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Preliminary study on vertical migrations of dinoflagellates in a dynamic coastal sea (Gulf of Trieste, northern Adriatic)

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The purpose of this preliminary study was to define the vertical migration pattern in the dinoflagellate community in the shallow coastal sea. Migrations were followed in an area of mussel farming, through two 24-hour samplings, first during mixed and second during stratified water column conditions. Despite variable physical environment we were able to follow vertical migrations of some autotrophic dinoflagellate species in the period of stratified water column. The results also suggest that Heterocapsa sp. may preserve its vertical migration pattern also under mixed conditions. Migrations were observed also for Dinophysis sacculus *that can cause DSP problems in the area.*

Key words: dinoflagellates, vertical migrations, dynamic environment, coastal sea, Adriatic

INTRODUCTION

Vertical migration as one of advantageous characteristics of dinoflagellates (SMAYDA, 1997) permits these organisms to access the water layer with an adequate quantity of inorganic nutrients, thereby improving their retrieval. Consequently, the benefit of migration is especially evident under stratified water column conditions where two principal goods, light and nutrients, are spatially separated. Such conditions in turn proved to be favourable for growth and blooming of several dinoflagellate species (SMAYDA, 2002) capable to surmount environmental barriers by swimming.

Few modelling studies (JI & FRANKS, 2007; RALSTON *et al.*, 2007) in conjunction with field data (FAUCHOT *et al.*, 2005; TOWNSEND *et al.*, 2005) confirmed the existence of different strategies of migratory behaviour. The majority of studies of vertical migration have been conducted under controlled conditions on isolated or cultured dinoflagellate species (HEANEY & EPPLEY, 1981; KAMYKOWSKI, 1981; MACINTYRE *et al.*, 1997), whereas *in situ* studies are fewer since they offer less understanding of dinoflagellates' physiological responses, but, on the other hand, present a true combination of environmental stresses. *In situ* studies are mainly constrained to bloom events (e.g. KOIZUMI *et al.*, 1996), during which dinoflagellates can be followed with fluorescence. Some species show different patterns of diel migration in the field when compared to laboratory studies (PASSOW, 1991). In the view of a great variety of species-specific migration patterns and site-specific control factors (FAUCHOT *et al.*, 2005) there is still need for new field investigations of diel vertical migrations of dinoflagellates.

In this work we aimed to examine the vertical migration pattern in the dinoflagellate community of the northern Adriatic (Gulf of Trieste), and to assess the importance of this migration in a rapidly changing shallow coastal sea. Dinoflagellate abundance constitutes only a small portion of the nanoflagellate-diatom dominated phytoplankton community of the area (around 4%; FRANCE, 2009) and dinoflagellate species rarely reach bloom abundances. Nevertheless, some dinoflagellate species are responsible for shellfish intoxication problems in the Gulf of Trieste (HONSELL *et al.*, 1996; CABRINI *et al.*, 2001; FRANCE & MOZETIČ, 2006) and it is therefore important to understand their biological properties as they can considerably impact public health and local economy.

MATERIAL AND METHODS

Diel vertical migrations of dinoflagellates were studied at a sampling station (12 m depth) in the mariculture area of the bay of Piran (SE part of the Gulf of Trieste, 45º29,49' N, 13º34,83' E) (Fig. 1). Two 24-hour samplings of the water column were performed: the first in the period of mixed water column in autumn (4th and 5th of November, 2002) and the second under stratified water column conditions in early summer (23rd and 24th of June, 2003). Sampling started at 10.00 hours and ended at 10.00 hours the next day. Seawater was pumped on board an anchored vessel every 4 hours, using a water pump and a Teflon hose. Five sampling depths were set at 3 m intervals in November 2002: at the surface, 3 m, 6 m, 9 m, and near the bottom (12 m). Sampling depths in June 2003 were set according to the CTD profile of the water column: at the surface, above the thermocline at 3.5 m, in the lower part of the thermocline at 5 m, below the thermocline at 7 m, and near the bottom (12 m). Phytoplankton samples were preserved in dark 1000 ml bottles and fixed with 2% neutralized formaldehyde (final concentration).

