



Grazing and Feeding Selectivity of *Oithona davisae* in the Black Sea: Importance of Cryptophytes

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Abstract

Feeding of non-indigenous cyclopoid copepod *Oithona davisae* in natural coastal phytoplankton assemblages from the Sevastopol Bay, the Black Sea, in late July and in late November 2013 and in artificial phytoplankton assemblage (mixture of 5 cultured microalgae) have been studied in laboratory experiments using flow cytometry. Three distinct main clusters of phytoplankton ($2 \mu\text{m} < \text{ESD} < 20 \mu\text{m}$) were identified in the natural water samples in both sampling period: photosynthetic picoeukaryotes, nanophytoplankton and nanoplanktonic cryptomonads (NanoPE). Female *O. davisae* grazed on all 3 phytoplankton groups but the highest ingestion rate in terms of carbon was on NanoPE with the value of $0.29 \pm 0.03 \mu\text{g C prey}^{-1} \text{d}^{-1}$ in July. Low abundance of 3 phytoplankton groups in late November resulted in a very low but significant carbon ingestion on cryptophytes ($0.008 \pm 0.002 \mu\text{g C prey}^{-1} \text{d}^{-1}$) than on the other groups. Clearance rates and electivity indices confirmed positive selection only for cryptophytes from natural phytoplankton assemblages, and for cryptophyte strain IBSS-Cr54 and *Tetraselmis suecica* from artificial plurialgal mixture. Highest daily specific carbon-rations of *O. davisae* females were found on cryptophytes (ESD 7 - 12 μm): up to 135% and 184% body carbon d-1 in late July and on artificial phytoplankton assemblage, respectively.

Keywords: Copepod; phytoplankton assemblages; ingestion rate

Introduction

While small planktonic marine oithonids exist ubiquitously in World Ocean (Paffenhöfer, 1993), the assessment of zooplankton using sampling technique with the mesh size larger than (or equal to) 150 μm resulted in a serious undervaluation of their abundance (Altukhov *et al.*, 2015; Dahms, Tseng & Hwang, 2015; Hwang, Kumar, Dahms, Tseng & Chen, 2007), misunderstanding of their role in trophic interactions in the sea (Turner, 2004) and of their grazing impact on phytoplankton primary production. Small marine cyclopoid *Oithona davisae* Ferrari and Orsi, 1984, is a typical neritic copepod (Uchima, 1988), which occasionally occurring high abundances in estuarine and coastal areas (Hirota, 1990; Nishida, 1985). Originally distributed in the west Pacific coastal waters it was considered endemic to the temperate coastal waters of East Asia, particularly in Japan coastal areas (Uchima, 1988; Uye & Sano, 1995). This species was suggested to be transferred with ballast waters of transoceanic ships (Choi, Kimmerer, Smith, Ruiz & Lion, 2005; Kasyan, 2010; Lawrence & Cordell, 2010), and thus invaded different areas of the World

Ocean coastal waters: off western USA (Ambler, Cloern & Hutchinson, 1985; Ferrari & Orsi, 1984), off southern Chile (Hirakawa, 1988), Mediterranean (Saiz, Calbet, Broglio & Mari, 2003) and Northern (Cornils & Wend-Heckmann, 2015) seas.

In the Black Sea, *O. davisae* was observed first time in December 2001 (mis-identified as *Oithona brevicornis*, Zagorodnyaya, 2002, and later re-identified as *O. davisae*, Temnykh & Nishida, 2012). This non-indigenous species quickly invaded the Black Sea waters, and since 2006 dominated during late summer - autumn periods in Sevastopol Bay and the nearest coastal ecosystem (Gubanova & Altukhov, 2007). Since 2008 *O. davisae* periodically contributed up to 99% of total copepod abundance in Sevastopol Bay (Altukhov, Gubanova & Mukhanov, 2014; Svetlichny *et al.*, 2016), similar to domination of *O. davisae* observed in Tokyo Bay where it contributed about 99% of the total number of copepods during the warm periods in 1980-s (Tsuda & Nemoto, 1988). It was registered later near Romanian (Timofte & Tabarcea, 2012), Bulgarian (Mihneva & Stefanova, 2013) and recently Turkish coasts (Üstün & Terbiyik Kurt, 2016; Yıldız, Feyzioğlu & Besiktepe, 2016), and

in Büyükçekmece Bay and in the Golden Horn Estuary located in the North-eastern (Doğan & Isinibilir, 2016; Isinibilir, Svetlichny & Hubareva, 2016).

Wide dissemination of *O. davisae* in variable climatic zones and its survival in wide range of temperature from + 6 °C (Svetlichny et al., 2016), and even from inconceivable - 1.8 °C in the North Sea (Cornils & Wend-Heckmann, 2015) up to + 28 °C in Pacific (Uye & Sano, 1998) and + 29 °C in the Black Sea (Svetlichny et al., 2016), was supposed due to the unique adaptive strategy of this species: flexible metabolism, high survival rate of overwintering fertilized females during long unfavorable cold winter, and emergence of the offsprings originated from these overwintering females in favorable conditions in late spring (Svetlichny et al., 2016).

The feeding of small oithonids is less studied than that of larger copepod species. The big oithonids, *Oithona* spp. can graze on diatoms (10 µm) in cold subantarctic waters (Atkinson, 1995). Small cyclopoids *O. nana* were grown in laboratory fed only diatom *Phaeodactylum tricorutum* (Haq, 1965). Evidence for *O. davisae* grazing on diatoms is still controversial. In particular, diatom cells were not found in *O. davisae* guts during diatom bloom in the Japan Sea (Uchima, 1988). On the contrary, the species ingested abundantly diatoms in natural phytoplankton assemblage of the NW Mediterranean Sea (Calbet et al., 2003). Diatoms were cleared by *O. davisae* at a high rate in late November in San Francisco Bay but were avoided during other months (Gifford, Rollwagen-Bollens & Bollens, 2007).

