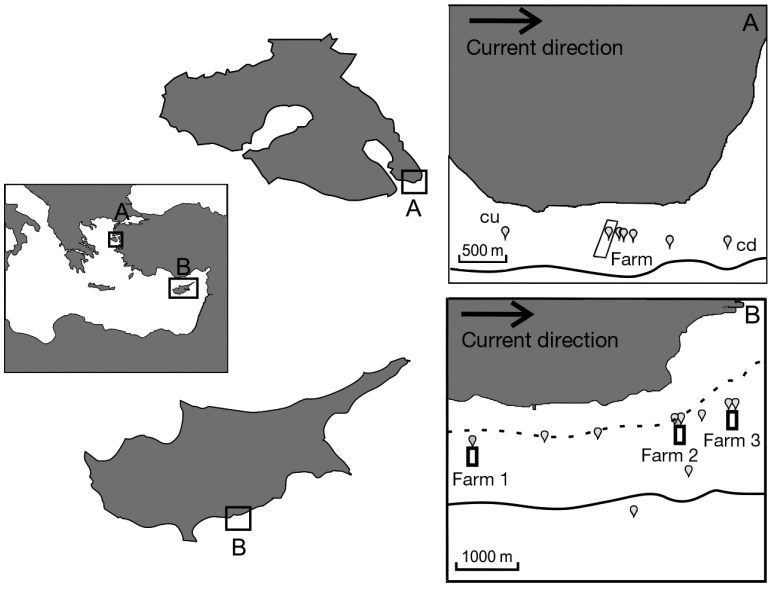

Fig. 1. Fish farm and sampling station locations in (A) Agrilia-Lesvos Island, Greece, and (B) Vasiliko, Cyprus. Station locations in (A) and (B) are indicated with grey placemarks (pins), and fish farm locations are outlined in black boxes. cu: control station upstream of cages; cd: control station downstream of cages. The 50 and 20 m isobaths are depicted with solid and dashed lines, respectively

The farm operating in Lesvos is located along the coast of the southern tip of the island on Cape Agrilia. The minimum depth at the site is 10 m and the maximum depth is 70 m. Despite its proximity to land, the site is characterized by strong hydrodynamic conditions. The annual production is 600 t of sea bream and seabass. Based on feeding records for the farm, a mass balance model was created and was described by Tsagaraki et al. (2011). Briefly, the farm at Lesvos releases 12.8 t of particulate organic nitrogen (PON), 11.3 t of particulate organic phosphorus (POP) and 5.7 t of dissolved phosphate (PO_4) into the water column annually.

The aquaculture development area in Cyprus was established in 2006, and all operating fish farms in Cyprus are located in that area. There were 3 in place at the time of sampling. Farm 1 had an annual production of 100 t, Farm 2 produced 700 t, and Farm 3 produced 600 t. According to the mass balance calculations, Farm 2 releases 14.7 t of PON, 12.9 t of POP and 6.5 t of PO_4 annually. The rest of the farms have similar outputs proportionate to their annual fish production rates.

Sampling design and stations

In order to sample the water mass flowing through the cages, emphasis was placed on real-time monitoring of the current direction. To this end, drifters were deployed (Davis 1982) in Lesvos. On the day of sampling, the drifters were deployed *in situ*, to verify the model prediction and to establish the location of the sampling stations. In Lesvos, sampling was done following the current indicated by the trajectory of the drifters. Samples were taken at the farm station and at 80, 100, 200 and 500 m downstream of the farm cages following the drifter direction. Two control stations were also sampled, one upstream of the cages (cu) and one downstream (cd) (Fig. 1A). In Cyprus, forecasts of current velocity and direction were extracted from the daily output of the Cyprus Coastal Ocean Forecasting and Observing System (CYCOFOS, www.oceanography.ucy.ac.cy/cycofos) hydrodynamic model in Cyprus. Samples were taken at the 3 farm cages (5 sampling points) and control stations (5 sampling points), shown in Fig. 1B.

Sampling and analysis

Samples at 2 discrete depths (0 and 15 m) were taken at all stations using a Niskin bottle, and the water collected was used for the analysis of particulate and dissolved nutrients, chlorophyll *a* (chl *a*), bacteria, nanoflagellates and microplankton. Water temperature and salinity were measured by deployment of a Seabird SBE-16 CTD. Mesozooplankton was collected by vertical tows (0–20 m layer) using a WP2 200 mm mesh size net equipped with a flowmeter.