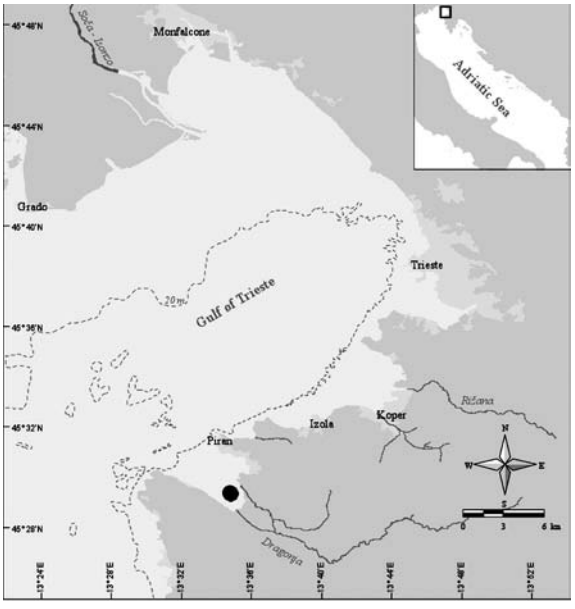

Fig. 1. Map of the Gulf of Trieste with the location of the sampling site in the Bay of Piran (black spot)

Concentrations of the main inorganic nutrients (ammonium, nitrite, nitrate and phosphate) in the water samples, collected at 10.00, 24.00 and 10.00 hours of each 24-hour sampling, were measured according to standard colorimetric methods (GRASSHOFF *et al.*, 1983) using a Perkin Elmer UV/VIS Lambda 14 Spectrophotometer. The temperature, salinity and density profiles of the water column, as well as profiles of photosynthetically available radiation (PAR), were measured at each sampling using a CTD probe (Centre for Water Research, Western Australia).

Dinoflagellate species in fixed samples were determined and counted on an inverted microscope ZEISS Axiovert 135 following UTERMÖHL method (UTERMÖHL, 1958). Subsamples of 50 or 100 ml were left to settle in the sedimentation chamber for 24 or 48 hours, respectively. The whole chamber bottom was counted at 200x magnification. Small species were counted at 400x magnification in 200 fields of the chamber. Vertical distribution of dinoflagellates in 24 hours is presented as their cumulative abundances in the upper, intermediate (November) or thermocline (June), and bottom layer. Absolute abundances were converted to indices of contribution to total abundance at a single sampling time.

RESULTS

During the first experiment in November 2002 the weather was prevalently calm with wind of variable direction not exceeding 6 m s^{-1}. CTD profiles showed homogenous conditions typical for the mixed water column; average temperature and salinity were 18.2°C and 37.7, respectively. These profiles were altered temporarily by a passage of a turbid, less saline water front originating from the nearby river mouth at 1530 h. This front provoked a modest decrease of salinity (35.8) in thin surface water layer. During the night the conditions normalized, so the last two profiles resembled those before the front passage. Maximum surface irradiance was 600 µE m^{-2} s^{-1} and water transparency was low (Secchi depth ≤ 4 m).

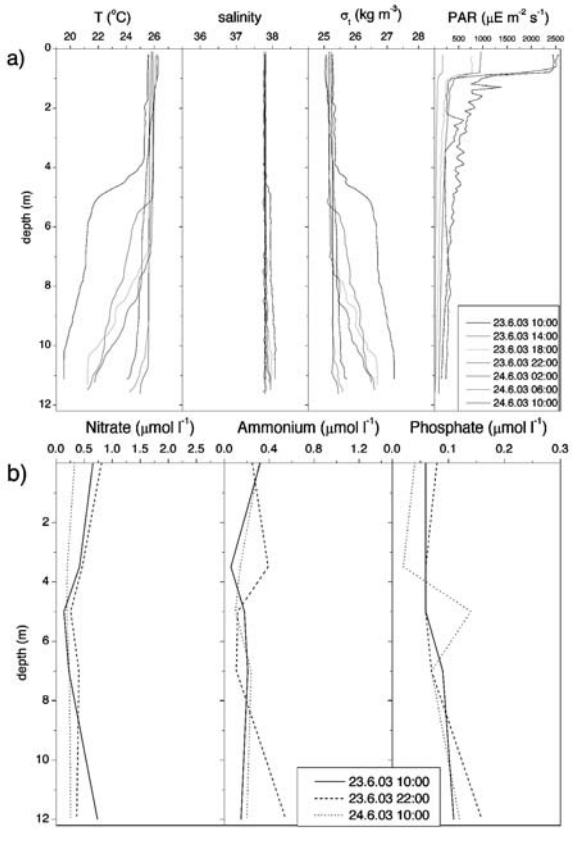

Fig. 2. Vertical profiles of a) temperature, salinity, density (sigma t) and PAR and b) nitrate, ammonium and phosphate concentrations during 24-hour sampling in June 2003