Neither in nature, nor in laboratory experiments feeding on only diatoms resulted in successful reproduction and growth of *O. davisae* (Uchima & Hirano 1986) proving that this prey is not suitable for the species recruitment. In different environments and experimental conditions *O. davisae* consume a diversity of prey, both phytoplankton and protozooplankton (Gifford, Rollwagen-Bollens & Bollens, 2007; Saiz, Griffell, Calbet & Isari, 2014). Despite the highest grazing rate of this cyclopoid was registered on ciliates and heterotrophic dinoflagellates (Saiz, Griffell, Calbet & Isari, 2014), laboratory-oriented method indicated that *O. davisae* can compete with microzooplankton for phytoplankton (Hiromi, 1996). Gut pigment analysis of *O. davisae* from a Japanese estuary confirmed that its diet was based mostly on Chl-a (Islam, Ueda & Tanaka, 2005). Numerically dominant in Tokyo Bay *O. davisae* consumed 3.69 mg Chl a m⁻² day⁻¹, accounting for 74% of the total consumption by the copepod community (Tsuda & Nemoto, 1988).

Though *O. davisae* is now highly abundant in the coastal Black Sea, its feeding ecology in the Black Sea environment was not studied yet. All-year-round presence of *O. davisae* implies the essential trophic linkage of this small cyclopoid to its potential prey. To be successful, the invasion and naturalization of *O. davisae* in the Black Sea had to be supported by

sufficient adequate food resources which allowed the alien copepod to produce high number and biomass during their dominance in zooplankton community during the warm seasons and survive during the cold periods. However, no significant shifts were observed in number and species composition of the main phytoplankton groups in Sevastopol Bay despite high *O. davisae* abundance. Meanwhile, it could be supposed that its invasion should have put a strong imprint on phytoplankton structure of Sevastopol Bay.

The goal of the present study was to investigate the grazing rate and feeding selectivity of *O. davisae* on the phytoplanktonic community in the Black Sea. We pursued this aim by using a combination of natural phytoplankton and artificial pluri-algal experiments.

Materials and Methods

Feeding experiments were carried out with the natural phytoplankton assemblages from the Sevastopol Bay in late July (Exp1) and in late November (Exp2) 2013, during the period of high egg production and during the period of low egg production by *O. davisae*, respectively (Svetlichny et al., 2016). Besides, a feeding experiment with mixed laboratory phytoplankton cultures was conducted (Exp3) at similar biomass carbon (C) level and close to proportion of the functional groups of nanophytoplankton as in the summer field assemblages. All experiments were performed at 21±1 °C, 12 h dimmed light : 12 h dark light cycle.

Field Collection of Copepods

Copepods were collected by slow horizontal hauls in the surface layer (0 - 1 m) in Sevastopol Bay at the central quay (depth about 3 m) using a plankton net (mouth diameter 0.3 m, mesh size 100 µm) fitted with a non-filtering cod-end (0.5 L). Samples were transported to laboratory within 0.5 h after collection. Once in the laboratory, undamaged vigorous *O. davisae* females were sorted from alive zooplankton sub-samples in Bogorov chamber under a stereomicroscope.

Copepods were maintained in 0.2 µm filtered sea water (FSW) 18 salinity, until the beginning of experiments to adapt to the experimental physical conditions and to assure that selected specimens were healthy, and their guts were cleared from any previously ingested particles. FSW was also used as a basis for preparation of microalgae mixture for grazing experiments.

Phytoplankton Assemblages

Experiments 1 (Exp1) and 2 (Exp2) were performed to assess grazing of *Oithona davisae* on natural microalgae assemblages. To obtain food suspension of natural phytoplankton, the natural sea water was back-siphoned gently through a submerged

20 µm mesh to remove all possible predators and the large prey; the obtained suspension was condensed by very mild reverse filtration on a track membrane with the pore size 3 µm to prevent fragile cells damage.

To assess grazing of *O. davisae* on artificial microalgae assemblage (Exp3), considered to mimic hypothetical natural phytoplankton, the food suspension was prepared by mixing aliquots of monospecific microalgae cultures. The mixture consisted of five nanoflagellate microalgae species from four different families: 3 species with only chlorophyll fluorescence (Prymnesiophyceae *Isochrysis galbana*, Prasinophyceae *Tetraselmis suecica*, Dinophyceae *Prorocentrum minimum*) and 2 species with both chlorophyll and phycoerythrin fluorescence (Cryptophyceae *Rhodomonas salina* and the Black Sea cryptophyte isolate - IBSS-Cr54 strain). Cultures of microalgae were grown semi-continuously at a light intensity $\approx 44 \text{ mE m}^{-2} \text{ s}^{-1}$ and diluted every 3-4 d by a half Walne medium (Andersen, 2005) depending on their growth rate to keep them in exponential phase. To obtain needed concentration of cells in mixed culture assemblage similar to Exp1, the mixture of cultures was diluted by FSW prior the experiment (Table 1, Exp 3). Before the experiment, the cells from each stock culture were analyzed by flow cytometry for their pigment contents (chlorophyll and phycoerythrin) and cell size (equivalent spherical diameter, ESD).

Grazing Experiments and Sample Processing

Grazing experiments were performed according to the food removal technique (Gifford, 1993), in 50 mL glass beakers during 24 hours. The control beakers (2 replicates) contained only phytoplankton assemblages, while the treatment beakers (3 replicates) contained phytoplankton prey assemblages and *O. davisae* females. Actively swimming females of *O. davisae* were transferred gently to the treatment beakers ($\sim 3 - 5 \text{ copepods mL}^{-1}$). Initial sub-samples (1 mL aliquots for flow cytometry analysis) were taken from the experimental and control beakers after 1 h incubation (Gifford, 1993). The beakers were sealed with Parafilm and incubated at room temperature $21 \pm 1 \text{ }^\circ\text{C}$, a 12 h dimmed light : 12 h dark cycle, and incubated at experimental conditions. Very low turbulence ($\sim 0.9 \text{ strokes min}^{-1}$) positively affecting *O. davisae* feeding activity (Saiz, Calbet, Broglio & Mari, 2003) was achieved by locating the beakers on a slowly shaking surface. At the end of the experiments, the final samplings of 1 mL aliquots were taken for the flow cytometry analysis, and all copepods were analyzed for their condition under a stereomicroscope. Copepod mortality during experimental periods was always negligible. For the morphological measurements of experimental copepods, minimum 20 females from each beaker were anesthetized using 300 ppm MS-222, their prosome length (PL) and width (PW) were measured by taking the digital pictures with

a camera attached to the inverted microscope Nikon Eclipse-200 at magnification 10 x 10, through image analysis by ImageJ software (<http://imagej.nih.gov/ij/>).