Samples for chl *a* analysis were filtered through glass fibre filters (GFF) and kept frozen until analysis; they were then extracted in 90% acetone for 24 h and fluorescence was measured by means of a Turner fluorometer according to Yentsch & Menzel (1963). Dissolved nutrient samples were preserved with chloroform and analysed for phosphate, silicate, nitrite and nitrate according to Strickland & Parsons (1972) and for ammonium according to Ivancic & Deggobis (1984). Particulate nutrients were analysed on GFF filters with X-ray fluorescence in a Bruker AXE S4 Pioneer WDXRF instrument. Dissolved organic carbon samples were collected in duplicates in pre-combusted (480°C, 12 h) glass ampoules, acidified with 2.5 N HCl to pH ~2 and flame-sealed as soon as possible. Analysis of total organic carbon was done using a commercially available automatic analyser (Shimadzu TOC-5000) according to Sugimura & Suzuki (1988).

Triplicates of bacteria and nanoflagellate samples were preserved in borax-buffered formalin (final concentration 2%), stained with DAPI (Porter & Feig 1980) and filtered onto 0.2 and 0.6 µm black polycarbonate filters, respectively. They were counted under epifluorescence microscopy on an Olympus BX 90 microscope.

Microplankton samples were preserved in 4% acid lugol solution and analysed according to Utermöhl (1931) on an inverted microscope (Olympus IX70) using an image analysis system after 24 h sedimentation.

Diatoms, dinoflagellates, naked ciliates and tintinnids were identified to genus and where possible to the species level. Naked dinoflagellates belonging to the genus *Gyrodinium* and *Gymnodinium* could not be distinguished and were grouped at the family level as Gymnodiniaceae.

The dimensions of each individual cell belonging to those phyla were measured using Image J and assigned an equivalent geometric shape according to Hillebrand et al. (1999). Carbon content was then calculated using the factors described by Davidson et al. (2002). Trophic status was assigned to each group/species according to Tomas (1997); additionally, recent literature suggests that most chloroplast-bear-

ing dinoflagellates are capable of mixotrophic nutrition via phagotrophy. Therefore, all dinoflagellate species were considered potentially heterotrophic and were assigned to the microzooplankton (Loder et al. 2011).

Mesozooplankton samples were preserved in 4% buffered formaldehyde. Half of the sample was used to determine biomass in dry weight (DW), after drying it at 60°C for 24 h. From the other half, a subsample of known volume was scanned to determine the abundance and size of dominant groups by image analysis (Image-pro plus 6.0).

Statistical analysis

One-way ANOVA was used for all results. In Lesvos, distance from farm was the factor and in Cyprus, all farm stations were grouped and compared to all control stations. Samples from both depths were considered in the analysis for each station in Lesvos, since the CTD data showed that the column at 15 m depth was mixed. In Cyprus, surface samples only were considered in the analysis, as CTD data showed that the water column was stratified above the sampled depth. For the comparison between Lesvos and Cyprus, all stations from each area were grouped together irrespective of depth and distance.

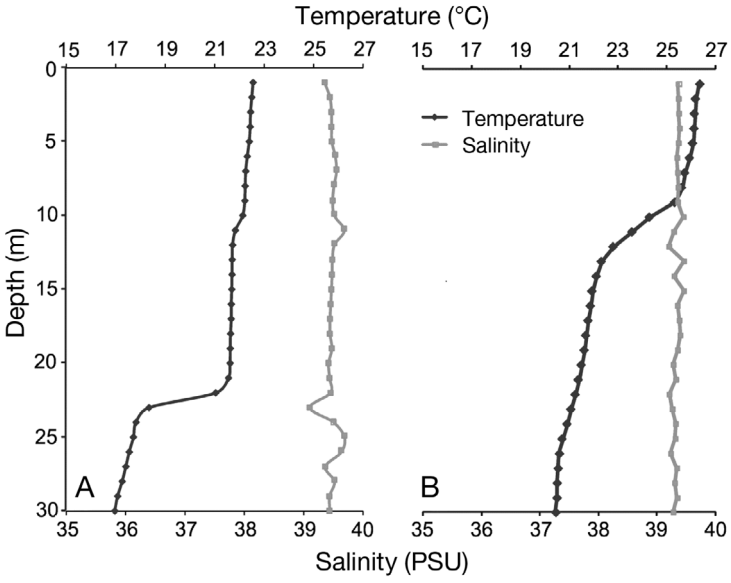

Fig. 2. Temperature (black) and salinity (grey) profiles in (A) Lesvos and (B) Cyprus

RESULTS

Lesvos

Physical parameters

In Lesvos, the average surface temperature and salinity were approximately 22.5°C and 39 (Fig. 2a). The water column appeared to be stratified and the thermocline was observed below 20 m depth. Average current speed in Lesvos on the day and time of sampling was measured at 0.23 m s^{-1} in a west to east direction.

