

# Degradation of the Adriatic medusa *Aurelia* sp. by ambient bacteria

Tinkara Tinta · Alenka Malej · Maja Kos ·  
Valentina Turk

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**Abstract** The decomposition of jellyfish after major bloom events results in the release of large amounts of nutrients, which can significantly alter nutrient and oxygen dynamics in the surrounding environment. The response of the ambient bacterial community to decomposing jellyfish biomass was evaluated in two marine ecosystems, the Gulf of Trieste (northern Adriatic Sea) and Big Lake (Mljet Island, southern Adriatic Sea). The major difference between these two ecosystems is that *Aurelia* sp. medusae occur throughout the year in the oligotrophic Big Lake, whereas in the mesotrophic Gulf of Trieste, they occur only seasonally and often as blooms. Addition of homogenized jellyfish to enclosed bottles containing ambient water from each of these systems triggered considerable changes in the bacterial community dynamics and in the nutrient regime. The high concentrations of protein, dissolved organic phosphorous (DOP), and  $\text{PO}_4^{3-}$  immediately after homogenate addition stimulated increase in bacterial abundance and production rate, coupled with  $\text{NH}_4^+$  accumulation in both ecosystems. Our preliminary results of the bacterial community structure, as determined with denaturing

gradient gel electrophoresis, indicated differences in the bacterial community response between the two ecosystems. Despite divergence in the bacterial community responses to jellyfish homogenate, increased bacterial biomass and growth rates in both distinctive marine systems indicate potentially significant effects of decaying jellyfish blooms on microbial plankton.

**Keywords** *Aurelia* sp. · Decomposition · Nutrients · Bacterioplankton · Jellyfish

## Introduction

The material from dead organisms (detritus) plays an important role in the cycling of organic matter in the marine environment (e.g., Alldredge, 1972, 1976, 2005). Bacterial decomposition of phytoplankton and numerous other organic matter sources has been demonstrated in field and laboratory experiments (e.g., Caron et al., 1982; Alldredge & Youngbluth, 1985; Alldredge et al., 1986; Simon et al., 1990; Passow & Alldredge, 1994; Passow et al., 1994). Gelatinous macrozooplankton often are regarded as nutritionally poor food sources due to their high water content and predation on them often disregarded; however, predation on jellyfish by fish and large marine animals, such as sunfish and leatherback turtles (reviewed in Arai, 2005; Houghton et al., 2006), indicates that these organisms are good organic sources and potential substrates for bacteria. In particular, during the decomposition of blooms,

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T. Tinta · A. Malej · M. Kos · V. Turk (✉)  
National Institute of Biology, Marine Biology Station,  
Fornace 41, 6330 Piran, Slovenia  
e-mail: turk@mbss.org

jellyfish carcasses can serve as major carbon sources to the environment (Billett et al., 2006; Yamamoto et al., 2008). The total organic content of jellyfish is 1–2% of wet weight (Larson, 1986; Clarke et al., 1992) and generally consists of low carbohydrate ( $7 \pm 5\%$ ), intermediate lipid ( $22 \pm 12\%$ ), and high protein ( $72 \pm 14\%$ ) contents (reviewed in Pitt et al., 2009).

The few studies that have addressed the fate of dead jellyfish (Titelman et al., 2006; West et al., 2009) indicated rapid decomposition and nutrient release, whether in the water column or on the sediment surface. Scavengers tend to enhance the decomposition of carcasses by increasing the available surface area, but most of the organic matter is ultimately processed by bacteria. Bacteria hydrolyze and utilize virtually all natural polymers by means of extracellular enzymes. The turnover rate of soluble proteins by bacteria in seawater is only hours (Hollibaugh & Azam, 1983; Hoppe et al., 1988) and similar to the turnover rate of free amino acids in solution (Keil & Kirchman, 1993). The end products of protein degradation, such as dissolved primary amines and free amino acids, contribute to the dissolved organic and inorganic nitrogen (e.g., ammonium) pools (Kremer, 1977). Degradation of lipids (phospholipid components of cell membranes) and nucleic acids (DNA and RNA) contributes to the dissolved organic phosphorous pool (DOP) (Kremer, 1975; Båmstedt & Skjoldal, 1980; Arai, 1997). Thus, the release of large amounts of nutrients into the marine environment from jellyfish bloom decomposition potentially causes substantial changes in substrate quality and quantity, and could affect the composition and activity of the bacterial community (Martinez et al., 1996; Pinhassi et al., 1999; Schafer et al., 2001; Carlson et al., 2002). This has been the subject of only one previous study (Titelman et al., 2006).

