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Feeding rates and abundance of marine invertebrate planktonic larvae under harmful algal bloom conditions off Vancouver Island

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ABSTRACT

The interactions between toxic phytoplankton and their potential grazers are poorly understood aspects of the ecology of harmful algal blooms. In this study, we determined the feeding rates, prey selection and trophic impact of different marine invertebrate planktonic larvae on the natural bloom of *Heterosigma akashiwo* and *Prorocentrum triestinum* which occurred on the west coast of Vancouver Island in July of 2006. Additionally, we estimated the abundance, biomass and composition of zooplankton before and during the harmful algal bloom.

Feeding experiments were performed with polychaete (*Serpula columbiana*), echinoderm (*Strongylocentratus purpuratus*) and cirripede (*Balanus crenatus*) larvae obtained from laboratory cultures, and bivalve and gastropod larvae collected from the study site by plankton tows. All larvae fed on *H. akashiwo* whereas only cirripede nauplii and echinoderm larvae fed on *P. triestinum*. *H. akashiwo* was the main component of all larval diets (>64%). We observed a positive relationship between prey availability in the food assemblages and their contribution to all larval diets. The potential trophic impact of meroplanktonic larvae on bloom forming phytoplankton species was low (<1.5%). The ingestion of bloom forming phytoplankton did not appear to have any adverse effects on the studied grazers after 48 h of incubation. In contrast, field abundance of planktonic larvae and other zooplankton continuously decreased throughout the progression of the bloom, with losses approaching 75% in comparison to their pre-bloom abundance.

The presence of *H. akashiwo* negatively affected the abundance of meroplanktonic larvae, despite efficient grazing of these larvae. Therefore, grazing pressure was reduced which likely contributed to the growth and persistence of the bloom. The reduction in meroplanktonic larvae and other zooplankton abundance associated with the *H. akashiwo* bloom may have potential impacts on benthic recruitment and energy transfers to higher trophic levels in marine food webs.

1. Introduction

Harmful algal blooms (HABs, commonly called 'red tides') are a common phenomenon in coastal waters and their occurrence has become more frequent worldwide over recent decades (Anderson, 1989; Smayda, 1990; Hallegraeff, 1993). The formation and persistence of HABs is partly controlled by several physical–chemical factors (often referred to as "bottom-up" controls) such as column stratification, nutrient concentration, temperature and salinity (Anderson and Lindquist, 1985; Connell and Jacobs, 1999). In addition, biological factors including viral or bacterial infection, parasitism and zooplankton grazing ("top-down" controls) may contribute to the decline of some HABs (Watras et al., 1985; Uye, 1986; Nagasaki et al., 1994; Mayali and Azam, 2004). Among these interactions, the grazing pressure of zooplankton on bloom-forming phytoplankton species remains poorly understood. Studies of zooplankton grazing on natural food assemblages during HABs are scarce (Turner and Anderson, 1983; Turner and Tester, 1997; Calbet et al., 2003; Turner and Borkman, 2005) and most of our knowledge on the interaction between HAB and zooplankton is provided from laboratory studies using unnaturally high concentrations of unialgal diets that are difficult to extrapolate to natural conditions (Turner et al., 1998). Some harmful bloom-forming phytoplankton exhibit chemical characteristics that make them less palatable to zooplankton (Huntley et al., 1986; Teegarden, 1999) or have an adverse effect on zooplankton abundance and survival (Turner et al., 1998; Turner, 2006). Reduced grazing pressure by zooplankton could contribute to the formation and persistence of some harmful phytoplankton blooms (Smayda and Villareal, 1989; Gilbert, 1990). Therefore,

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information on the effects of HABs on grazer populations and zooplankton–toxic phytoplankton trophic interactions (e.g. grazing rates, feeding selection patterns, trophic impact) are required for a better understanding of the ecology and dynamics of harmful phytoplankton blooms.

Most available information on the interactions of zooplankton grazers with harmful phytoplankton blooms has focused on copepods and recently on microprotozoans (Turner and Tester, 1997; Turner, 2006). Little is known regarding the interactions between marine invertebrate planktonic larvae (meroplankton) and harmful bloom-forming phytoplankton. It is important to study these interactions because meroplanktonic larvae may frequently be the dominant component of the coastal metazooplankton during the reproductive season (Blanner, 1982; Anger et al., 1986; Andreu and Duarte, 1996) and are the key factor that determines the success of adult populations of benthic invertebrates (Thorson, 1946, 1950). For example, phytoplankton blooms act to directly induce the release of gametes or larvae in some benthic invertebrate species either through direct contact (phytoplankton particle settlement) or chemical triggering (Starr et al., 1990, 1991, 1994). This results in a high abundance of meroplanktonic larvae during phytoplankton blooms (Thorson, 1946; Olson, 1987; Andreu and Duarte, 1996). Low abundance of copepods and a high abundance of meroplanktonic larvae (polychaete larvae) have been observed during blooms of some toxic phytoplankton species (e.g. *Alexandrium* spp.) (Turner and Anderson, 1983; Watras et al., 1985). Therefore, the associated feeding interactions and the potential capability of marine invertebrate planktonic larvae to control harmful phytoplankton blooms should be not neglected.

In this study, we examine the trophic interactions between representative marine invertebrate planktonic larvae and natural food assemblages during a phytoplankton bloom that occurred on the west coast of Vancouver Island in July 2006. The specific objectives of this study were: (1) to determine the abundance, biomass and composition of zooplankton before and during the HAB; (2) to estimate the feeding rates and food selection behavior of different meroplanktonic larvae during the HAB; and (3) to evaluate the potential trophic impact (top-down control) of meroplanktonic larvae on the HAB.

2. Materials and methods

2.1. Bloom event and identification of bloom-forming phytoplankton species

Sampling was conducted at the mouth of Bamfield Inlet (N 48 50.343′ W 123 08.152′), in Barkley Sound, Vancouver Island, Canada during a coastal phytoplankton bloom in July 2006 (Fig. 1). Chlorophyll concentration was used as an indicator of phytoplankton biomass during the phytoplankton bloom. Chlorophyll and water temperature data were extracted from SeaWifs satellite images. Seawater samples were collected with a Niskin bottle at 5 m depth from the sampling site to identify the dominant bloom-forming phytoplankton species. Seawater subsamples (200 ml) were fixed with Lugol's solution (1%) for light field microscopy and with glutaraldehyde (1%) for epifluorescence microscopy (see procedure details below). Cells were examined at 200× with a Nikon Diaphot TMD inverted microscope and at 1000× magnification with an Olympus BX61 epifluorescence microscope under blue and UV wavelength excitation. Aliquots from Lugol's samples (20 ml) were filtered using a vacuum pump onto polycarbonate Nuclepore filters (0.8 μm pore size and 25 mm diameter) for Scanning Electron Microscopy (SEM). The filters were air dried and stored under partial vacuum in hermetically closed boxes until preparation for the SEM. A part of the filter was placed on a SEM stub and coated with a film (of about 150 Å) of gold–palladium to avoid electric charges; the sputter coater used was a Polaron SC-500. The examination and microphotographs of the specimens were made with a Hitachi S-3500N scanning electron microscope operating at 5 kV. The identification of bloom-forming phytoplankton species was based on morphology and ultrastructure (Hara et al., 1985; Hara and Chihara, 1987; Dodge, 1982; Steidinger and Tangen, 1996).