Flow Cytometry

To assess the grazing effect of *O. davisae* on phytoplankton assemblages, phytoplankton concentration at the initial and at the end of the experiment were determined; analysis (identification of dominant clusters and estimation of their cell concentration and biomass) was carried out by flow cytometry in fresh subsamples. Phytoplankton groups were distinguished by combination of a taxonomic (by the two major autofluorescent pigments, chlorophyll and phycoerythrin) and allometric (size) types of cytometry analysis (Cucci, Shumway, Brown & Newel, 1989). Photoautotrophic cells were classified to size and taxonomical groups and enumerated using a Cytomics™ FC 500 flow cytometry system (Beckman Coulter Inc., USA), equipped with a 488-nm (20 mV) argon-ion output laser, and CXP acquisition and analysis software (Beckman Coulter Inc., USA). Gating and sizing the cells were performed using Flowing Software v. 2.5.0 (Perttu Terho, Turku Centre for Biotechnology, University of Turku, Finland, www.floowingsoftware.com). The standard forward light scatter (FSC), orange fluorescence (FL2, 575 nm), and red fluorescence (FL4, 675 nm) channels were used for the acquisition and analysis. At least 3,000 cells were analyzed in each cell population at the flow rates of 15 to 60 $\mu\text{L min}^{-1}$ and the acquisition time of 360 to 480 s.

Phytoplankton groups were gated in chlorophyll-*vs*-size (FL4/FSC) and chlorophyll-*vs*-phycoerythrin (FL4/FL2) cytograms (4 decades, log scale) on the basis of their autofluorescence properties and scattering characteristics. Picoeukaryotes had intermediate FL4 and FSC signals lower than nanophytoplankton and were distinguished clearly by zero FL2 signal. As a part of nanophytoplankton, cryptomonads were identified by their bright orange fluorescence (the highest FL2 signal). Light microscopy observation of cryptomonad cells confirmed flow cytometry data. Other nanophytoplankton from natural assemblages, and microalgal species from pluri-algal mixtures were clearly gated from each other in the FL4/FSC cytograms.

Cell size measurements were calibrated in terms of equivalent spherical diameter (ESD, µm) using a set of standard polystyrene beads (Polysciences, Inc.) in the size range between 0.5 and 10 µm, calibration curves were obtained for each flow cytometry protocol by plotting the ESD as a function of the FSC signal (coefficient of determination $r^2 > 0.8$ in all cases). Average ESD, cell volume and biovolume were calculated for every cell population from the calibration curves (Olson, Zettler & DuRand, 1993).

Calculations

Clearance (F, mL cop⁻¹ d⁻¹) and ingestion (I, cells cop⁻¹ d⁻¹) rates of *Oithona davisae* were calculated according to Frost (1972) from the differences in phytoplankton cell concentrations in control and treatment beakers at start and at the end of experiment. If the calculated values of clearance rates were negative they were interpreted as zero ingestion (Nejstgaard, Gismervik & Solberg, 1997). Phytoplankton cell biovolumes obtained from flow cytometry data (ESD) were converted to carbon equivalent biomass using specific taxon conversion factor (Verity *et al.*, 1992). Using proportion of carbon ingestion rate and mean copepod carbon content, ingestion rates were calculated as carbon ingestion rate ($\mu\text{g C}_{\text{prey}} \text{ cop}^{-1} \text{ d}^{-1}$), or carbon-specific ingestion rate ($\mu\text{g C}_{\text{prey}} \mu\text{g C}_{\text{cop}}^{-1} \text{ d}^{-1}$) expressed as daily ration of copepod as a percentage of body carbon (% body C d⁻¹). Carbon equivalents of copepods were calculated from sizes by applying carbon-size relationships (Uye, 1982) for the same species: female ($\mu\text{g C}$) = $1.26 \cdot 10^{-4} \times \text{PL}^{1.31}$, where PL is a prosome length of *O. davisae* females obtained by direct measurements of the specimens at the end of each experiment. Selective feeding of *O. davisae* females was assessed using electivity index E_i^* (Vanderploeg & Scavia, 1979) recommended in cases where the different food types are not equally abundant, as in our experiments. Statistical evaluation of data was conducted by 1-way analysis of variance and Student's *t*-test (STATISTICA) prior to the calculations to find out whether grazing coefficients were significantly different from zero, to test the possible effect of trophic cascade, and to assess statistical significance between the means. Average values presented in the figures and table are means \pm SD.

The copepod concentrations used in the experiments may seem too high for extrapolation of the resulting grazing rates to natural community, however, considering high phytoplankton cell concentrations in concentrated natural and in artificial microalgae assemblages (see Table 1), an equally high abundance of predators was needed to detect the cell reduction by grazing effect.

Results

Phytoplankton Groups in Natural and Artificial Assemblages

Figure 1 shows the clusters of natural particles with chlorophyll fluorescence and the forward scattering (Figure 1A), and chlorophyll vs phycoerythrin fluorescence (Figure 1B) obtained by flow cytometry. On the flow cytometer plots, three distinct main clusters of phytoplankton (size range $2 \mu\text{m} < \text{ESD} < 20 \mu\text{m}$) were identified in the natural water samples collected in late July and late November 2013 from Sevastopol Bay: photosynthetic

picoeukaryotes (PicoEu), nanophytoplankton (Nano1) and nanoplanktonic cryptomonads (NanoPE). Overall cell abundance and carbon biomass of PicoEu, Nano1 and NanoPE in natural seston, before concentrating to experimental concentrations, exceeded 10^8 cells L⁻¹ and 0.5 mg C L^{-1} in July and 2×10^6 cells L⁻¹ and $15 \mu\text{g C L}^{-1}$ in November, respectively. The most significant inter-seasonal difference was observed in Nano1 cell abundance and carbon biomass, being approximately 2-fold higher in July than in November. The lowest inter-seasonal variation was observed in NanoPE abundance, about an order of magnitude lower in cell number and biomass.

We considered the certain species from artificial microalgae assemblage (Table 1, Exp 3) to mimic probable phytoflagellates from hypothetical natural phytoplankton: 1) *Isochrysis galbana* imitated group Prymnesiophyceae “*Isochrysis*-like”, or “*Chrysochromulina*-like”, the latter occasionally dominate Nano1 fraction; 2) *Tetraselmis suecica* imitated nanoplankton Prasinophyceae occasionally contributing significantly Nano1 during late summer; 3) *Rhodomonas salina* and 4) cryptophyte IBSS-Cr54 strain mean imitated cryptophytes fraction (Nano2-PE); 5) *Prorocentrum minimum*, simulated Dinophyceae group present permanently in phytoplankton as numerically minor component. Five species of microalgae were clearly detected as 5 clusters on chlorophyll fluorescence vs forward scattering (Figure 2A) and chlorophyll vs phycoerythrin fluorescence cytogram (Figure 2B).