Chlorophyll, dissolved and particulate nutrients

Chl *a*, phaeopigments and dissolved and particulate nutrients showed no statistically significant differences between stations ($p > 0.05$). The only exception was the chl *a* to phaeophytin ratio, which was significantly lower 100 and 200 m downstream ($p < 0.001$) than all other stations. Significantly higher values ($p < 0.001$) were observed at both controls (cu and cd) and the farm station.

Pico- and nanoplankton abundance

The bacterial and nanoflagellate abundance were examined upstream and downstream of the farm cages (Fig. 3). For bacteria, ANOVA showed significant differences between stations ($p < 0.001$). The 100 m downstream station had a significantly higher abundance of bacteria (~7 × 10^5 cells ml^{-1}) than all other stations (apart from 200 m downstream). The upstream and downstream controls had a significantly lower abundance of bacteria ($p < 0.001$, ~4 × 10^5 cells ml^{-1}) compared to the stations 100, 200 and 500 m downstream. A similar trend was observed for nanoflagellates; we found statistically significant differences between the stations ($p < 0.005$), with the 100 m downstream station presenting a higher abundance (346 cells ml^{-1}) than all other stations.

Microplankton community composition and size frequency distribution

The dominant microplankton genus in Lesvos was the diatom *Hemiaulus*, which was observed at all stations, but in particular at the control stations. Its abundance was significantly higher (5400 cells l^{-1}) at

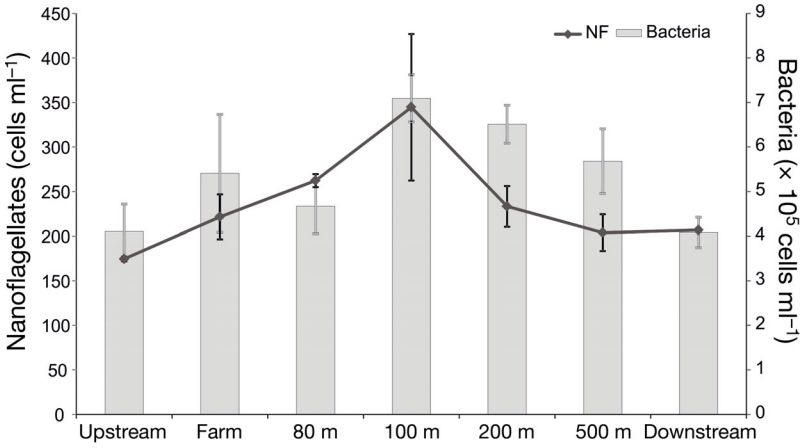

Fig. 3. Bacteria and nanoflagellate (NF) abundance (cells ml^{-1}) at each station in Lesvos

the upstream control station than at all other stations ($p < 0.005$). Other abundant diatom genera were *Nitzschia* and *Guinardia*, although no significant differences in their abundance or biomass were observed between stations. Among dinoflagellates, the genera *Gyrodinium* and *Gymnodinium* were the most abundant. No significant differences were found between stations for these genera. Ciliates mostly belonged to the genus *Strombidium*, followed by *Tontonia*.

When considering species richness (Margalef's diversity index) at each station, based on the species distribution of diatoms, dinoflagellates and ciliates, we found a significant difference ($p < 0.05$) between the upstream control and all downstream stations. Diversity upstream of the farm was 6.7, whereas it ranged between 8.1 and 10.5 at all other stations with no significant differences between them. The same was the case for Pielou's evenness ($p < 0.001$), which was lower at the upstream control (0.47) than at all other stations (0.75 to 0.92). The expected number of species (ES100) was also lower at the upstream control ($p < 0.01$) at 20.6, while all other stations had an expected number of species between 29 and 42.

Size frequency distributions were also analysed for diatoms, dinoflagellates and ciliates. In Lesvos, tintinnid abundance was low (<50 ind. l^{-1} in total), so they were not included in the analysis. Individuals from all groups were separated according to their equivalent spherical diameters (ESD) and grouped into 2 size classes, small (<20 ESD) and large (>20 ESD). In the large category, at least 95% of cells were between 20 and 40 ESD. At both control stations, the diatom community consisted of cells larger than 20 µm. Only 7 and 9% of cells fell within the small category at the upstream and downstream controls, respectively. At the farm and at 80 m downstream, this changed considerably. Smaller cells became more abundant, and at those 2 stations, the community was divided almost exactly in half. At 100 m downstream, the percentage of large diatoms increased again to 85% of the community and remained higher than the smaller individuals up to the downstream control. Small dinoflagellate cells were more abundant at all stations. However, large dinoflagellates increased downstream from the cages from 13% in the upstream control to ca. 30% at all other stations including the downstream control. An exception to this was the 100 m downstream station where large dinoflagellates decreased to 19% of individuals measured. For ciliates, the contribution of small cells to the community was higher at all stations. It appeared that more larger ciliates were present downstream of the fish cages; at the upstream control, medium and large ciliates comprised 25% of the community, and this percentage went up to 45% at 500 m downstream of the farm.