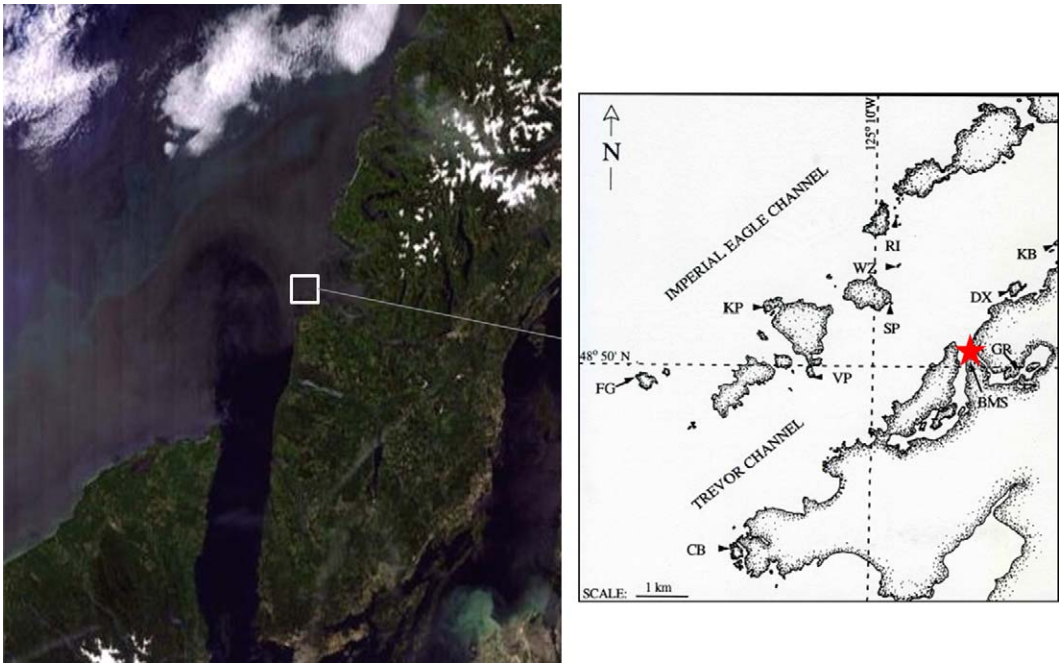

Fig. 1. (A) Satellite image of the west coast of Vancouver Island during the harmful phytoplankton bloom in July of 2006. Note that besides the *Heterosigma akashiwo*/*Prorocentrum triestinum* bloom (red color), we can also observe some remains of a coccolithophorid bloom (green color) which previously occurred in the same area. (B) Sampling station in the Bamfield inlet (★). Map courtesy of Canadian Hydrographic Services. Ottawa, Canada. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Table 1
Length vs. carbon content regressions used to provide biomass estimates for the different groups of zooplankton. Individual biomass ($\mu g\, C$) = $a \times L\ (\mu m)^b$.

Organism	Measurement	a	b	Reference
Copepods	Length of prosome	4.27×10^{-9}	3.07	Uye (1982)
Copepodites	Length of prosome	1.11×10^{-8}	2.92	Berggreen et al. (1988)
Copepod nauplii	Body length (without setae)	3.18×10^{-9}	3.31	Berggreen et al. (1988)
Appendicularia	Trunk length (without tail)	7.33×10^{-8}	2.63	King (1980)
Cladocera	Total length	4.57×10^{-10}	3.46	Uye (1982)
Rotifers	Body length	1.06×10^{-7}	2.74	Hansen et al. (1997a)
Polychaeta larvae	Maximal length	1.58×10^{-4}	1.38	Hansen (1999) as *Polydora sp*
Bivalvia larvae	Maximal length	3.06×10^{-8}	2.88	Fotel et al. (1999)
Gastropoda larvae	Maximal length	2.31×10^{-5}	2.05	Hansen and Ockelmann (1991)
Echinoderma larvae	Total length	3.06×10^{-8}	2.88	Fotel et al. (1999) as bivalve
Cirripedia nauplii	Body length (without spine)	2.20×10^{-10}	3.72	Turner et al. (2001)
Decapoda larvae	Carapace length	4.01×10^{-12}	4.43	Hirota and Fukuda (1985)*
Other larvae	Total length	3.06×10^{-8}	2.88	Fotel et al. (1999) as bivalve

* Calculated using length and carbon content data from Table 1 ($r^2 = 0.91$).

2.2. Estimation of abundance, biomass and composition of zooplankton during the HAB

The abundance, biomass and composition of zooplankton in the study site (Bamfield Inlet) were determined periodically throughout July 2006. Zooplankton samples were obtained by duplicate vertical tows from ~30 m to the surface using a microplankton net (64 μm mesh, 30 cm diameter). During sample collection, the plankton net was rinsed and the entire cod end sample was poured into a 500 ml plastic bottle and preserved in borax-buffered formaldehyde at 4% final concentration. To estimate the abundance and composition of metazooplankton, these samples were divided into two nominal size fractions (64–200 μm, >200 μm) by filtering through a 200 μm sieve. Two aliquots per fraction (at least 350 organisms per aliquot) were counted under a stereomicroscope. Body length measurements were made for >60 randomly selected individuals from each zooplankton size fraction to determine biomass. Measurements were taken from digital images from a stereomicroscope using image analysis (ImageJ software). Each individual's carbon weight was calculated by applying body size–carbon content relationships from the literature (Table 1).

2.3. Sources of larvae for meroplankton feeding experiment

We obtained larvae of *Serpula columbiana* (Polychaeta), *Stronglyocentrotus purpuratus* (Echinoderma), and *Balanus crenatus* (Cirripedia) through laboratory cultures. Mature individuals of each species were collected from different locations in Trevor Channel; *S. columbiana* specimens were obtained from the intertidal zone of Dixon Island, *S. purpuratus* from the subtidal zone in Scott's Bay, and *B. crenatus* from the subtidal zone in Bamfield Inlet.

Bivalve and gastropod larvae were collected 24 h before the start of the experiment from Trevor Channel using a 64 μm-mesh size plankton net. Bivalve larvae of similar size (mainly *Mytilus* spp.) were carefully pipetted from each plankton sample, repeatedly rinsed by transferring them through a series of Petri dishes filled with 0.2 μm filtered seawater (FSW), and maintained in autoclaved 0.2 μm FSW at 16 °C until commencement of the experiments. A similar procedure was used for the collection of gastropod larvae. Some types (morphology) and sizes of gastropod veligers (mainly nudibranchia) were used in the feeding experiment.

To obtain *S. columbiana* larvae, 5 adult worms of each sex were induced to spawn by breaking their tubes, starting at the posterior end, until the soft body of the polychaete could be touched. This caused the animal to contract and releases its gametes. An aliquot of sperm suspension was mixed with oocytes for 5 min for fertilization. The remaining sperm were removed by filtering the suspension though a 25 μm screen and the eggs were rinsed in a bath of 0.2 μm autoclaved filtered seawater (FSW). The eggs were then placed in a beaker with FSW for 24 h to allow the embryos to develop to the trochophore stage. *S. columbiana* larvae were provided with the flagellate Isochrysis galbana as a food source for 4 d until the beginning of the experiments.

Adult *B. crenatus* were exposed to bright light after 6 h of emersion resulting in the release of nauplius I larvae. The barnacle larvae were transferred to a beaker of autoclaved 0.2 μm FSW for 24 h before the beginning of the experiment, during which time they molted to the nauplius II stage. No food was provided during this period because these larvae develop through the nauplius I stage without feeding (Strathmann, 1987).

To induce spawning in *S. purpuratus*, 0.5 M KCL solution was injected into the intracoelomic cavity in 3–6 adults of each sex (Strathmann, 1987). Sperm were collected with a glass pipette and kept at 4 °C until use and the oocytes were collected and transferred to autoclaved 0.2 μm FSW. An aliquot of sperm was added to the oocytes for 5 min. The remaining sperm were removed by filtering the suspension though a 25 μm screen and then rinsing the eggs in a bath of 0.2 μm filtered seawater (FSW). The eggs were then placed in a beaker with 0.2 μm FSW and maintained for 76 h to allow development to the early pluteus stage (four-armed stage).

2.4. Design of the feeding experiment

The feeding experiment consisted of bottle incubations of the naturally blooming microplanktonic community with and without the addition of meroplanktonic larvae as grazers. Details of the larvae and the larval densities used in the feeding experiments are described in Table 2. At the start of the experiment, subsamples of larvae of each group were fixed with formaldehyde at 4% final concentration for later biomass determinations. Approximately 40 photographs of each group of larvae were taken using an inverted microscope (100×), from which body lengths (L, μm) were measured by image analysis (Image J software). The average individual carbon weight was calculated by applying the body size–carbon content relationships given in Table 1.