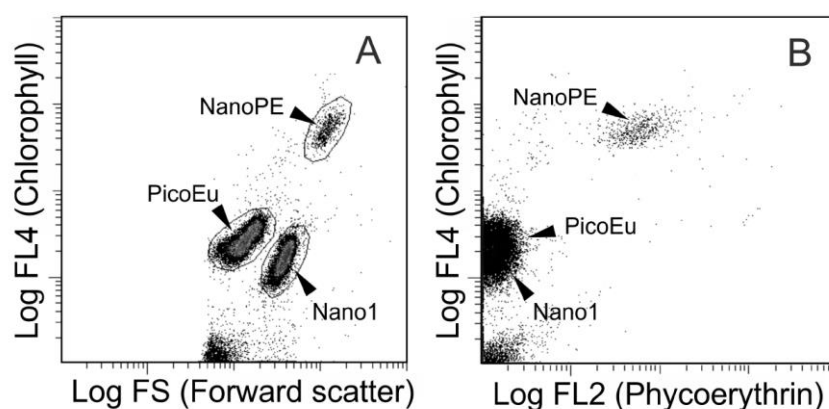
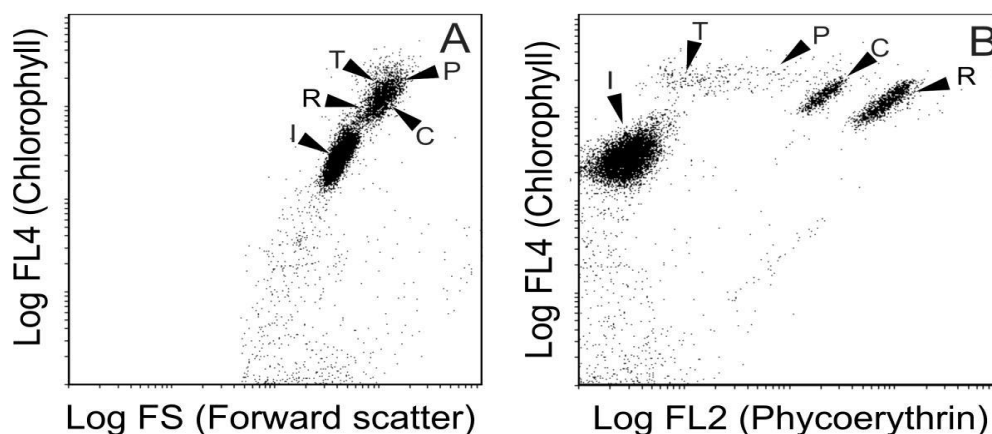
Grazing on Natural Phytoplankton Assemblages

Experimental cell concentrations and prey sizes are given in Table 1. In all experiments the concentration of phytoplankton groups in the treatment beakers were significantly ($p < 0.05$) lower than those in the final control beakers. In late July experiment, total food concentration was 5.1 mg C L^{-1} and Nano1 represented almost 55% of food resources in terms of biomass (as mg C L^{-1}), PicoEu and NanoPE contributed 8 and 37% to the carbon pool, respectively (Table 1). The filtration rate in July (Figure 3A) was significantly higher for NanoPE in comparison with other phytoplankton but ingestion rate in terms of cells number for this group was the lowest (Figure 3B). Total ingestion rate in terms of carbon of *O. davisae* on phytoplankton in size range $2 \mu\text{m} - 20 \mu\text{m}$ was $0.36 \pm 0.04 \mu\text{g C}_{\text{prey}} \text{ cop}^{-1} \text{ d}^{-1}$ (Figure 3C). Copepods grazed on all 3 phytoplanktonic groups but the highest ingestion rate in terms of carbon was on cryptophytes (NanoPE) with the value of $0.29 \pm 0.03 \mu\text{g C}_{\text{prey}} \text{ cop}^{-1} \text{ d}^{-1}$ which was significantly ($P < 0.05$) higher than that of other groups. Thereby, cryptophytes contributing only 3% to cell abundance in late July Exp 1, were removed as 14% of cumulative cell ingestion; and contributing 37% of carbon biomass to overall phytoplankton biomass, reached 80% of daily ingested carbon by *O. davisae* females (Figure 4A). Cytometry data were

Table 1. Initial abundance (mean values) of the different prey groups in phytoplankton assemblages in grazing experiments. ESD=Equivalent spherical diameter, Conc.=Concentrations.

Experiments	Algal groups/species	ESD (\pm SD) μ m	Conc. 10^6 cells L^{-1}	Conc. $mg C L^{-1}$
Exp1 with natural phytoplankton in July 2013	PicoEu*	2.1 (0.1)	465	0.4
	Nano1*	4.3 (0.1)	373	2.8
	NanoPE*	9.2 (1.6)	25	1.9
	Total		863	5.1
Exp2 with natural phytoplankton in November 2013	PicoEu*	1.9 (0.1)	9.4	0.006
	Nano1*	5.0 (0.1)	3.1	0.036
	NanoPE*	7.7 (1.3)	1.6	0.072
	Total		14.1	0.114
Exp3 with mixture of cultivated microalgae	Iso**	4.9 (0.2)	135	1.5
	Rho**	9.7 (0.6)	19	1.6
	Cr-54**	10.9 (1.4)	6	0.7
	Tet**	7.7 (0.9)	7	0.3
	Pro**	13.1 (1.5)	2	0.4
	Total		169	4.6

* PicoEu - photosynthetic picoeukaryotes, Nano1- photosyntheticnanoplankton excluding nanoplankton cryptomonads (NanoPE).
** Iso - *Isochrysis galbana*, Rho - *Rhodomonas salina*, Cr-54 - cryptophyte IBSS-CrPr54, Tet - *Tetraselmis suecica*, Pro - *Prorocentrum minimum*.