The size of individuals counted was also used to establish whether the size of the most abundant genera from each group differed relative to distance from cages. The Gymnodiniaceae showed significant differences in their size distribution ($p < 0.001$). The Student-Neuman-Keuls test grouped stations into 2 homogeneous subsets consisting of (1) the upstream control, 100 and 500 m stations and (2) the farm, 80, 200, 500 m and downstream control. The mean ESD of the Gymnodiniaceae was significantly lower (13.8 µm) at the upstream control and 100 m stations ($p < 0.005$). At all other stations, apart from 500 m downstream, these genera had a higher mean ESD, ranging from 16 to 16.7 µm.

The percentage contribution of carbon in each group to the total microplankton carbon biomass is shown in Fig. 4. Large diatoms dominated the biomass in the upstream control, but were replaced by smaller diatoms at the farm station, although they still made up 30% of the total carbon biomass. The contribution of diatoms to the carbon biomass started decreasing 80 m downstream and remained very low until the downstream control. Ciliates both increased their contribution to the total carbon biomass downstream from the farm and also increased in size, the same applied for dinoflagellates, although larger cells in this group increased their contribution farther downstream from the farm.

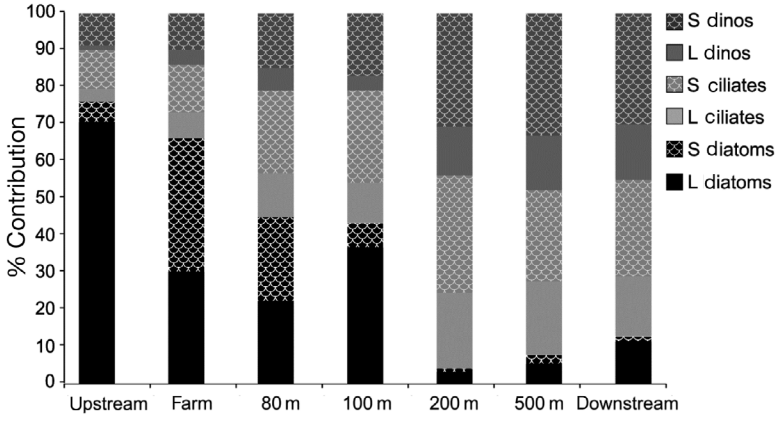

Fig. 4. Percentage contribution of diatoms, dinoflagellates (dinos) and ciliates to the total microplankton carbon in Lesvos at each station. S: small cells (<20 equivalent spherical diameter, ESD); L: large cells (>20 ESD)

Finally, the ratio of large to small cells for these groups in terms of abundance showed a very interesting pattern. The ciliates and dinoflagellates presented similar trends in the ratio of small to large cells for all stations (Fig. 5). Diatoms presented a different pattern that appeared to be inversely correlated with the microzooplankton and especially the dinoflagellates.

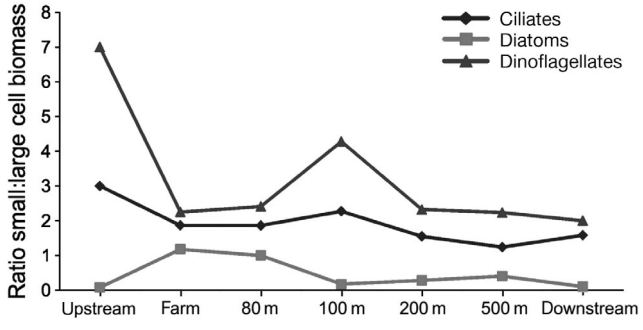

Fig. 5. Ratio of small (<20 equivalent spherical diameter, ESD) to large (>20 ESD) microplankton cells at Lesvos stations