Concentrations of inorganic N and P were high throughout the experiment. Nitrate concentrations were the highest (11.65 µmol l^{-1}) in the surface layer, while phosphate concentrations were the highest (0.25 µmol l^{-1}) in the bottom layer. A total of 42 dinoflagellate taxa were determined, *Heterocapsa* sp. being the most abundant species. Dinoflagellates were the most abundant in the lower part of the water column, especially during dark period. Nevertheless due to low dinoflagellates abundances (maximum 4500 cells l^{-1}), vertical movements of single species were hardly detectable. *Heterocapsa* sp. was the only species whose vertical distribution pattern could infer a migration through the water column: cells were mostly grouped in the surface layer during the day and accumulated near the bottom at night (maximum 1000 cells l^{-1}).

The second experiment was performed in June 2003, in the period normally characterized by thermal stratification. A light breeze up to 3 m s^{-1} was blowing from the NW during daytime. The first CTD cast showed a typical profile with a thermocline at the depth of 4-5 m with temperature ranging from 19.5°C at the bottom to 25.6°C at the surface (Fig. 2a). A midnight storm provoked firstly, deepening of the thermocline and finally, breaking down of the stratification. Nevertheless, this rain did not affect surface salinity (Fig. 2a). The surface irradiance was very high during the first day, with maximum values of around 2500 µE m^{-2} s^{-1} (Fig. 2a). The Secchi disk depth ranged from 8 to 11 m.

Concentrations of nitrate (0.14-0.81 µmol l^{-1}) and ammonium (0.06-0.55 µmol l^{-1}) were on average an order of magnitude lower than in November, whereas those of phosphate (0.02-0.16 µmol l^{-1}) were lower but more comparable (Fig. 2b). Vertical profiles of nitrate and ammonium were quite uniform while phosphate concentrations were the highest near the bottom.

Among 66 dinoflagellate taxa identified in June 2003, the most abundant species was *Heterocapsa* sp. with the mean abundance of 7000 cells l^{-1}. Abundances of *Heterocapsa* sp. were relatively constant in the layer above thermocline during most of the samplings, while in the thermocline and bottom layer the evolving of

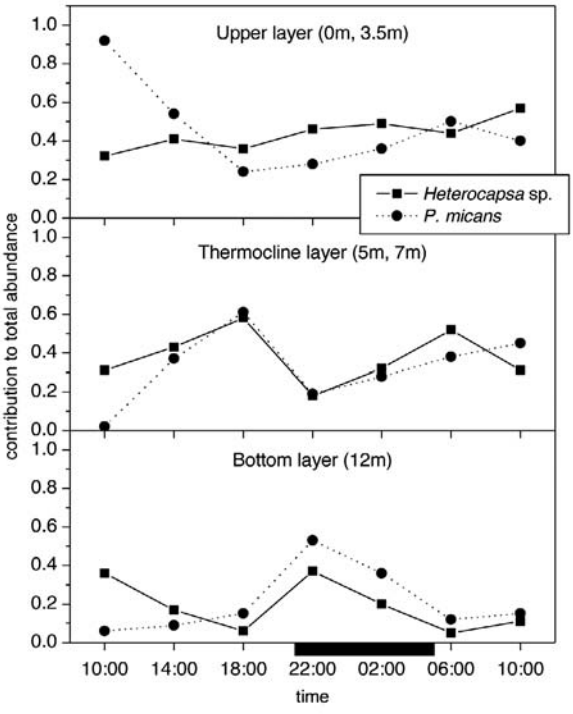

Fig. 3. Temporal evolution of Heterocapsa *sp. and* Prorocentrum micans *abundance in three water layers (upper, intermediate and bottom) expressed as contribution to their total abundance at a single sampling time during 24-hour sampling in June 2003. Black bar indicates dark period*