We have conducted incubation experiments to evaluate the responses of native bacterial communities to the addition of dead jellyfish biomass. Homogenized tissue of *Aurelia* sp. was used as the jellyfish substrate. This genus of scyphomedusae occurs worldwide in numerous coastal and shelf sea environments (Lucas, 2001) where it frequently forms large blooms (Hamner & Dawson, 2009). We examined the degradation of *Aurelia* sp. and its effects on the ambient bacterial community during

spring of 2008 and 2009 in the Gulf of Trieste (northern Adriatic Sea), where they often form blooms in the winter–spring period (Kogovšek et al., this volume) and in Veliko jezero (Big Lake, Mljet Island, southern Adriatic Sea), where they are abundant throughout the year (Benović et al., 2000). Effects of jellyfish tissue on bacterial abundance and production, and on the bacterial community structure, were monitored concurrently with changes in protein and nutrient concentrations.

## Materials and methods

### Sampling and locations

Experiments were performed in the Bay of Piran (Gulf of Trieste, northern Adriatic Sea) and in the marine lake Veliko jezero (Big Lake) at Mljet Island (southern Adriatic Sea). Experiments were conducted at both locations during spring of 2008 and 2009. The two sets of results from each location were treated as replicates for the site. Seawater samples were collected with a Niskin sampler at 3-m depth at the station BF ( $45^{\circ}32.804\text{N}$ ;  $13^{\circ}33.034\text{E}$ ) in the Bay of Piran, and at 3- and 30-m depths at station Buza ( $42^{\circ}46.477\text{N}$ ;  $17^{\circ}23.304\text{E}$ ) in Big Lake. Before each sampling, seawater temperature and salinity profiles were determined with a CTD fine-scale probe (Microstructure Profiler MSS90, Sea & Sun Technology GmbH). The water temperatures in spring 2008 and 2009 were 14 and 11°C, respectively, in the Bay of Piran (3-m depth), between 17 and 19°C in Big Lake at 3-m depth, and  $\sim 10^{\circ}\text{C}$  at 30-m depth in both years. The ambient ammonium concentration varied in the spring 2008 and 2009 between 0.5 and 0.82  $\mu\text{M}$  in the Bay of Piran and between 0.19 and 0.43  $\mu\text{M}$  in Big Lake. The ambient orthophosphate concentration was low and similar in both years in both ecosystems, between 0.1 and 0.17  $\mu\text{M}$  at 3-m depth, and 0.03 and 0.04  $\mu\text{M}$  at 30-m depth (Table 1).

Immediately after sampling, seawater was filtered through a 200- $\mu\text{m}$  mesh net and subsequently through GF/F filters (Whatman) to yield a filtrate containing microorganisms  $<0.8 \mu\text{m}$  in size. The filtered seawater was collected in 8-l acid-washed, autoclaved polycarbonate bottles (Nalgene) and was used for the jellyfish decomposition experiments as described

**Table 1** Summary of jellyfish degradation experiments with details of the incubations and ambient seawater temperature ( $T$ ), ammonium ( $\text{NH}_4^+$ ), and orthophosphate ( $\text{PO}_4^{3-}$ ) concentrations

Exp. code	Incubation time	Depth (m)	$T$ ( $^\circ\text{C}$ )	Inoculate ( $\text{g l}^{-1}$ )	$\text{NH}_4^+$ ( $\mu\text{M}$ )	$\text{PO}_4^{3-}$ ( $\mu\text{M}$ )
Piran 2008	17 Apr–5 May 2008	3	14	30.0	0.50	0.10
Piran 2009	24 Mar–15 Apr 2009	3	11	12.5	0.82	0.17
Mljet 2008	8 May–12 May 2008	3	17	15.0	0.30	0.11
		30	10	15.0	0.19	0.03
Mljet 2009	15 May–22 May 2009	3	19	5.5	0.32	0.15
		30	11	3.1	0.43	0.04

below. At the same time, subsamples of filtrate were frozen at  $-20^\circ\text{C}$  for nutrient analysis.

### Jellyfish decomposition experiments

*Aurelia* sp. medusae were collected by dip net from the surface during bloom events (April 2008 and March 2009) in the Bay of Piran and by divers in Big Lake. Bell diameter and wet weight were recorded for each individual. Bell diameters ranged from 6.3 to 18.5 cm, and a mean wet weight was 15 g. In each experiment, 3–5 jellyfish of different sizes were selected and their whole bodies homogenized with an Ultra-Turrax TP 18/10 (Janke & Kundel<sup>®</sup>) at 20,000 rpm for several minutes. The whole tissue was homogenized to enhance its degradation by the ambient bacterial community in the sampled sea water. Sea water samples were inoculated with different concentrations of the homogenate (from 3 to 30  $\text{g l}^{-1}$ , Table 1). The volume of added homogenate was recalculated according to the average organic content of *Aurelia* sp. and approximate jellyfish concentration during the bloom (Alvarez-Colombo et al., 2008; Turk et al., 2008). Part of the homogenate was frozen and stored for CHNS elemental analysis.