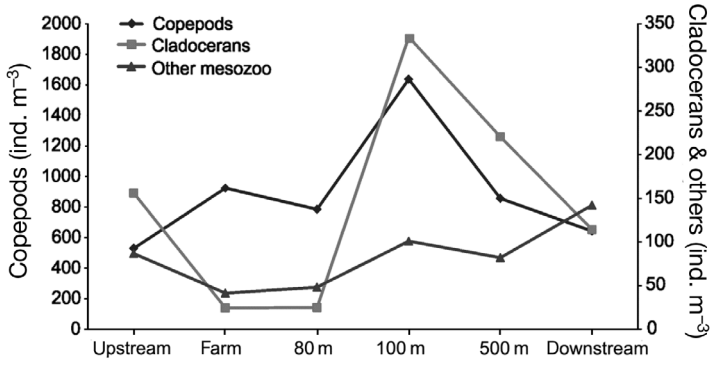

Fig. 6. Abundance of copepods, cladocerans and other mesozooplankton (ind. m^{-3}) at Lesvos stations. No data are available for the 200 m station

Mesozooplankton community composition and size frequency distribution

The mesozooplankton biomass and abundance showed an increasing trend closer to the cages beginning from stations upstream of the farm. The biomass doubled from 10.17 mg DW m^{-3} upstream to 20.79 mg DW m^{-3} at the cages. It peaked at 80 m downstream at 29.94 mg DW m^{-3} and subsequently started to decrease as distance from the farm increased, to 8.87 mg DW m^{-3} at the downstream control. An interesting observation was a striking, almost an order of magnitude, decrease of cladoceran abundance (the second largest group after copepods), from >100 ind. m^{-3} upstream, to values <25 ind. m^{-3} at the farm cages and at 80 m downstream, recovering back to >100 ind. m^{-3} farther downstream (Fig. 6). The opposite trend was observed for copepod abundance, which almost doubled 100 m downstream and faded back to background values farther downstream. Small differences in mesozooplankton size were observed among stations; however, the smallest individuals were found 100 m downstream of the farm (~570 µm body size for both copepods and cladocerans).

Cyprus

Physical parameters and picoplankton

Average surface temperature at the Cyprus site (Fig. 2b) was approximately 26.3ºC and salinity was approximately 39 PSU. The water column appeared to be stratified and the thermocline was observed quite close to the surface at slightly less than 10 m. Based on model outputs, the average monthly current speed in Cyprus was 0.15 m s^{-1}, and the surface current direction was west to east.

Bacterial abundance in Cyprus was significantly (20%) lower ($p < 0.05$) at the control stations compared to the farm stations.

Microplankton community composition and size frequency distributions

The dominant microplankton genera in Cyprus were *Gymnodinium* and *Gyrodinium*. Other abundant genera were the diatoms *Hemiaulus* and *Navi-*

cula, although no significant differences in the abundance of any dominant genus were observed between stations. At the group level, the diatom biomass was significantly lower at the farm stations ($p < 0.05$), although there were no significant differences in abundance.

Margalef's diversity index did not present any marked differences between stations in Cyprus; the highest species richness was 12.4 at the largest fish farm site, although this was followed closely by a species richness of 13.2 at 1 of the control stations. However, the total number of individuals was significantly higher at the farm stations ($p < 0.05$) for all microplankton groups.

Size frequency distributions in Cyprus showed no differences between small and large cells for ciliates. The percentage of small cells was higher for both the farm and control stations at 85% of the individuals measured. This was not the case for dinoflagellates where it appears that larger cells increased by 10% at the farm stations, making up 35% of the individuals measured. There was also a marked difference in the size frequency distribution of diatoms. At the control stations, cells larger than 20 µm ESD comprised 61% of the total individuals counted, whereas at the farm stations they were equally divided between small and large.

Table 1. ANOVA results of comparison (all stations pooled) between Lesvos and Cyprus (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

Parameter	Area	Mean	*F*	p
Chl *a* (µg l^{-1})	Cyprus	0.05	14.1	**
	Lesvos	0.11		
Phaeophytin (µg l^{-1})	Cyprus	0.02	24.1	***
	Lesvos	0.05		
Chl *a*/Phaeophytin	Cyprus	3.03	32.0	***
	Lesvos	5.58		
PO_4 (µM)	Cyprus	0.04	44.3	***
	Lesvos	0.14		
NH_4 (µM)	Cyprus	0.52	32.4	***
	Lesvos	2.51		
Total dissolved N (µM)	Cyprus	1.32	10.5	**
	Lesvos	0.70		
SiO_2 (µM)	Cyprus	1.37	68.8	***
	Lesvos	0.61		
Particulate C (µM)	Cyprus	2.76	80.0	***
	Lesvos	11.43		
Particulate N (µM)	Cyprus	0.241	44.2	***
	Lesvos	0.992		
Particulate P (µM)	Cyprus	0.016	42.1	***
	Lesvos	0.049		
N/P	Cyprus	15	9.5	**
	Lesvos	20		
Bacterial abundance (cells ml^{-1})	Cyprus	440353	7.3	*
	Lesvos	536466		
Ciliate biomass (pg C l^{-1})	Cyprus	340269	64.0	***
	Lesvos	879410		
Diatom biomass (pg C l^{-1})	Cyprus	173776	6.3	*
	Lesvos	980381		
Tintinnid biomass (pg C l^{-1})	Cyprus	364232	11.4	**
	Lesvos	86222		
Diatom abundance (cells l^{-1})	Cyprus	324	7.5	**
	Lesvos	1973		
Dinoflagellate abundance (cells l^{-1})	Cyprus	1289	4.9	*
	Lesvos	943		
Microplankton abundance (cells l^{-1})	Cyprus	2302	4.5	*
	Lesvos	3747		