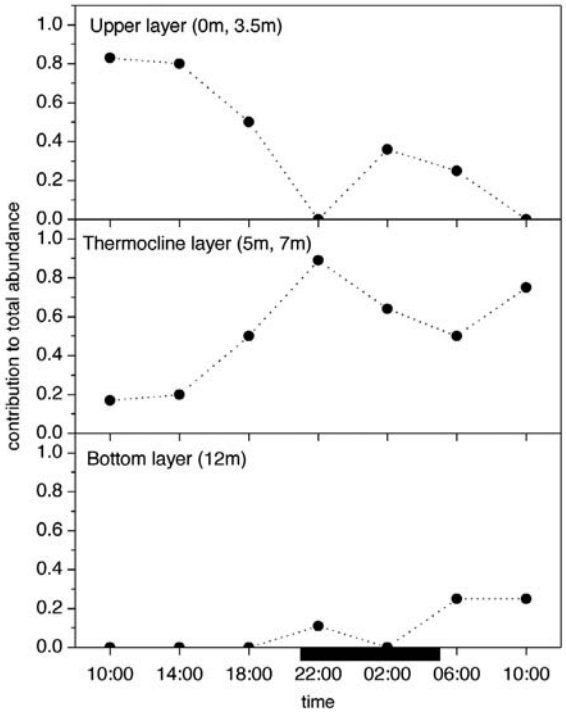

Fig. 4. Temporal evolution of D. sacculus *abundance in three water layers (upper, intermediate and bottom) expressed as contribution to their total abundance at a single sampling time during 24-hour sampling in June 2003. Black bar indicates dark period*

abundances in time indicates a vertical migration pattern (Fig. 3; solid line). The abundance peak in the thermocline layer was observed at 1.800 h, when the surface irradiance dropped down to 770 $\mu E\ m^2\ s^{-1}$. Approximately two thirds of these cells moved towards the bottom afterwards and four hours later the abundance peak was detected in the bottom layer. At 02.00 h the abundance near the bottom decreased but the expected upward migration at dawn was not detected.

A similar migration pattern was detected in *Prorocentrum micans* with the mean abundance of 500 cells l^{-1} (Fig. 3; dotted line). Cells of *P. micans* were aggregated in the upper layer during the first sampling at 1000 h. During the highest surface irradiance at 14.00 h approximately one third of the cells moved to the depth of thermocline where the abundance peak was observed four hours later. Cells then continued to descend and probably reached the bottom layer before sunset as indicated by the bottom abundance peak at 22.00 h. As with *Heterocapsa* sp., no ascending of the cells was recorded during the next morning.

The most abundant among harmful species, *D. sacculus* (maximum abundance 100 cells l^{-1}) exhibited downward migration during the first day (Fig. 4) but only in the upper part of the water column (surface – depth of thermocline). The abundance in the bottom layer was mostly under the detection limit. The upward migration next morning was not observed.

DISCUSSION

Non-bloom conditions, when dinoflagellates constitute just a minor part of phytoplankton community, offer less opportunity to investigate properly their vertical migration. Indeed, we were able to follow the movements through the water column only in June, notwhitstanding that we performed the same experiment during mixed and stratified water column periods.

Vertical migration that we observed in some autotrophic dinoflagellates are in concordance with the results of many field studies worldwide (KAMYKOWSKI, 1981; OLSSON & GRANELI, 1991; FIGUEROA *et al.*, 1998; OLLI, 1999; FAUCHOT *et al.*, 2005; SCHOFIELD *et al.*, 2006). The migratory behaviour of different species under same environmental conditions is distinguished with regard to the velocity and timing (OLSSON & GRANELI, 1991). This fact was observed also during our June experiment: *Prorocentrum micans* and *Heterocapsa* sp. migrated through the whole water column, while migration of *Dinophysis sacculus* was apparently limited to the distance from surface to the thermocline layer. These differences can be associated with different circadian rhythms of individual species (KAMYKOWSKI, 1995; JI & FRANKS, 2007) and may also result from swimming speeds different species can achieve (LEVANDOWSKY & KANETA, 1987). On the other hand, the environmental factors that govern the dinoflagellate' movements in the natural water column are the nutrient availability, light intensity and gravity (KAMYKOWSKI & YAMAZAKI, 1997), together with physical forcing caused by winds and currents (BASTERRETXEA *et al.*, 2005).