Seawater and plankton for the feeding experiment were collected at 5 m depth with a Niskin bottle. The seawater samples were gently transferred to carboys and transported to the laboratory within 1 h of collection. Seawater was screened through a 25 μm mesh by reverse filtration to exclude metazoan consumers. The filtered seawater was carefully siphoned into 700 ml acid-washed glass bottles in a three-step filling procedure to ensure homogeneity between replicates. The experiment comprised three types of treatments: (1) initial plankton conditions

Table 2
Characteristics of the marine invertebrate larvae used in the feeding experiment at commencement of incubation. Each larval type was used separately.

Taxon	Larval stage	Species	Length ± SE (μm)	Biomass ± SE (μg C Ind^{-1})	Density (Ind l^{-1})
Polychaeta	Trochophore	*Serpula columbiana*	149.2 ± 2.1	0.16 ± 0.01	50
Echinoidea	Four-armed echinopluteus	*Strongylocentrotus purpuratus*	261.9 ± 2.2	0.28 ± 0.01	30
Cirripedia	Nauplii II	*Balanus crenatus*	287.8 ± 6.1	0.33 ± 0.02	30
Bivalvia	Late veliger	*Mytilus* spp.	298.5 ± 11.2	0.45 ± 0.05	29
Gastropoda	Late veliger	Unidentified	362.1 ± 18.9	4.26 ± 0.42	7

(3 replicate bottles of the above 25 μm filtered seawater, sampled immediately at the start of the experiment); (2) control (3 replicate bottles of 25 μm filtered seawater, sampled after 48 h); and (3) experimental (25 μm filtered seawater, with added larvae, sampled after 48 h, 3 replicate bottles for each larval type). The experimental treatments therefore involved a total of 15 bottles (5 larval types × 3 replicate bottles per larval type).

The bottles were placed in situ in mesh bags suspended at a 5 m depth in Bamfield Inlet off of the Bamfield Marine Science Center dock. The incubations were carried out for 48 h and the bottles were gently mixed by hand every 2–3 h. The water temperature during incubation was recorded every 24 h. Water subsamples were taken from the bottles at the beginning and at the end of the experiments to estimate the initial and final abundance and biomass of planktonic organisms.

2.5. Sample processing and calculations

Grazing was measured by quantitative microscopic analysis of the microbial community abundance in initial, control and experimental treatments. The microbial components studied in the feeding experiments included the bloom-forming phytoplankton species, heterotrophic nanoflagellates (HNF), phototrophic nanoflagellates (PNF), dinoflagellates, diatoms and ciliates.

For nanoflagellates, 50 ml samples were preserved in glutaraldehyde (1% final concentration). Duplicate 20 ml subsamples were gravity-filtered at low pressure onto 0.8 μm pore-size black polycarbonate membrane filters and stained with DAPI (5 μg ml^{-1} final concentration) for 5 min (Porter and Feig, 1980). Filters were mounted on glass slides and stored at −20 °C until analysis. At least 600 cells were counted by epifluorescence microscopy at a magnification of 1000× and classified as auto- or heterotrophic according their fluorescence for chlorophyll under blue light. Fifty cells were sized and converted into carbon using a conversion factor of 0.22 pgC μm^{-3} for HNF (Børsheim and Bratbak, 1987) and the equation pgC cell^{-1} = 0.109 × volume$^{0.991}$ for PNF (Montagnes et al., 1994).

To determine the concentration of dinoflagellates, ciliates and diatoms, 200 ml samples were fixed with 1% acidic Lugol's solution and allowed to settle for 48 h in 50 ml Utermöhl chambers. An inverted microscope (Nikon DIAPHOT 200) at 200× magnification was used to count all ciliates and dinoflagellates in each chamber and diatoms were counted in at least 50 microscopic fields (or 200 cells). Sixty randomly chosen cells for each group were sized and converted into carbon using the conversion factors of 0.19 and 0.053 pgC μm^{-3} for oligotrich ciliates (Putt and Stoecker, 1989) and tintinnids (Verity and Langdon, 1984), respectively, and the equations of pgC_{Dino} cell^{-1} = 0.760 × volume$^{0.819}$ for dinoflagellates and pgC_{Diat} cell^{-1} = 0.288 × volume$^{0.811}$ for diatoms (Menden-Deuer and Lessard, 2000). Because microzooplankton were preserved with acidic Lugol's solution, no distinction between strict heterotrophs and auto-/mixotrophs was made for ciliates and dinoflagellates.

Clearance and ingestion rates were calculated for each prey type according to Frost (1972) after verification that prey growth rates in the grazing bottles were different and lower than in the controls (one-way ANOVA test, $p < 0.05$).

Selective feeding by metazoan microzooplankton was evaluated using the electivity index (E^*) of Vanderploeg and Scavia (1979). Electivity index of the i food type (E_i) was calculated as

$$E_i = \frac{W_i - (1/n)}{W_i + (1/n)}$$

with n as the total number of prey types in a given experiment, and the coefficient W_i defined by

$$W_i = \frac{F_i}{\sum F_i}$$

where F_i is the clearance rate of the i food type, and ΣF_i is the sum of clearance rates on all food types. The electivity index (E) ranges between −1 and +1, where 0 signifies no electivity (no selective grazing), negative values correspond to avoidance and positive values represent selection. The use of this index has been especially recommended in cases where different food types are not equally abundant (Lechowicz, 1982). The selectivity coefficients were computed within each experiment based on average clearance values (between replicate bottles) for each considered prey type.

The trophic impact of meroplanktonic larvae on each prey type was calculated as the % of biomass of the standing stock grazed daily assuming a homogenous prey and predator distribution in the water column. We calculated the potential impact from in situ larvae biomass and their specific ingestion rates (d^{-1}) obtained from the feeding experiments.

3. Results

3.1. Bloom event and bloom-forming phytoplankton species

The bloom event that ocurred on the west coast of Vancouver Island in July 2006 (Fig. 1) was composed of the raphydophicean *Heterosigma akashiwo* (Hada) Hada ex Y. Hara & Chihara (= *Heterosigma carterae*, previously referred to as *Olisthodiscus luteus*) and the dinoflagellate *Prorocentrum triestinum* Schiller (Fig. 2). The cell density of *H. akashiwo* and *P. triestinum* reached values higher than 10^6 and 10^4 cells l^{-1}, respectively (Table 3). During the phytoplankotn bloom, water temperature increased from ∼13 to 17 °C and chlorophyll (Chl) concentration increased from ∼2 to 6 μg Chl l^{-1} (Fig. 3A and B).

3.2. Abundance, biomass and composition of zooplankton during the HAB

Zooplankton abundance decreased progressively from ∼40 to ∼10 ind l^{-1} (Fig. 3C) as the bloom developed. The zooplankton biomass was dominated by the mesozooplankton fraction (Fig. 4A). Meroplanktonic larvae represented 22–37% of the total metazooplankton biomass (Fig. 4B). Adult calanoid copepods and copepod