Mesozooplankton biomass

The mesozooplankton biomass showed no clear trend; the average biomass was 5.55 mg DW m^{-3} with no noticeable differences near the fish farms.

Area comparison

In order to assess the characteristics of the environments studied during the summer season, a comparison between the 2 study areas was also necessary. This included all stations considered in statistical analyses for both areas (Table 1). Briefly, total chl *a* values were significantly lower in Cyprus, as were the dissolved phosphate and all particulate nutrients measured (Table 1). The biomass of ciliates and diatoms was higher in Lesvos, and dinoflagellate abundance was higher in Cyprus (Table 1). Additionally, both the species richness (Margalef) and the expected number of species (ES100) were higher in Cyprus ($p < 0.05$). Zooplankton biomass was higher in Lesvos. The size frequency distributions showed that small ciliates made up 84% of the total ciliate population in Cyprus, whereas in Lesvos small ciliates were 20% less. No differences between dinoflagellate size were observed between the 2 areas.

DISCUSSION

In a review of the current literature on aquaculture effects in the water column, Sarà (2007a) concluded that the ecosystem type appears to be one of the causal factors determining differences among the literature reviewed. One would expect to detect more of an effect in more enclosed water bodies.

The study sites, which were both exposed and with relatively high current velocity, showed that the trophic status and background community of the farm also play an important part in changes detected. Comparison between the 2 areas and between stations showed a plankton community response of different degrees. The response seen in the water column in the present study illustrates what many authors have concluded in the past, viz. that knowledge of the area hydrography is paramount to detection of farm influence, especially at exposed sites (Maldonado et al. 2005, Sarà et al. 2006). In the present study, we were able to monitor the water mass that was known to flow through the farm especially in the case of Lesvos. It proved that due to high current velocities, considering the farm station as impacted would have proven futile; indeed, it was the intermediate distances that were 'impacted,' at least in terms of the measured parameters. This is in agreement with Sarà et al. (2006) and Sarà (2007a, and references therein), who found that dispersal patterns from aquaculture indicate that the greatest effects are found 100 to 300 m downstream of cages and, depending on current velocity, could reach even larger distances. This was the case in Lesvos, with the distance being approximately 100 to 500 m downstream and possibly more. Modelling the dispersal of nutrients in both study areas showed an accumulation of effluents much farther downstream (Tsagaraki et al. 2011).

When comparing the 2 areas, it is apparent that Cyprus was more oligotrophic and specifically phosphate limited compared to Lesvos. This is indicated both by the lower dissolved and particulate phosphate levels, as well as by the increased availability of dissolved nitrogen species and silicic acid. As far as the general community structure goes, Cyprus presented a higher abundance of dinoflagellates, whereas Lesvos had more ciliates and diatoms. Both communities seemed to be composed mostly of small phytoplankton and grazers under 20 µm ESD, and a larger percentage of cells in Cyprus was under 20 µm ESD. Despite the lower microplankton abundance in Cyprus, the overall diversity was higher. This presents us with the 'paradox of the plankton' (Hutchinson 1961) which, briefly, questions how a limited amount of resources can support a much wider range of organisms contrary to the competitive exclusion principle, which states that when competing for the same resources, eventually, fewer species will persist. Results are in agreement with Ignatiades et al. (2009), who found higher diversity in plankton communities in the Eastern Mediterranean basin, attributing this to the greater number of diversified niches found in this warmer and saltier environment.