During June experiment, depth profile of phosphate only conformed to the picture of a stratified water column, while nitrate and ammonium were measured in the limiting concentrations throughout the water column. Unfortunately, we don't have data on organic nutrients concentrations during our experiments that can represent an important source for dinoflagellates (SMAYDA, 1997). Nevertheless, if additional experiments will confirm that migrating species adopted the strategy of dark phosphate uptake near the bottom, this could indicate vertical migration as an important advantage in the phosphorus limited environment (SOLIDORO *et al.*, 2009).

It is worth stressing, however, that some of our results have low confidence due to low abundances and need to be verified in the field by additional experiments. Especially important would these experiments be for *Dinophysis* species as its migration may have an impact on the incidence of DSP intoxications. In a previous study from the same area, the June abundance peak of *Dinophysis* species (which was mostly constituted of *D. sacculus*) was especially evident in the surface layer (FRANCE & MOZETIČ, 2006). In that study, authors suggested vertical migrations to be the cause of such distribution. Such migration pattern of toxic species can have implications for the toxicity of mussels, which grow on ropes that extent from the surface to the depth of approx. 5 m. If the cells of *D. sacculus* migrate along the upper part of the water column only, then the mussels are continuously exposed to them and can accumulate greater amounts of toxins than in the case cells would migrate to the bottom layer during night.

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REFERENCES

BASTERRETXEA, G., E. GARCÉS, A. JORDI, M. MASÒ & J. TINTORÉ. 2005. Breeze conditions as a favoring mechanism of *Alexandrium taylori* blooms at a Mediterranean beach. Est. Coast. Shelf Sci., 62: 1-12.

CABRINI, M., S. ČOK, I. PECCHIAR, A. BERAN & S. PREDONZANI. 2001. *Alexandrium* distribution and toxicity in the Gulf of Trieste (Northern Adriatic Sea). In: G.M. Hallegraeff, S.I. Blackburn, C.J. Bolch and R.J. Lewis (Editors). Harmful Algal Bloom 2000. IOC UNESCO, Paris, pp. 22-25.

FAUCHOT, J., M. LEVASSEUR & S. ROY. 2005. Daytime and nighttime vertical migrations of *Alexandrium tamarense* in the St. Lawrence estuary (Canada). Mar. Ecol. Prog. Ser., 296: 241-250.

FIGUEROA, F.L., F.X. NIELL, F.G. FIGUEIRAS & M.L. VILLARINO. 1998. Diel migration of phytoplankton and spectral light field in the Ria de Vigo (NW Spain). Mar. Biol., 130: 491-499.

FRANCE, J. & P. MOZETIČ. 2006. Ecological characterization of toxic phytoplankton species (*Dinophysis* spp., Dinophyceae) in Slovenian mariculture areas (Gulf of Trieste, Adriatic Sea) and the implications for monitoring. Mar. Pollut. Bull., 52: 1504-1516.

FRANCE, J. 2009. Long-term structural changes of the phytoplankton community of the Gulf of Trieste. Ph.D. Thesis, University of Ljubljana, 160 pp.

GRASSHOFF, K., M. EHRHARDT & K. KREMLING. 1983. Methods of Seawater Analysis. Verlag Chemie. Weinheim, 419 pp.

HEANEY, S.I. & R.W. EPPLEY. 1981. Light, temperature and nitrogen as interacting factors affecting diel vertical migrations of dinoflagellates in culture. J. Plankton Res., 3: 331-344.

HONSELL, G., R. POLETTI, M. POMPEI, L. SIDARI, A. MILANDRI, C. CASADEI & R. VIVIANI. 1996. *Alexandrium minutum* Halim and PSP contamination in the Northern Adriatic Sea (Mediterranea Sea). In: T. Yasumoto, Y. Oshima and Y. Fukuyo (Editors). Harmful and toxic algal blooms. Intergovernmental Oceanographic Commission of UNESCO, pp. 77-80.

JI, R. & P.J.S. FRANKS. 2007. Vertical migration of dinoflagellates: model analysis of strategies, growth, and vertical distribution patterns. Mar. Ecol. Prog. Ser., 344: 49-61.

KAMYKOWSKI, D. 1981. Laboratory experiments on the diurnal vertical migration of the marine dinoflagellate through temperature gradients. Mar. Biol., 62: 57-64.

KAMYKOWSKI, D. 1995. Trajectories of autotrophic marine dinoflagellates. J. Phycol., 31: 200-208.