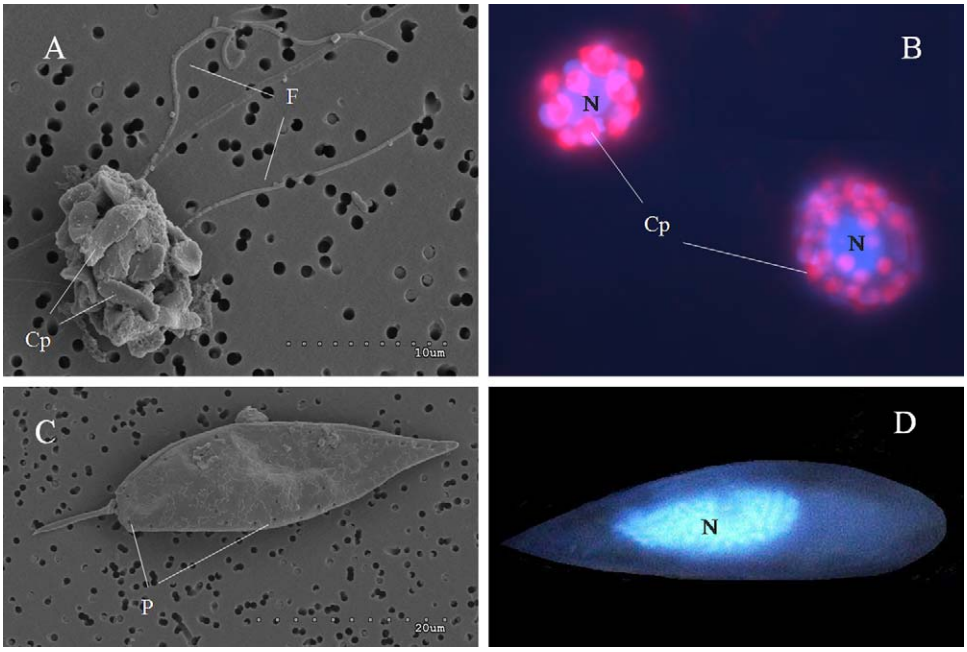

Fig. 2. Electronic and fluorescence microscope images used for the identification of the bloom-forming phytoplankton species. (A) and (B) *Heterosigma akashiwo*; (C) and (D) *Prorocentrum triestinum*. F: flagella; N: nucleus; Cp: chloroplasts; P: pores.

larvae (nauplii and copepodites) were the most important groups in terms of carbon biomass (Fig. 4C). Polychaete, gastropod and bivalve larvae were the major contributors of biomass to the meroplankton community (Fig. 4D). All zooplankton groups/fractions decreased in abundance and biomass with the development of the HAB (Fig. 4), although this decrease was more drastic in some groups (e.g. rotifers, appendicularia) than others (Fig. 4).

3.3. Microbial community abundance and compostion in the feeding experiment

The abundance, size and biomass of the different microbial groups (excluding bacterioplankton) at the beginning of the feeding experiment (July 16, 2006), are shown in Table 3. The initial microbial community was dominated, in terms of biomass, by *H. akashiwo* and *P. triestinum*, which comprised ~74% of total carbon biomass (Table 3). Other important contributors to the microbial community were pico- and nanoflagellates (~20%). In contrast, ciliates (represented by non-loricate ciliates such as *Strombidium* spp., *Mesodinium* spp.), dinoflagellates (mostly *Heterocapsa* spp., *Scripsiella* spp. *Protoperidinium bipes*) and diatoms (mainly *Leptocylindrus* spp. and) represented a small percentage of total microbial biomass (<6%, Table 3).

3.4. Feeding rates, feeding selectivity and trophic impact

There were no apparent adverse effects of *H. akashiwo* on larvae after the 48 h incubation period, grazer survival being 100%. Clearance and ingestion rates varied significantly (ANOVA, $p < 0.05$) depending on the larvae and the prey type (Tables 4 and 5). Clearance rates ranged from 0.34 ml larvae^{-1} d^{-1} for early polychaete larvae feeding on HNF to 6.36 ml larvae^{-1} d^{-1} for late gastropod larvae feeding on ciliates (Table 4). All studied larvae fed on *H. akashiwo* but only *B. crenatus* nauplii and *S. purpuratus* echinopluteus grazed on *P. triestinum* (Tables 4 and 5). We did not detect significant feeding on pico-flagellates (Tables 4 and 5). The carbon-specific ingestion rate of *B. crenatus* nauplii was significantly (ANOVA, $p < 0.01$) higher than the ingestion rates observed in other larvae (Fig. 5).

Heterosigma akashiwo was the main component of the diet (>64%) of all studied larvae (Fig. 5). *Prorocentrum triestinum* represented ~25 and 20% of the diet of *B. crenatus* nauplii and *S. purpuratus* echinopluteus, respectively. The biomass contribution of ciliates, dinoflagellates and diatoms to the larval diet was low (<8%), whereas HNF and PNF represented ~20–25% of the diet of bivalve, gastropod and polychaete larvae (Fig. 5). We observed a positive relationship between prey availability in

Table 3
Initial abundance, size, and biomass of the different studied potential prey from the microbial community. HNF: heterotrophic nanoflagellates; PNF: phototrophic nanoflagellates except *Heterosigma akashiwo*; Dinoflag.: dinoflagellates except *Prorocentrum triestinum*; M: major axis, m: minor axis. The volume of the organism was assumed to be equivalent to that of the corresponding ellipsoid except for HNF, PNF and picoflagellates which were considered spherical [$M = m$ = equivalent spherical diameter, ESD (μm)]. A nominal size of 1.5 μm was assumed for picoflagellates (<2 μm).

Prey type	Abundance ± SE (cells ml^{-1})	M ± SE (μm)	m ± SE (μm)	Biovolume ± SE (μm^3 cell^{-1})	Biomass ± SE (μg C l^{-1})
Picoflagellates	9297 ± 489	1.5	1.5	1.77	3.6 ± 0.2
HNF	3579 ± 155	2.4 ± 0.1	2.4 ± 0.1	7.3 ± 0.01	5.7 ± 0.2
PNF	6965 ± 539	4.0 ± 0.3	4.0 ± 0.3	33.5 ± 0.01	24.6 ± 1.9
Diatoms	102 ± 7	31.2 ± 2.1	2.5 ± 0.1	107 ± 16	1.6 ± 0.2
H. akashiwo	1027 ± 48	14.2 ± 0.3	9.3 ± 0.2	661 ± 33	98.5 ± 4.6
Dinoflag.	23 ± 2	15.8 ± 0.5	11.4 ± 0.4	1159 ± 99	5.6 ± 0.5
Ciliates	5.2 ± 0.2	19.2 ± 1.2	14.6 ± 0.9	2318 ± 393	2.7 ± 0.1
P. triestinum	64 ± 5	36.4 ± 0.4	10.7 ± 0.3	2323 ± 132	26.9 ± 2.1

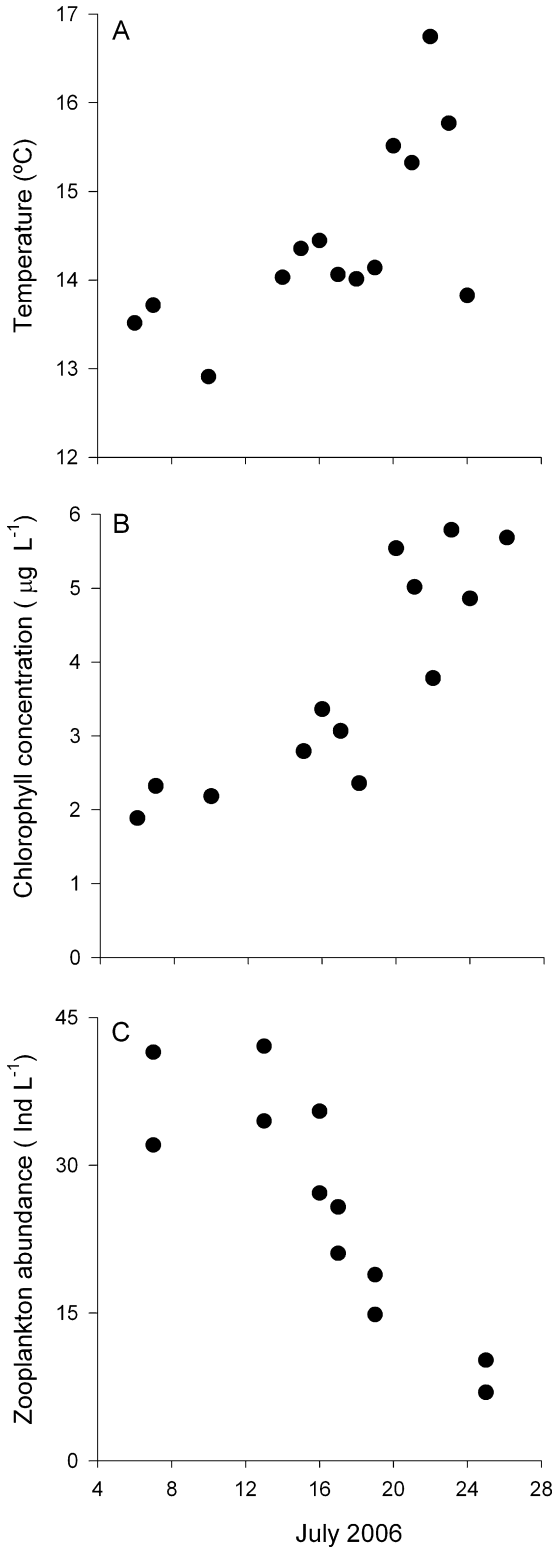

Fig. 3. Temporal progression of temperature (A), chlorophyll a concentration (B) and zooplankton abundance (C) under the harmful phytoplankton bloom conditions during the study period.

the food assemblages and their contribution to larval diets (Fig. 6).