All experiments were carried out in 8-l polycarbonate bottles with GF/F pre-filtered sea water, as described above. A measured weight of homogenized jellyfish tissue was added to each experimental bottle (Table 1). Bottles with filtered sea water without jellyfish homogenate served as controls. The bottles were incubated in situ at each study site to provide ambient conditions of irradiance and water temperature. Locations, dates, durations, and depths of the incubations are in Table 1.

The same sampling procedure was followed at both locations throughout the experiments. Bottles

were sampled immediately after inoculation ( $T_0$ ), and subsamples for nutrient and bacterioplankton analysis were taken on days 1, 2, 4, 6, 8, and 18 in 2008 and on days 1, 2, 3, 6, 9, and 22 in 2009 Bay of Piran experiments. During the experiments in Big Lake, the subsamples were taken daily. Prior to sampling, the enclosed fluid was mixed gently. Subsamples were taken by pouring 500 ml of seawater into acid-washed, autoclaved flasks. Some air was introduced into incubation bottles during subsampling.

Subsamples (5 ml) for bacterial carbon production measurements were incubated immediately after sampling. 300-ml subsamples were filtered (GF/F Whatman) and kept frozen for nutrient analysis. 30 ml of seawater was fixed for bacterial counts and stored at  $-4^\circ\text{C}$ . At the beginning and end of the experiments, sea water from the bottles was filtered in triplicate onto 0.2- $\mu\text{m}$  polyethersulfone membrane filters (PALL Inc.) for bacterial community analysis. Those filters were stored at  $-80^\circ\text{C}$  for DNA extraction.

### Bacterial abundance and carbon production

Bacterial abundance was determined from formaldehyde-fixed ( $<0.2 \mu\text{m}$  pre-filtered, 2% final concentration) seawater samples. The samples were filtered onto 0.2- $\mu\text{m}$  black polycarbonate filters (Millipore), and bacteria were stained with DAPI (4',6-diamino-2-phenylindole,  $1 \mu\text{g ml}^{-1}$ , final, Sigma) (Porter & Feig, 1980). From each sample, more than 200 bacteria or at least 10 fields per filter were counted using an Olympus BX51 epifluorescence microscope. Pictures were taken using an Olympus Microscope Digital DP70 camera and analyzed with computer software Olympus DP-Soft BX-51. A carbon-to-cell ratio of  $19.8 \text{ fg C bacterium}^{-1}$  (Lee & Fuhrman, 1987) was used to estimate bacterial biomass.

Bacterial carbon production (BCP) was measured by  $^3\text{H}$ -leucine incorporation (Kirchman et al., 1985). Each sample was incubated in triplicate with  $^3\text{H}$ -leucine (20 nM, final, Amersham) for 1 h. Samples with trichloroacetic acid (TCA) added to 5% final concentration prior to addition of  $^3\text{H}$ -leucine served as controls. Incubation was stopped by adding TCA (5% final concentration) to all samples. The samples were washed according to the centrifugation protocol (Smith & Azam, 1992). Radioactivity was measured using a liquid scintillation counter (Canberra Packard and TriCarb Liquid Scintillation Analyzer, model 2500 TR). BCP was calculated as described by Simon & Azam (1989). Bacterial community growth rate,  $\mu$ , was calculated as the ratio of BCP to bacterial biomass (B).