KAMYKOWSKI, D. & H. YAMAZAKI. 1997. A study of metabolism-influenced orientation in diel vertical migration of marine dinoflagellates. Limnol. Oceanogr., 42: 1189-1202.

KOIZUMI, Y., T. UCHIDA & T. HONJO. 1996. Diurnal vertical migration of *Gymnodinium mikimotoi* during a red tide in Hoketsu Bay, Japan. J. Plankton Res., 18: 289-294.

LEVANDOWSKY, M. & P.J. KANETA. 1987. Behaviour in dinoflagellates. In: F.J.R. Taylor (Editor). The biology of dinoflagellates. Blackwell Scientific Publications, Oxford, pp. 360-398.

MACINTYRE, G.J., J.J. CULLEN & A.D. CEMBELLA. 1997. Vertical migration, nutrition and toxicity in the dinoflagellate *Alexandrium tamarense*. Mar. Ecol. Prog. Ser., 148: 201-216.

OLLI, K. 1999. Diel vertical migration of phytoplankton and heterotrophic flagellates in the Gulf of Riga. J. Mar. Syst., 23: 145-163.

OLSSON, P. & E. GRANELI. 1991. Observations on diurnal vertical migration and phased cell division for three coexisting marine dinoflagellates. J. Plankton Res., 13: 1313-1324.

PASSOW, U. 1991. Vertical migration of *Gonyaulax catenata* and *Mesodinium rubrum*. Mar. Biol., 110: 455-463.

RALSTON, D.K., D.J. MCGILLICUDDY JR. & D.W. TOWNSEND. 2007. Asynchronous vertical migration and bimodal distribution of motile phytoplankton. J. Plankton Res., 29: 803-821.

SCHOFIELD, O., J. KERFOOT, K. MAHONEY, M.

MOLINE, M. OLIVER, S. LOHRENZ & G. KIRKPATRICK. 2006. Vertical migration of the toxic dinoflagellate *Karenia brevis* and the impact on ocean optical properties. J. Geophys. Res., 111, C06009, doi:10.1029/2005JC003115.

SMAYDA, T.J. 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol. Oceanogr., 42: 1137-1153.

SMAYDA, T.J. 2002. Adaptive ecology, growth strategies and the global bloom expansion of dinoflagellates. J. Oceanogr., 58: 281-294.

SOLIDORO, C., M. BASTIANINI, V. BANDELJ, R. CODERMATZ, G. COSSARINI, D. MELAKU CANU, E. RAVAGNAN, S. SALON & S. TREVISANI. 2009. Current state, scales of variability and decadal trends of biogeochemical properties in the Northern Adriatic Sea. J. Geophys. Res., C, 114: C07S91.

TOWNSEND, D.W., S.L. BENNETT & M.A. THOMAS. 2005. Diel vertical distributions of the red tide dinoflagellate *Alexandrium fundyense* in the Gulf of Maine. Deep-Sea Res. II, 52: 2593-2602.

UTERMÖHL, H. 1958. Zur Vervollkommung der Quantitativen Phytoplankton-Metodik. Mit. Int. Verein. Theor. Angew. (na engl) Limnol., 9: 1-38.

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Preliminarna studija o vertikalnim migracijama dinoflagelata u dinamičnom obalnom moru (Tršćanski zaljev, sjeverni Jadran)

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SAŽETAK

Svrha ove preliminarne studije je definirati obrasce vertikalnih migracija zajednice dinoflagelata u plitkom obalnom moru. Migracije su praćene na području uzgoja dagnji, kroz dva 24-satna uzorkovanja, prvo za vrijeme pomiješanih i drugo za vrijeme stratificiranih uvjeta vodenog stupca. Unatoč promjenjivom fizičkom okolišu bili smo u mogućnosti slijediti vertikalne migracije nekih autotrofnih vrsta dinoflagelata u razdoblju stratificiranog vodenog stupca. Rezultati također sugeriraju da *Heterocapsa* sp. može očuvati obrazac svoje vertikalne migracije čak i u pomiješanim uvjetima. Migracije su promatrane i za vrstu *Dinophysis sacculus* koja može uzrokovati dijaretičko trovanje školjkama (DSP) na tom području.

Ključne riječi: dinoflagelati, vertikalne migracije, dinamično okruženje, obalno more, Jadran