Selection indexes (E^*) varied depending on the larvae and prey type (Fig. 7). All larvae showed positive selection of *H. akashiwo* (Fig. 7). Polychaete larvae preferentially fed on nanoflagellates whereas echinoderm larvae and cirripede nauplii preferentially fed on dinoflagellates and ciliates. Gastropod and bivalve veligers exhibited positive selection for nanoflagellates, ciliates and dinoflagellates except for *P. triestinum*.

The trophic impact (% of biomass of the standing stock grazed daily) of each larval type was very low (<1%) for all prey types (Table 6). The cumulative potential trophic impact on *H. akashiwo* and *P. triestinum* by all studied larvae was 1.29% and 0.99% per day, respectively.

4. Discussion

4.1. Bloom event and bloom-forming phytoplankton species

Heterosigma akashiwo is a noxious flagellate that frequently causes heavy and extensive blooms in temperate coastal waters in Pacific and Atlantic Oceans (Hara and Chihara, 1987). Blooms of *H. akashiwo* are frequent in early summer on the west coast of Vancouver Island where they can become extensive (Taylor and Haigh, 1993). Consistent with previous field observations (Taylor and Haigh, 1993), the increased water temperature observed during this study period seemed to be a crucial factor in bloom formation since the germination of *H. akashiwo* cysts is known to be successful above 15 °C (Imai and Itakura, 1999). The extent and intensity of a bloom of this species depends on several factors, including water stratification, salinity and nutrient concentration (Honjo, 1990, 1993; Taylor and Haigh, 1993). Taylor and Haigh (1993) indicate that strong HABs occur after early spring diatom blooms that cause surface water to become nitrate depleted. A bloom of coccolithophorids did occur in Trevor Channel (Fig. 1) previous to our study; this may have aided in the removal of nitrate, providing optimal conditions for the intense bloom of *H. akashiwo* observed in July 2006. *Prorocentrum triestinum* is a cosmopolitan bloom-forming dinoflagellate considered as potentially harmful (Anderson, 1995).

4.2. Effects of harmful algal bloom on the plankton community

Heterosigma akashiwo is known mainly for its lethal effects on wild and cultivated fish and shellfish (Black et al., 1991; Honjo, 1993; Smayda, 1998), resulting in large economic losses for aquaculture industries and further depletion of endangered wild species (Horner et al., 1991). In fact, fish kills (farmed salmon) due to *Heterosigma akashiwo* were documented in close areas (North Puget Sound, BC) during the studied bloom (Rensel, 2007). However, information concerning the effects of naturally occurring *H. akashiwo* blooms on zooplankton communities is scarce. Our field results show that the abundance of meroplanktonic larvae and other zooplankton are negatively affected by the *H. akashiwo* bloom; this may impact benthic recruitment and energy transfers to higher trophic levels in marine food webs. Previous fields studies that were focused on microzooplankton have also documented negative effects of *H. akashiwo* blooms on copepod nauplii (Kamiyama, 1995) and tintinnids (Verity and Stoecker, 1982; Kamiyama, 1995; Kamiyama et al., 2000), which is consistent with our findings. The effects of *H. akashiwo* on zooplankton are expected to be site-specific since toxicity level of this species depends on strain and environmental conditions (Ono et al., 2000; Haque and Onoue, 2002).

Heterosigma akashiwo produces an allelopathic substance, identified as a polysaccharide-protein complex (Yamasaki et al., 2009) that may cause dramatic changes in the abundance and composition of the phytoplankton community (Pratt, 1966; Honjo et al., 1978; Yamasaki et al., 2009). The allelopathic substance produced by *H. akashiwo* can inhibit diatom growth (e.g. *Skeletonema costatum*) and stimulate or enhance dinoflagellate growth, for example, *Prorocentrum* spp. (Pratt, 1966; Honjo et al.,

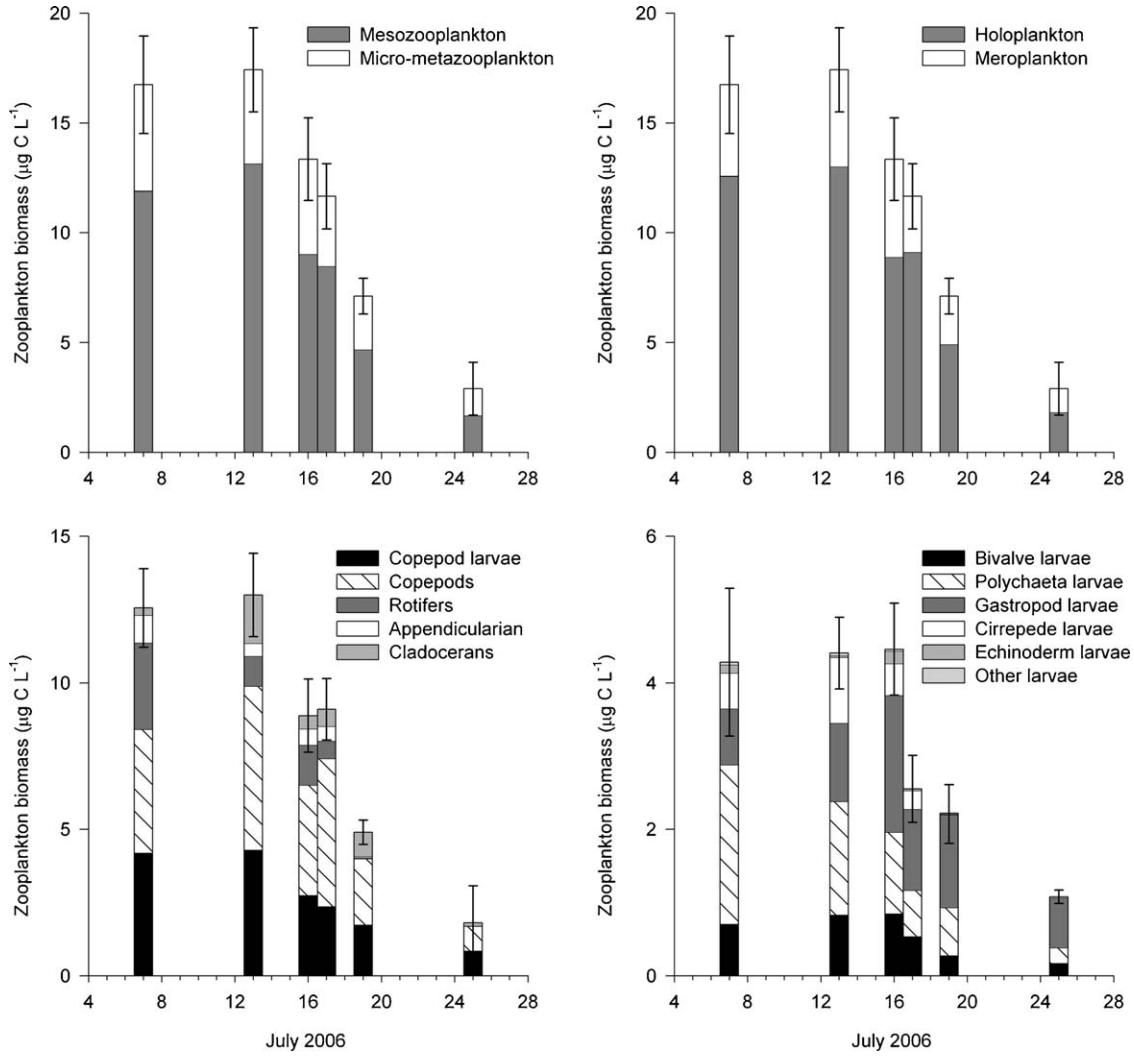

Fig. 4. Zooplankton biomass and composition under the harmful phytoplankton conditions during the study period. Error bars represent the standard error.