**Figure 1.** Chlorophyll fluorescence vs forward scatter (A) and chlorophyll vs phycoerythrin fluorescence (B) cytograms of natural phytoplankton assemblage. Abbreviations: PicoEu – photosynthetic picoeukaryotes; Nano1 - photosynthetic nanoplankton; NanoPE - nanoplankton cryptomonads.**Figure 2.** Chlorophyll fluorescence vs forward scatter (A) and chlorophyll vs phycoerythrin fluorescence (B) cytograms of the artificial phytoplankton assemblage at the start of the experiment 3. Abbreviations: I - *Isochrysis galbana*; T - *Tetraselmis suecica*; P - *Prorocentrum minimum*; R - *Rhodomonas salina*; C - cryptophyte IBSS-Cr54.

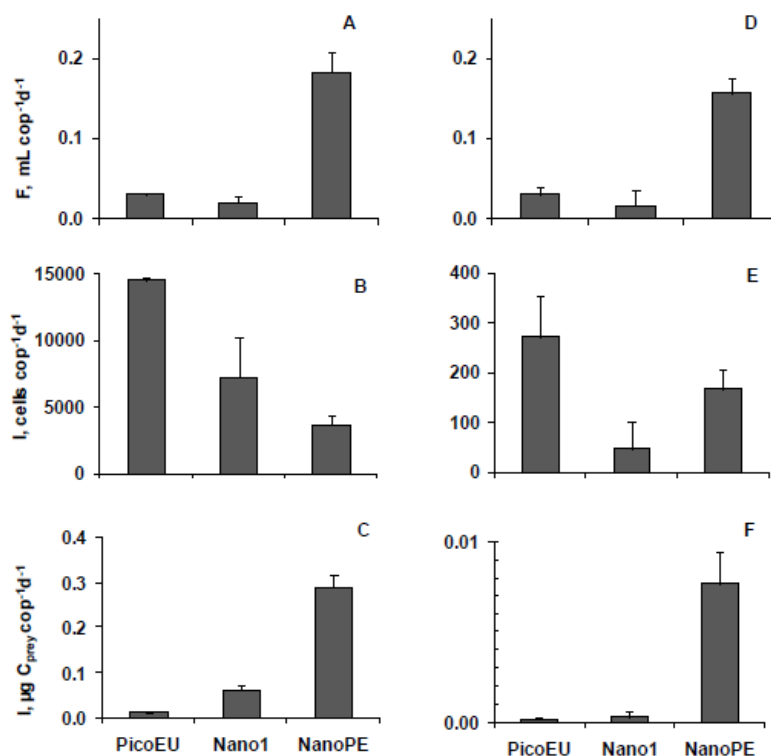


Figure 3. Clearance (F, mL cop⁻¹ d⁻¹) (3A and 3D) and ingestion rates of *O. davisae* females in cell number (I, cells cop⁻¹ d⁻¹) (3B and 3E) and carbon biomass (μg C_{prey} cop⁻¹ d⁻¹) (3C and 3F) on phytoplankton fractions: piceoplankton (PicoEu), nanoplankton (Nano1) and cryptophytes (NanoPE) of natural phytoplankton assemblages in late July (3A - 3C) and late November (3D - 3F). Error bars represent Mean ± SD

confirmed by light microscopy: numerous cryptophytes (8 clearly distinguished cell forms) observed at start of experiment were eliminated to few cells in experimental beakers while were present in significant amounts in final control beakers to the end of feeding experiment. In July, the body carbon of female was 0.199 ± 0.003 μg C, daily ingestion rate of *O. davisae* ranged from the equivalent of 7% (PicoEu) to 135% (the cryptophytes) of the body carbon. The total daily ingestion rate was 169% of the body carbon.

In late November (Exp2), the total microalgae concentration was 0.114 mg C L⁻¹ and the contribution of PicoEu, Nano1 and NanoPE to food phyto-resources were 5, 32 and 63%, respectively (Table 1). The overall water volume swept by *O. davisae* females in late November phytoplankton assemblage was comparable with that in late July. The only phytoplankton prey in the size range $2 \mu\text{m} < \text{ESD} < 20 \mu\text{m}$ for which *O. davisae* elicited significant clearance rate 0.16 ± 0.02 mL cop⁻¹ d⁻¹ was NanoPE fraction (Figure 3D). Low cell abundance of 3 fractions in late November assemblage resulted in a very low the only significant among other phytoplankton cells, cryptophytes carbon but ingestion of 0.008 ± 0.002 μg C_{prey} cop⁻¹ d⁻¹ (Figure 3F). Thereby, even in poor late November phytoplankton assemblage, cryptophytes were removed as 43% of cumulative phytoplankton cell ingestion and made up 92% of daily ingested carbon of

O. davisae whereas they contributed 11% to overall cell number and 63 % to overall carbon biomass of 3 fractions (Figure 4B). While clearance rates of *O. davisae* in late July and late November for certain phytoplankton fractions were comparable, ingestion rates in phytoplankton cells and carbon biomass appeared to be roughly two orders of magnitude higher in late July than in late November. In November, the body carbon of female was 0.210 ± 0.009 μg C, daily ingestion rate of phytoplankton for *O. davisae* ranged from the equivalent of 0.01% (PicoEu) to 0.65% (NanoPE) of the body carbon.

Grazing on Artificial Phytoplankton Assemblages

In the mixed phytoplankton culture experiment, the total food carbon concentration offered to *Oithona* was 4.6 mg C L⁻¹ (Table 1). The contributions of 5 different microalgae, *I. galbana*, *R. salina*, cryptophyte IBSS-Cr54 strain, *T. suecica* and *P. minimum* were 33, 36, 16, 7 and 9% to the total carbon food biomass (Table 1). The clearance rates of *O. davisae* on the cryptophyte IBSS-Cr54 strain and on *T. suecica* were the highest with the values of 0.20 ± 0.06 and 0.21 ± 0.09 mL cop⁻¹ d⁻¹, respectively (Figure 5A), and the cell ingestion on these algae were not significantly different (Figure 5B). The lowest clearance rate (0.05 ± 0.01 mL cop⁻¹ d⁻¹) was observed