It appears that the influence of fish farming was more pronounced in Lesvos. Initially this seems counter-intuitive; because Cyprus was more P-limited, it was expected that effluents from farms would trigger a more detectable and immediate response in the surrounding area. Also, current velocity in Lesvos was higher, suggesting a higher removal rate, making it harder to detect differences between stations. A possible explanation for the community response observed in both areas can be found when considering phosphate turnover time. Zohary & Robarts (1998) reported very rapid phosphate turnover times for the eastern Mediterranean; Flaten et al. (2005) reported a phosphate turnover time of 2 to 3 h in the Levantine Sea. Based on results from the eastern Mediterranean, turnover time in the area is less than 5 h and could be as little as 2 min (Ivancic et al. 2012). Results from a phosphate addition experiment in the Levantine led to the proposal of uptake mechanisms which were not mutually exclusive (Krom et al. 2005) but could explain the apparent transfer of energy to higher trophic levels. One was termed the 'trophic bypass' where the phosphate goes directly to the grazers through the bacteria bypassing the phytoplankton, a mechanism also observed in freshwater environments by Kerner et al. (2003). The second is the 'tunneling' mechanism where phosphate is absorbed in excess by bacteria and larger plankton, causing changes in the quality of food available to grazers and shifting the stoichiometric composition of prey. This mechanism has also been observed in freshwater environments by Hessen (1992), who found that zooplankton biomass was best correlated with particulate P instead of phytoplankton biomass or chl *a* values. It appears that in both areas, effects observed near the fish farms suggest a trophic bypass in conjunction with a tunneling effect. In Cyprus, ciliates and dinoflagellates could replace mesozooplankton as they are often more efficient predators, especially on smaller size fractions (Sherr & Sherr 2007), and are more abundant relative to zooplankton on a gradient from the northern Aegean, to southern regions of the eastern Mediterranean

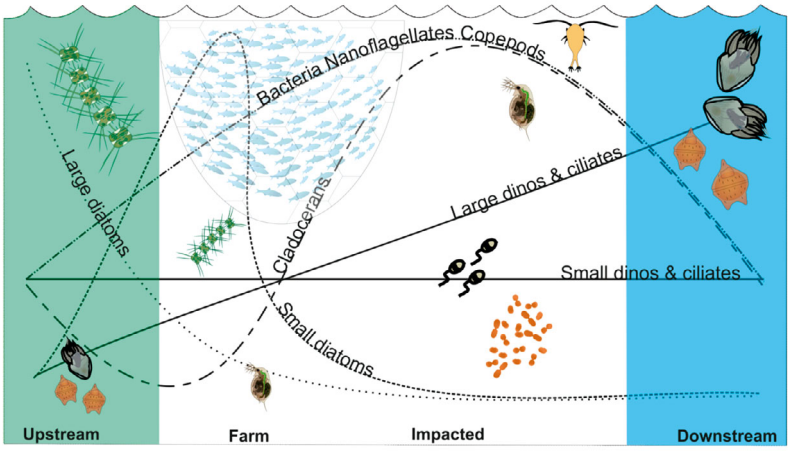

Fig. 7. Conceptual diagram of changes in plankton community biomass at upstream, farm, impacted and downstream stations in Lesvos. Each line represents the trend shown by groups of organisms; therefore, grouping into 1 line indicates a common pattern of response. Solid lines: ciliates and dinoflagellates (dinos; small and large), dotted lines: diatoms (small and large), dashed lines: cladocerans, copepods, bacteria and nanoflagellates

(Siokou-Frangou et al. 2002, Ignatiades et al. 2009). In Lesvos, the higher background nutrient values and community dynamics illustrated these responses more clearly.

Although water column changes in bacterial populations are seldom reported near fish cages, they were detected in both areas. However, Maldonado et al. (2005) and Pitta et al. (2005) measured an increase in bacterial populations as a response to added nutrients. Such a response was also found by Weisse (1991), who suggested that the increase in bacterial abundance he observed eventually leads to a prevalence of more linear food chains. Results from both study sites suggest a potential common pattern in response to fish farming in oligotrophic areas. This consists of an immediate response of some groups (nanoplankton, picoplankton and mesozooplankton) to the added nutrients (tunneling) and an increase in microzooplankton size, capitalizing on available resources (bypass). Similar findings in the Mediterranean were reported by Pitta et al. (2005, 2009).

Based on the measurements of the present work, we can attempt to paint a picture of the planktonic community near the fish cages, a visual representation of which is shown in Fig. 7. The community upstream of the farm appeared to be typical of the oligotrophic conditions of the area in the summer. Microplankton carbon was dominated by large diatoms and smaller microzooplankton cells. The dominant diatom, *Hemiaulus*, is known to be very efficient at utilizing low-nutrient conditions (Werner 1977). At the same time, even though dinoflagellates are considered major grazers of diatoms (Calbet & Landry 2004), they also often display selective feeding (Lawrence & Menden-Deuer 2012), which does not necessarily include diatoms even if they are available.