1978; Yamasaki et al., 2009). The phytoplankton composition observed during the studied bloom is in agreement with the expected composition due to these allelopathic effects.

Laboratory studies have reported that the effects of *Heterosigma akashiwo* on zooplankton may vary depending on species, developmental stage, cell concentration and exposure time. *H. akashiwo* has adverse effects on the survival, feeding, growth and/or reproduction of some species of copepods (Tomas and Deason, 1981; Yu et al., 2010), rotifers (Xie et al., 2008) and on early stages of gastropod larvae (Wang et al., 2006). However, other zooplankters such as the copepods *Calanus pacificus* and *Acartia omorii* do not exhibit apparent adverse effects from ingesting *H. akashiwo* (Uye and Takamatsu, 1990). Egg hatching success in some invertebrate species is negatively related to the *H. akashiwo* cell concentration and the embryos and early larvae of some invertebrates seem to be more susceptible to *H. akashiwo* than later stages (Wang et al., 2006). The negative effects of *H. akashiwo* on zooplankton are frequently time-dependent (Wang et al., 2006; Yu et al., 2010). Little (<10%) to no lethal effects of *H. akashiwo* on invertebrate larvae have been observed after incubation ≤48 h (Botes et al., 2003; Wang et al., 2006). In contrast, mortality increased significantly (>70%) after 96 h exposure of gastropod

Table 4
Clearance rates (ml larvae^{-1} d^{-1}) ± standard error (SE) of the studied meroplanktonic larvae on the different potential prey. HNF: heterotrophic nanoflagellates; PNF: phototrophic nanoflagellates except *Heterosigma akashiwo*; Dinoflag.: dinoflagellates except *Prorocentrum triestinum*. 0 indicates no significant ingestion (ANOVA, $p > 0.05$) and (*) that the concentration in the experimental bottles was significantly higher than in the control bottles (ANOVA, $p < 0.05$).

Prey	Polychaeta larvae	Echinoderm larvae	Cirripede larvae	Bivalve larvae	Gastropod larvae
Picoflagellates	*	*	*	*	*
HNF	0.336 ± 0.030	0	*	1.859 ± 0.315	4.077 ± 1.394
PNF	0.534 ± 0. 142	0	0.974 ± 0.131	1.898 ± 0.187	4.442 ± 0.570
Diatoms	0	1.292 ± 0.154	*	0	*
H. akashiwo	0.475 ± 0.045	1.135 ± 0.214	3.556 ± 0.351	1.615 ± 0.227	5.775 ± 0.831
Dinoflag.	0	2.158 ± 0.453	3.935 ± 0.422	1.689 ± 0.323	5.636 ± 1.164
Ciliates	0	2.579 ± 0.415	4.471 ± 0.499	1.571 ± 0.397	6.305 ± 0.462
P. triestinum	0	1.224 ± 0.230	5.123 ± 0.501	0	0

Table 5
Ingestion rates (cells larvae^{-1} d^{-1}) ± standard error (SE) by the studied meroplanktonic larvae on the different potential prey. HNF: heterotrophic nanoflagellates; PNF: phototrophic nanoflagellates except *Heterosigma akashiwo*; Dinoflag.: dinoflagellates except *Prorocentrum triestinum*. 0 indicates no significant ingestion (one way-ANOVA, $p > 0.05$) and (*) that the concentration in the experimental bottles was higher than in the control bottles (one way-ANOVA, $p < 0.05$).

Prey	Polychaeta larvae	Echinoderm larvae	Cirripede larvae	Bivalve larvae	Gastropod larvae
Picoflagellates	*	*	*	*	*
HNF	1549 ± 135	0	*	8044 ± 1247	18580 ± 7169
PNF	5832 ± 1509	0	10647 ± 2543	19829 ± 1775	48393 ± 5949
Diatoms	0	195 ± 22	*	0	*
H. akashiwo	698 ± 64	1635 ± 290	4571 ± 375	2275 ± 294	8250 ± 609
Dinoflag.	0	52 ± 10	88 ± 8	42 ± 5	142 ± 34
Ciliates	0	9.4 ± 1.5	15.2 ± 1.4	5.9 ± 1.4	24.0 ± 1.7
P. triestinum	0	122 ± 21	428 ± 28	0	0

larvae (Wang et al., 2006). This is consistent with our feeding experiment results, in which no meroplankton mortality was observed after 48 h of exposure. The larvae produced in our laboratory cultures had never been exposed to any toxic phytoplankton and the larvae collected in plankton nets (bivalve and gastropod larvae) were washed and maintained in filtered seawater for 24 h before the experiment. Zooplankton that ingest harmful phytoplankton species may become inhibited only after several days of continuous ingestion (da Costa and Fernández, 2002; Guisande et al., 2002); therefore, long-term incubation experiments are required to more effectively evaluate the harmful effects of *H. akashiwo* on zooplankton.

The mechanisms of *Heterosigma akashiwo* toxicity remain controversial and unresolved (Twiner et al., 2001; Rensel and Whyte, 2003). The toxic mechanisms of *H. akashiwo* include the discharge of mucus or other lectin-like polysaccharide substances (Nakamura et al., 1998; Oda et al., 1998; Smayda, 1998), the production of brevetoxin-like neurotoxins (Khan et al., 1997; Haque and Onoue, 2002) and the production of reactive oxygen species (Honjo, 1994). Recently, it has been reported that *H. akashiwo* produces extracellular organic compounds that can alter the metabolic activity of mammalian cells and induce apoptotic cell death by inhibiting the plasma membrane Ca^{2+}-ATPase transporter (Twiner et al., 2004, 2005). As previously mentioned, *H. akashiwo* produces polysaccharide-protein complexes (APPCs), analogous to a glycocalyx, which has allelopathic effects on the phytoplankton community (Yamasaki et al. (2009). The inhibitory effect of APPCs on the growth of diatoms has been attributed to APPCs that bind to the cell surfaces of target species. Similarly, several studies have demonstrated that these APPCs on cell membranes (Honjo, 1993; Oda et al., 1998) cause adherence of *H. akashiwo* cells to the zooplankton body, resulting in strongly inhibited swimming ability and consequently decreased food ingestion, development, reproduction and survival (Yan et al., 2003; Wang et al., 2006; Xie et al., 2008; Yu et al., 2010). The attachment of *H. akashiwo* cells to zooplankton might promote or activate the release of endogenous toxic substances from the algae. Additional field and laboratory studies are required to better understand the effects and mechanisms of *H. akashiwo* toxicity on zooplankton.

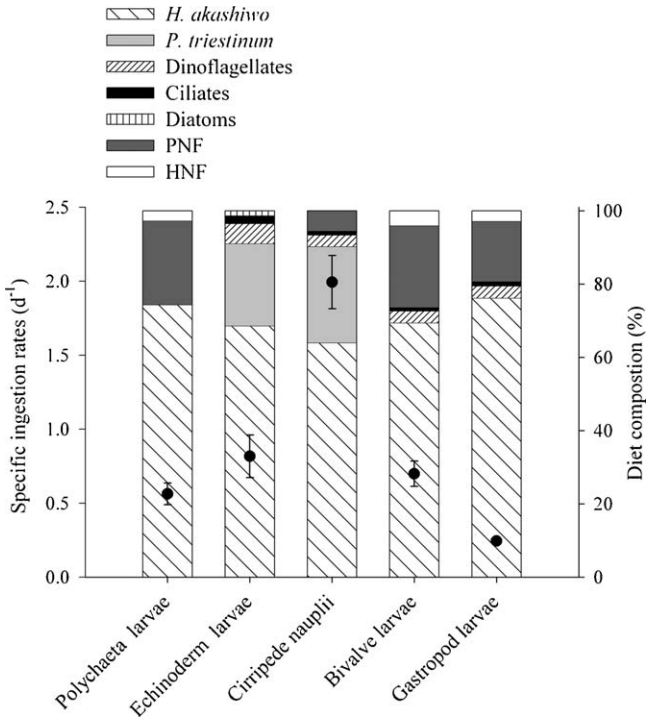

Fig. 5. Carbon-specific ingestion rates (d^{-1}, left axis) and diet composition (% biomass, right axis) of the studied larvae feeding on natural food assemblages under the harmful phytoplankton bloom conditions.