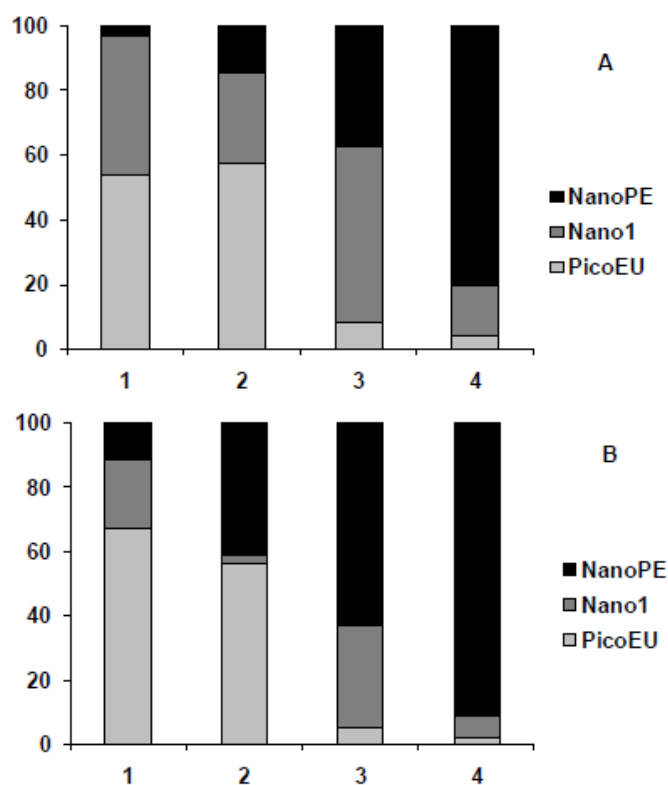


Figure 4. Relative contribution (%) of phytoplankton fractions: picoeuplankton (PicoEu), nanoplankton (Nano1) and cryptophytes (NanoPE) to the sum of their abundance (cells mL⁻¹) and carbon biomass ($\mu\text{g C mL}^{-1}$) in late July (4A) and late November (4B) natural phytoplankton assemblages versus percent contribution (%) of these fractions in copepods ingestion rate in cell number (cells copepod⁻¹ day⁻¹) and carbon biomass ($\mu\text{g C}_{\text{prey}} \text{cop}^{-1} \text{day}^{-1}$) of ingested prey. 1 - % cells mL⁻¹; 2 - % I, cells cop⁻¹ day⁻¹; 3 - % $\mu\text{g C L}^{-1}$; 4 - % I, $\mu\text{g C}_{\text{prey}} \text{cop}^{-1} \text{day}^{-1}$.

when feeding on *I. galbana* but it resulted in the maximum cell ingestion rate (around 8400 ± 3100 cells cop⁻¹ d⁻¹) as a consequence of high cell concentration of this microalgae (Table 1). No grazing of *O. davisae* on *P. minimum* was detected at all (Figure 5A, B).

Ingestion rate in terms of carbon insignificantly differed between 2 species of cryptophytes (0.21 ± 0.01 vs 0.16 ± 0.04 $\mu\text{g C}_{\text{prey}} \text{cop}^{-1} \text{d}^{-1}$, for *R. salina* and IBSS-Cr54 strain, correspondingly) and were comparatively lower when feeding on *I. galbana* 0.10 ± 0.04 $\mu\text{g C}_{\text{prey}} \text{cop}^{-1} \text{d}^{-1}$ and on *T. suecica* 0.05 ± 0.04 $\mu\text{g C}_{\text{prey}} \text{cop}^{-1} \text{d}^{-1}$ (Figure 5C). Hereby, two cryptophytes, *R. salina* and cryptophyte IBSS-Cr54 strain, contributing about 15 % to cell abundance in mixed culture assemblage, were removed as about 30 % of cumulative cell ingestion of *O. davisae*; and contributing about 50% to carbon biomass of pluralgal assemblage, made up to 70 % of copepod daily ingested carbon (Figure 6).

In artificial phytoplankton assemblage cumulative daily ration of copepods (for 4 microalgae, except *P. minimum*), reached 260% body C per day when we considered copepod body carbon of 0.198 $\mu\text{g C}$. The cumulative contribution of two cryptophytes amounted to 184 % body C, and that of *T. suecica* -

28% body C. Consistent with the cell-biomass-based ingestion rates, most of the cumulative carbon daily ration resulted from the ingestion of cryptophytes.

Selective Feeding

In both experiments with natural phytoplankton assemblages in late July and late November (Exp1 and Exp2), *O. davisae* showed persistent and the only positive values of electivity index E_i^* (Vanderploeg & Scavia, 1979) for cryptophytes, not significantly modulated by changes neither in proportion, nor in cell abundance in phytoplankton assemblages (Figure 7). The values of E_i^* for cryptophytes varying in the range of $+0.59$ ($\pm\text{SD } 0.03$) (late July, Figure 7A) and $+0.51$ ($\pm\text{SD } 0.11$) (late November, Figure 7B) suggested that *O. davisae* selected cryptophytes actively over other microalgae prey. On the contrary, persistent negative values of E_i^* for both Nano1 and PicoEu ($-0.2/-0.4$) below the threshold line, both in July (Figure 7A) and November (Figure 7B) indicated that copepods ignored, or avoided them.

In artificial pluralgal assemblage *O. davisae* demonstrated positive selective behavior only for two microalgae species among five (Figure 8): $E_i^* = +0.28$ ($\pm\text{SD } 0.03$) for cryptophyte strain IBSS Cr54

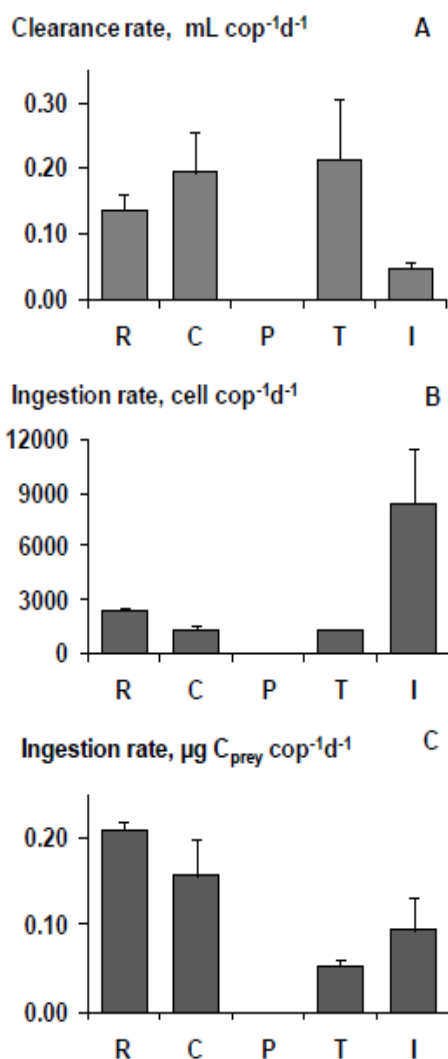
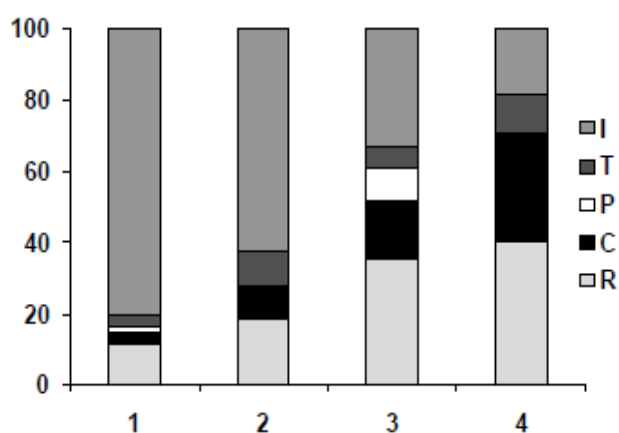