At the farm cages and 80 m downstream, picoplankton abundance started to increase, the contribution of diatoms to the carbon biomass decreased, and concurrently, small diatom cells made up a larger fraction of the carbon biomass compared to large cells. Ciliate contribution (small and large) was higher while the dinoflagellate contribution to the biomass increased, but appeared to be lagging. This is expected, as under the same nutrient conditions ciliates have been shown to grow faster than dinoflagellates of the same size (Neuer & Cowles 1995, Strom & Morello 1998). On the other hand, cladoceran abundance dropped by an order of magnitude; this is counterintuitive since this group grazes very efficiently on picophytoplanton compared to other mesozooplanton groups (Atienza et al. 2006), typically on prey under 10 µm ESD (Hansen et al. 1994). Therefore, the drastic decrease in their abundance is more likely to come either from top-down control, possibly by juvenile fish using the farm cages as settlement sites as shown by Fernandez-Jover et al. (2009), or increased competition by copepods which feed size selectively when food availability increases (Kleppel 1993, Sommer & Stibor 2002), whereas cladocerans are obligate filter feeders (Sommer & Stibor 2002).

At the 'impacted' stations 100 m downstream, the highest abundances of bacteria, nanoflagellates, cladocerans and copepods were observed (trophic bypass effect). Concurrently at this distance, the carbon contribution of larger ciliates and dinoflagellates continued to increase (tunneling effect), although the ratio of small to large cells at this station was higher than at all other downstream stations. A similar picture was present 200 m downstream. The contribution of larger ciliates and dinoflagellates to the microplankton carbon was higher at these distances, a trend which continued all the way to the downstream control stations, even though abundance and biomass of picoplankton and mesozooplankton returned to background levels. Overall, it appeared that downstream from the farm, the heterotrophic fraction dominated the biomass, which is an indication towards a shift from the microbial loop to a linear food chain.

In interpreting the results, it is useful to keep in mind that the concentration of any emitted substance is inversely proportional to the square of the distance from the emission point. Therefore, samples taken at intermediate stations are expected to have 'incubated' their communities under high nutrient content with enough time to observe changes. At the same time, the water masses are not so diluted as to achieve a composition identical to that of the ambient water.

Changes in the community structure were also made apparent when considering species diversity at the sampling stations. The farm vicinity tended to support a higher diversity of microplankton. Here, given the opportunity, more species are able to take advantage of the micronutrients supplied by the farm cages. This is in contrast to observations in fish farm sediments where the diversity is substantially decreased close to farm cages (Kalantzi & Karakassis 2006). It is also in contrast with effects of adding nutrients in the water column which can induce a monospecific bloom (Coale et al. 1996, Egge et al. 2009). The intermediate disturbance hypothesis (Grime 1973, Dial & Roughgarden 1998) offers a plausible explanation as to the changes in biodiversity observed near the farm. Very simply put, the hypothesis predicts that intermediate frequency or intensity of disturbance will increase diversity. This is mainly because of the coexistence of competitive dominants and rapid colonizers (Svensson et al. 2012). In this case, the disturbance consists of the changes in available resources and the physical environment as defined by Pickett & White (1985) but could also be a result of increased grazing pressure (Sommer 1995). Microplankton response to intermediate disturbance has been shown in lakes (Grover & Chrzanowski 2004) and in plankton communities of reservoirs (Hambright & Zohary 2000), and the daily nutrient supply from fish farms falls within the frequency described by Reynolds (1988) (ca. 200 h or less) capable of preserving high species diversity in phytoplankton. Additionally, Tilman (1996) observed that diversification also acts as a mechanism to ensure that some species will be able to compensate for other species' responses to disturbance or change.

Interpretations of community structure aside, the fact remains that the community is changed, and this change persists downstream of the cages and up to the highest trophic level measured. In this respect, we have more clues as to the way energy is channeled going up to juvenile (Fernandez-Jover et al. 2009) and adult (Bacher et al. 2012) fish close to the cages and wild fish assemblages at mesoscale distances (Machias et al. 2004) or even top level predators at regional scales (Piroddi et al. 2011).

CONCLUSIONS

Our study represents one of the very few cases where a community-level response to fish farming was detected in the field. It appears that oligotrophic areas respond to perturbations fast and often without much trace, unless site-specific sampling designs and measurements are implemented. The range of potential mechanisms in place cannot be feasibly measured in one study. It would be very interesting to see whether this pattern persists seasonally, to monitor autotroph groups more closely and to establish whether trophic modes within the microzooplankton also change in response to the point source.

Maldonado et al. (2005) suggested that farms at exposed sites may have fewer environmental impacts than traditionally located fish farms. In our study areas, this was not the case, at least in terms of change. Continuous input of nutrients from fish farms provides us with the unique opportunity to study community interactions in a dynamic environment. The data emerging from studies such as the present one show that far more information remains to be extracted about natural processes, interactions and community responses to change.

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