Although there are no reports on the toxicity of *Prorocentrum triestinum*, this species is considered as potentially harmful (Anderson, 1995). This species is not noxious for some copepods (da Costa et al., 2005) but negatively affects the survival of bivalve larvae after 4 days of incubation (Lee, 2003). More research is needed to assess the mechanism by which *P. triestinum* is harmful to some marine invertebrate larvae.

4.3. Feeding rates, food selection and diet compostion

Clearance and ingestion rates of zooplankton vary widely depending on many factors including body size, predator density, prey size, food concentration, food quality and temperature (Frost, 1972; Hansen, 1991a; Hansen et al., 1997; Almeda et al., 2009, 2010). The clearance rates measured in this study are in agreement with the range of values reported in the literature for this type of meroplanktonic larvae feeding on natural food assemblages (Rassoulzadegan and Fenaux, 1979; Baldwin and Newell, 1991; Turner et al., 2001; Vargas et al., 2006) and in the upper limit or higher than those commonly reported from laboratory studies (Strathmann, 1971; Jeong et al., 2004; Almeda et al., 2009). The high C-specific ingestion rates of cirripede nauplii as compared to other meroplanktonic larvae are not only related to body size, since larvae of similar size differed significantly (ANOVA, $p < 0.01$) in specific ingestion rates. These differences in ingestion rates may reflect important differences in feeding behavior and physiology between crustacean and ciliated meroplanktonic larvae as supported by previous studies (Hansen et al., 1997; Vargas et al., 2006)

Information regarding zooplankton feeding rates on *P. triestinum* is limited, although this species is considered as a non-toxic food suitable for some zooplankton (da Costa et al., 2005). The

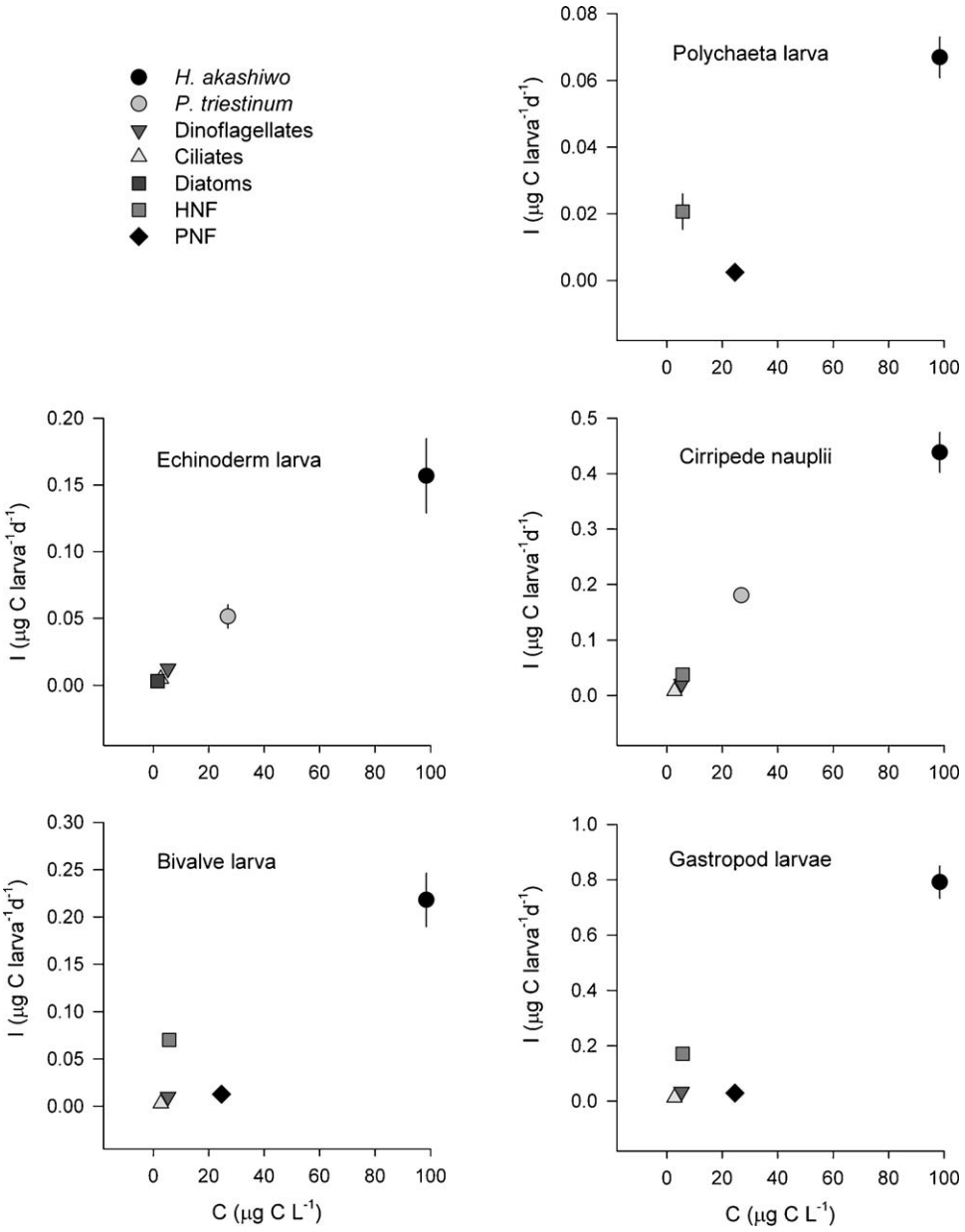

Fig. 6. Relationships between ingestion rates of each prey type and biomass concentration.

interactions between zooplankton grazers and *H. akashiwo* seem to be species-specific. Some species of copepods (e.g. *Acartia hudsonica*, *A. tonsa*—Tomas and Deason, 1981), rotifers (e.g. *Synchaeta cecilia*—Egloff, 1986) and tintinnids (*Favella* sp.—Verity and Stoecker, 1982) show no or little feeding on *H. akashiwo*. However, in agreement with our results for meroplanktonic larvae, *H. akashiwo* is accepted as food by some species of copepods (e.g. *Acartia omorii*—Uye and Takamatsu, 1990; *Schmackeria inopinus*—Yu et al., 2010), and rotifers (e.g. *Brachionus plicatilis*—Xie et al., 2008). Although in some cases feeding on *H. akashiwo* results in

Table 6
Potential trophic impact (% biomass of the standing stock grazed daily) by the studied larvae upon the different prey during the harmful phytoplankton bloom. The last column indicates the total trophic impact by the studied larvae as a whole. HNF: heterotrophic nanoflagellates; PNF: phototrophic nanoflagellates except *Heterosigma akashiwo*; Dinoflag.: dinoflagellates except *Prorocentrum triestinum*.