Figure 5. Clearance (mL cop⁻¹ d⁻¹) (5A) and ingestion rates of *O. davisiae* females in cell number (cells cop⁻¹ d⁻¹) (5B) and carbon biomass (µg C_{prey} cop⁻¹ d⁻¹) (5C) on different microalgae from artificial phytoplankton assemblage (R- *Rhodomonas salina*; C - cryptophyte IBSS-Cr54 strain; P - *Prorocentrum minimum*; I - *Isochrysis galbana*; T- *Tetraselmis suecica*).



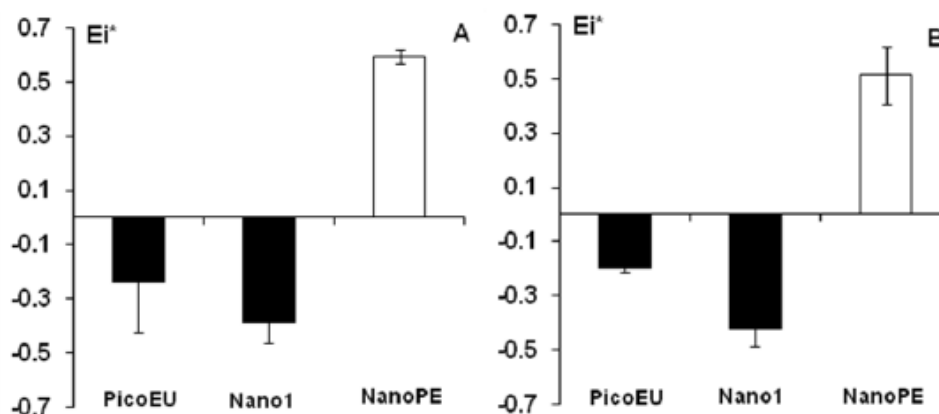


Figure 7. Electivity indices (E_i^*) for phytoplankton fractions: piceoplankton (PicoEu), nanoplankton (Nano1) and cryptophytes (NanoPE) consumed by female *O. davisae* from natural phytoplankton assemblages in late July (A) and in late November (B).

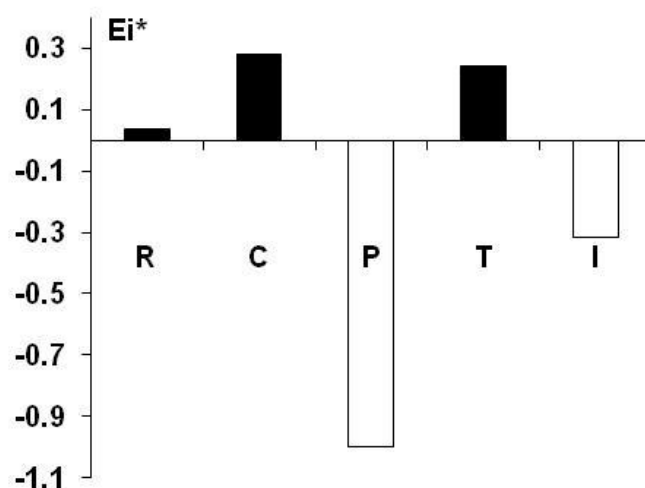


Figure 8. Electivity indices (E_i^*) for microalgae species: R – *Rhodomonas salina*; C – cryptophyte strain IBSS-Cr54; P – *Prorocentrum minimum*; T – *Tetraselmis suecica*; I – *Isochrysis galbana* consumed by female *O. davisae* from artificial microalgae assemblage.

and $E_i^* = +0.24$ (\pm SD 0.03) for *T. suecica*. Negative value of electivity index ($E_i^* = -0.3$) for *Isochrysis galbana* pointed that the copepods prefer others than this species cells, while $E_i^* = -1$ for *Prorocentrum minimum* is the evidence of its absolute avoidance.

Discussion

The results of our grazing experiments confirmed that *O. davisae* from the coastal waters of the Black Sea can ingest a broad range of phytoplankton prey including small cells, with exception of diatoms, in correspondence with Uchima and Hirano (1986) and Uchima (1988). Weight specific ingestion rates of *O. davisae* on phytoplankton corresponded seasonal changes in abundance, size and taxonomic class of phytoplankton, with cumulative values for all fractions

from as low as 1.7 % in late November up to 170 % of the body carbon ingested per day in late July. However, *O. davisae* females did not remove phytoplankton cells in proportion to their abundance neither in natural, nor in artificial assemblages. Similarly to field observations on congeneric *O. nana* (Lampitt & Gamble, 1982), it appears unlikely that *O. davisae* feeding on the cells $\leq 5 \mu\text{m}$ could be significant even if they are highly abundant, and their clearance rate is not above $0.03 \text{ mL cop}^{-1} \text{ d}^{-1}$. In agreement with Saiz, Griffell, Calbet and Isari (2014), we suppose that negligible clearance rates of a small prey which cannot be detected by mechanoreception, might occur occasionally, simultaneously with the capture of a larger prey. Our data support the idea that the feeding behavior of *O. davisae* in phytoplankton assemblages, both natural and artificial, is not centered on the most

abundant cells but should be described as an active selection for preferred food items, yet not for the major size class as in Tsuda and Nemoto (1988), but for exclusively motile and elongated cells (Atkinson, 1995). High clearance rates and selective feeding of *O. davisae* in our experiments occurred only in a narrow flagellate prey spectrum: cryptomonads (both in natural and artificial assemblages) and prasinophytes (artificial assemblage) at maximum among tested microalgae clearance rates (0.14 - 0.19 mL female⁻¹ d⁻¹).

Highest clearance rates were obtained when *O. davisae* fed on larger prey, 1 - 7 mL female⁻¹ d⁻¹ on heterotrophic dinoflagellate *Oxyrrhis marina* (Zamora-Terol & Saiz, 2013), and 1 -13 mL female⁻¹ d⁻¹ on plankton ciliates (*Strombidium* sp.) (Saiz, Griffell, Calbet, & Isari, 2014) provided at low concentrations. Saiz, Griffell, Calbet, and Isari (2014) reported the clearance rate of *O. davisae* was 0.2 - 0.6 mL ind⁻¹ d⁻¹ fed on larger photosynthetic dinoflagellate *P. minimum* and attained daily ration on this microalgae up to 100% body C d⁻¹. However in our experiments *P. minimum* was the only prey totally avoided by *O. davisae*. Our finding corresponds to the absence of significant feeding of congeneric *O. similis* on *Prorocentrum*-like cells (Nakamura & Turner, 1997) and in agreement with our own recent experimental data (Khanaychenko, Aganesova, Mukhanov, Sakhon & Rauen, 2016). Grazing rate of *O. davisae* females on *T. suecica* (about 2000 cells cop⁻¹ d⁻¹) in our experimental artificial microalgae assemblages are in good agreement with reported earlier, ingestion rates of the same copepod on the same microalgae species ranging from 1630 cells d⁻¹ (Vogt *et al.*, 2013) to 4000 cells d⁻¹ (Cheng, Akiba, Omura & Tanaka, 2014). High cell ingestion rate of a small prymnesiophyte *I. galbana* by *O. davisae* is most likely the effect of its high cell concentration in our experimental phytoplankton artificial assemblage, and implemented as a side effect in the course of capturing a larger prey. However, our recent grazing experiments with the cultured *O. davisae* showed total avoidance of both *P. minimum* and *I. galbana* in presence of two cryptophyte prey (Khanaychenko, Aganesova, Mukhanov, Sakhon & Rauen, 2016). In the literature, rejection of *R. salina* by *O. davisae* has been reported (Vogt *et al.*, 2013). This could be attributed to loosing motility, cell-clumping and settling in the absence of aeration, typical for the cells of this microalgae in experimental conditions. Mucilage generated by the cells of *R. salina* can somehow affect negatively *O. davisae* feeding. Actually, grazing rates of *O. davisae* females on cryptomonads in our late July and artificial phytoplankton assemblages' experiments accounted for the most of their phytoplankton daily rations. Daily rations of *O. davisae* females only on cryptophytes (135% in late July and up to 184 % body C in artificial microalgae assemblages) from our experiments are comparable with reported earlier as 180 - 250% body C d⁻¹ obtained when adult *O. davisae*

fed on natural phyto- and micro-plankton assemblages during toxic dinoflagellate *Alexandrium minutum* bloom (Calbet *et al.*, 2003), 160% body C d⁻¹ on *Heterocapsa* sp., > 180% body C d⁻¹ on *O. marina* and up to 250% body C day⁻¹ on *Strombidium* sp., the last suggested by the authors to be sloppy feeding (Saiz, Griffell, Calbet, & Isari, 2014). Sloppy feeding strategy was possibly also implemented to a certain degree when *O. davisae* was feeding at high phytoplankton abundance in our experiments.

Our results showed that *O. davisae* fed selectively on cryptophytes both in natural and artificial assemblages with positive electivity indices, Ei* insignificantly varying regardless of season, species composition and cell abundance. We found rare information in the literature on interactions between oithonids and cryptophytes in natural environment. However, the growth of photosynthetic cryptophytes under nutrient-enriched conditions in estuarine system in Japan was assumed almost balanced with the grazing losses caused by a microzooplankton population dominated by cyclopoid copepods (Nakamura & Hirata, 2006). Correlation between *Oithona* spp. and cryptophytes was found in a mesotrophic bayou estuary suggesting the possibility of cyclopoids to utilize these microalgae as food sources (Moshiri *et al.*, 1978). In addition, in Tokyo Bay where *O. davisae* dominated among copepods (Tsuda & Nemoto, 1988), cryptophytes

(*Hemiselmis*, *Cryptomonas/Chroomonas*, *Plagioselmis* spp.) were registered as dominant phytoplankton contributing 10⁵ - 10⁶ cells L⁻¹ during April - November (Han & Furuya, 2000). In San-Francisco Bay where introduced *O. davisae* was one of the dominant copepods (Ambler *et al.*, 1985; Ferrari & Orsi, 1984), mixotrophic cryptophytes (third microalgae group after diatoms and dinoflagellates) contributed minimum 5% to cumulative phytoplankton biomass (Cloern & Dufford, 2005). In many coastal marine environments mixotrophic cryptophytes (*Plagioselmis*, *Teleaulax*, *Rhodomonas* and *Cryptomonas*) are part of key components of phytoplankton communities (Cloern & Dufford, 2005; Han & Furuya, 2000). In the Black Sea, only few studies have reported the distribution of cryptophytes in the coastal regions under the influence of Danube (Eker-Develi *et al.*, 2012) and the Kızılırmak River (Baytut & Gonulol, 2016). However, the role of nutritionally rich cryptophytes with the highest organic content per cell volume among microalgae (Moal, Martin-Jezequel, Harris, Samain & Poulet, 1987) is apparently underestimated by cell volume to carbon conversion factors usually used for microalgae groups (Menden-Deuer & Lessard, 2000; Montagnes, Berges, Harrison & Taylor, 1994; Verity *et al.*, 1992). Temporal and spatial distributions of cryptophytes in the Black Sea deserve further investigation.

In summary, this study is the first to raise the question of interaction, and possibly a tight coupling between nanoplanktonic cryptophytes and cyclopoid

O. davisae. Cryptophytes were selectively grazed by *O. davisae* females at the highest among other microalgae clearance rates regardless of their concentration in various natural phytoplankton assemblages in different seasons and in artificial microalgae mixtures.

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