Prey	Polychaeta larvae	Echinoderm larvae	Cirripede larvae	Bivalve larvae	Gastropod larvae	All larvae
HNF	0.18	0.00	0.00	0.18	0.10	0.46
PNF	0.34	0.00	0.20	0.23	0.14	0.91
Diatoms	0.00	0.12	0.00	0.00	0.00	0.12
H. akashiwo	0.28	0.10	0.58	0.18	0.16	1.29
Dinoflag.	0.00	0.14	0.49	0.15	0.12	0.90
Ciliates	0.00	0.11	0.38	0.09	0.09	0.67
P. triestinum	0.00	0.12	0.87	0.00	0.00	0.99

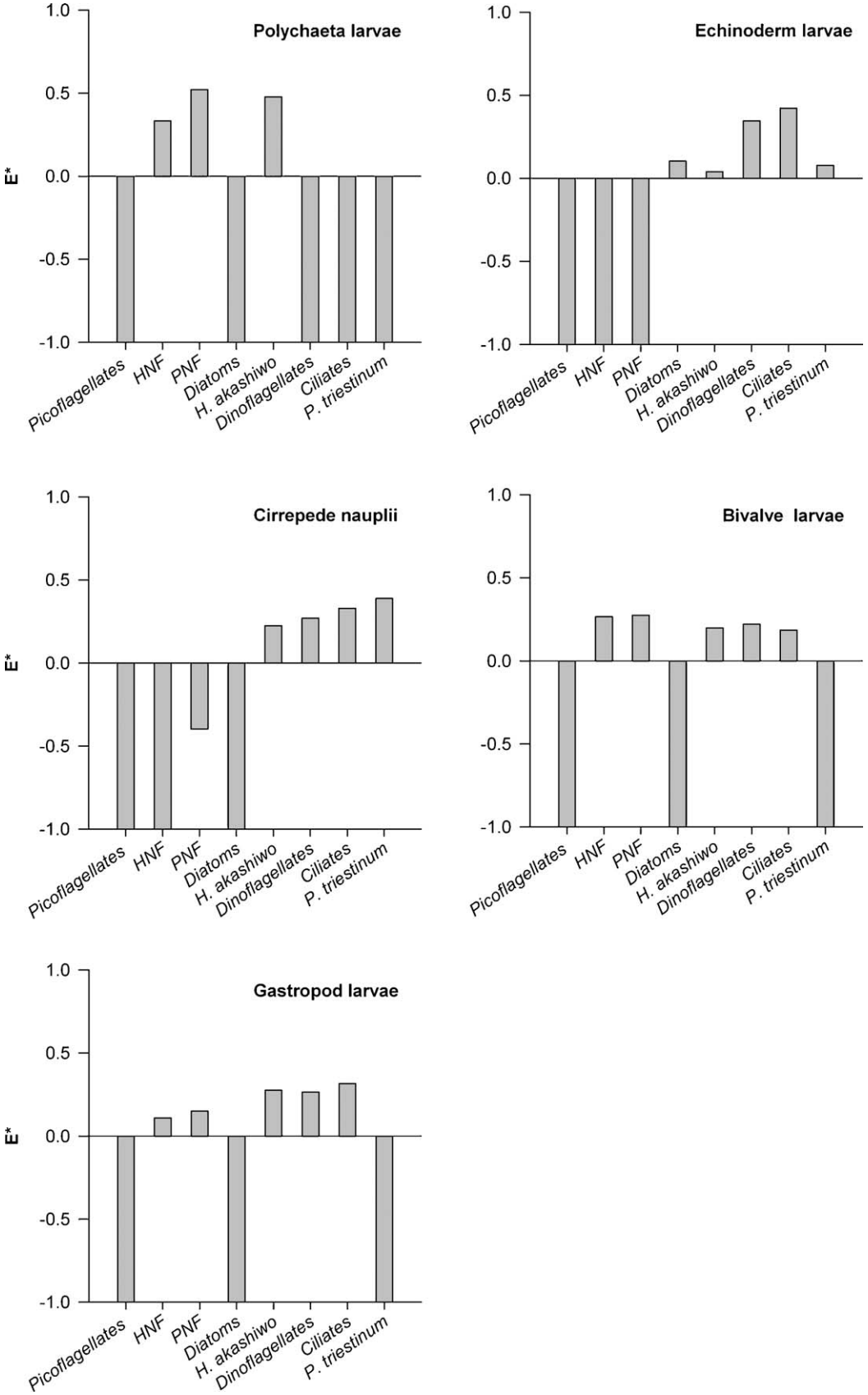

Fig. 7. Electivity index (E^*) of the studied meroplanktonic larvae feeding on natural food assemblages under harmful phytoplankton bloom conditions.

low ingestion efficiencies (Xie et al., 2008; Yu et al., 2010), the specific ingestion rates observed in this study were in the range of those commonly found for meroplankton feeding on non-toxic prey (White and Roman, 1992; Hansen et al., 1997).

Prey selectivity by zooplankton may be the result of differential vulnerability of prey species (passive selection, e.g. size-based selection) or of choice exercised by the predator in accepting or rejecting a prey type (active selection). In contrast to other zooplankton species that actively reject *H. akashiwo* as food (Taniguchi and Takeda, 1988; Uye and Takamatsu, 1990), the larvae examined in this study selectively ingested this species. However, this selection may due to the high abundance of this prey (passive selection) instead of an active selection. Passive selection is frequently a size-based selection that could be explained partly by the morphology of the predator's filtering structures (Boyd, 1976). The size of *H. akashiwo* is within or at the upper limit (depending on larvae type) of the spectrum of particle sizes commonly ingested by meroplanktonic larvae (Rassoulzadegan and Fenaux, 1979; Hansen, 1991a; Raby et al., 1997; Jeong et al., 2004; Vargas et al., 2006; Hansen et al., 2010). However, *P. triestinum* seems to be too large to be captured by veliger and trochophore larvae, whereas the available diatom species may be too thin to be efficiently captured by cirripede nauplii and too long to be ingested by veliger and trochophore larvae. In agreement with other studies for some zooplankton (Parsons et al., 1967; Raby et al., 1997; Turner and Borkman, 2005), when prey were of the appropriate size and were not actively avoided, the diet composition was concentration dependent. Biochemical composition (food quality) plays an important role in active food selection by zooplankton (Gallagher, 1988; Paffenhöfer and Lewis, 1990). Some authors have proposed that the ability to reject *H. akashiwo* by some zooplankters is chemically mediated (Uye and Takamatsu, 1990). However, these mechanisms and the intra- and extracellular compounds have not been fully elucidated (see below).

Picoplankton concentrations significantly increased in all our experimental treatments, suggesting a positive effect of these larvae on picoplankton growth. The mechanism responsible for this effect is not clear. However, the larvae used in this study may have removed other potential grazers of picoplankton (e.g. ciliates, dinoflagellates, HNF—Hansen, 1991b; Sherr and Sherr, 2002), resulting in relaxed protozoan grazing pressure on these small prey, in turn, overshadowing the effects of meroplanktonic larval grazing. Although some of the meroplanktonic larvae that we have studied may feed on picoplankton, the ingestion rates of this food source as well as other prey, may be masked by a more significant cascade of trophic effects caused by the removal of other predatory groups. It is also important to note that picoplankton can be an important part of the diet of some meroplanktonic larvae (Baldwin and Newell, 1991; Raby et al., 1997).

4.4. Trophic impact

The negative effect of *H. akashiwo* on both meroplanktonic larvae and holoplankton abundance may contribute to the reduction of grazing pressure on the harmful bloom. The impact of zooplankton grazing on harmful algal blooms is quite variable and depends on several factors including the bloom stage, the abundance of grazers and the grazing rates of individual zooplankters. In some instances, the effect of metazooplankton grazing on HABs has been reported to be minimal (Calbet et al., 2003; Turner and Borkman, 2005; Turner, 2006) or only important during the inital phase of the bloom (Uye, 1986). However, other studies have reported that the grazing impact of metazooplankton may cause or significantly contribute to the decline of the bloom when zooplankton abundance is very high [polychaete larvae: 855 ind l^{-1} (Turner and Anderson, 1983); *Acartia hudsonica*: 50 mg C m^{-3} (Campbell et al., 2005)]. Since bloom-forming phytoplankton species were accepted as food by the meroplankton larvae in the present study, the small trophic impact may mainly be due to low grazer abundance. In conclusion, meroplanktonic larvae seem to have a minimal effect on *H. akashiwo*/*P. triestinum* blooms on the west coast of Vancouver Island.

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