### Bacterial community structure

DNA was extracted from the filters as described in Boström et al. (2004), with slight modifications. DNA was precipitated at  $-20^\circ\text{C}$  for 1 h, with 0.1 volume of ammonium acetate (3 M NaAc, pH 5.2) and 0.6 volume of isopropanol; 5- $\mu\text{l}$  baker's yeast tRNA (stock 9.7 mg  $\text{ml}^{-1}$ ) was added as co-precipitant. The pellet was washed with 70% ice-cold ethanol and air dried. Precipitated DNA was re-suspended in 0.02- $\mu\text{m}$  pre-filtered, autoclaved  $\text{H}_2\text{O}$ , and kept at  $-20^\circ\text{C}$ . Bacterial 16S rDNA was amplified using a universal primer complementary to position 907–927 (Muyzer & Smalla, 1998) and a bacterial primer complementary to position 341–358, with a 40-bp GC-clamp (Muyzer et al., 1993). The PCR reaction mix contained 1 $\times$  reaction buffer (Tris KCl), 2 mM  $\text{MgCl}_2$ , 0.2 mM dNTP, 1  $\mu\text{M}$  of each primer, Taq polymerase (5 U  $\mu\text{l}^{-1}$ , Fermentas), and 1  $\mu\text{l}$  of DNA (50–100 ng). The PCR touchdown protocol of Don et al. (1991) was used; entailing initial denaturation at  $94^\circ\text{C}$  for 5 min, followed by a thermal cycler program: denaturation for 1 min at  $94^\circ\text{C}$ , primer annealing for 1 min at an initial  $65^\circ\text{C}$ , decreasing  $1^\circ\text{C}$  every two cycles to a final  $50^\circ\text{C}$ , primer extension for 3 min at  $72^\circ\text{C}$ . Ten touchdown cycles were run followed by 20 standard cycles (denaturation for 1 min at  $94^\circ\text{C}$ , primer annealing for 1 min at  $55^\circ\text{C}$ , primer extension for 3 min at  $72^\circ\text{C}$ ). The last cycle was followed with a 10-min final incubation at an annealing temperature of  $72^\circ\text{C}$ . The size and quality of PCR products were confirmed by agarose gel electrophoresis. DNA concentration was measured fluorometrically using PicoGreen quantification reagent

(Molecular Probes). PCR products were analyzed by DGGE electrophoresis (C.B.S. Scientific Co.). 60 ng  $\mu\text{l}^{-1}$  of PCR product was loaded onto 6% polyacrylamide gels (acrylamide: $N,N'$ -methylbis-acrylamide 37:1) containing a denaturant gradient top to bottom of 29–52% (100% denaturant is defined as 7 M urea and 40% (v/v) formamide). Electrophoresis was run at 150 V for 6 h using  $0.5\times$  TAE running buffer at  $60^\circ\text{C}$ . The concentration and volume of DNA loaded per lane were constant, which enabled quantitative comparison between bands. A dendrogram was constructed from DGGE banding patterns by the software Quantity One 4.6.2 (Bio-Rad) using Dice coefficient and cluster analysis by the unweighted-pair group method using arithmetic average. DGGE bands were visually detected, and the presence and position of bands were employed in the similarity analysis.

### Nutrient analysis

All nutrient analyses were performed on GF/F pre-filtered water samples. Samples were analyzed for total dissolved nitrogen (TDN), ammonium ( $\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), total dissolved phosphorus (TDP), and orthophosphate ( $\text{PO}_4^{3-}$ ), using the standard protocols (Grasshoff et al., 1983). Dissolved organic phosphorus (DOP) was calculated as the difference between TDP and  $\text{PO}_4^{3-}$ . Dissolved oxygen (DO) concentration was measured in triplicate by the standard Winkler method.

Protein concentrations were determined by the standard Bradford method (Bradford, 1976) using the Bio Rad Protein Assay Kit. Seawater samples for protein assays were filtered through GF/F filters and stored at  $-20^\circ\text{C}$  prior to analysis. For each sample, triplicate 2-ml aliquots were centrifuged for 10 min at  $20,000\times g$  at  $4^\circ\text{C}$ .  $\text{OD}_{595}$  was measured versus reagent blanks (0.22- $\mu\text{m}$  pre-filtered seawater) and protein concentrations were determined using a standard curve prepared by dilutions of a bovine serum albumin standard in the range 1–20  $\mu\text{g}$  protein  $\text{ml}^{-1}$ .

## Results

### Bacterial and nutrient dynamics

Bacterial colonization of jellyfish micro-particles was observed by microscope within the first 24 h of the

experiment, and bacterial numbers increased substantially in the following days. In experiments in the Bay of Piran in 2008, the abundance of bacteria increased from an initial value of  $4.1 \times 10^8$  to  $2.3 \times 10^{10}$  cells  $l^{-1}$  by day 2. At the same location in spring 2009, the bacterial abundance increased from  $2.8 \times 10^8$  to  $2.7 \times 10^{10}$  cells  $l^{-1}$  by day 6, showing a much longer lag phase (Fig. 1A). Bacterial carbon production (BCP), measured only in 2009, showed a pattern similar to that of bacterial abundance. At the beginning of the experiment, BCP was low ( $0.1 \mu\text{g C } l^{-1} \text{ d}^{-1}$ ) in the bottles with added jellyfish, but increased by day 3 to  $24.7 \mu\text{g C } l^{-1} \text{ d}^{-1}$ . After an initial lag period, BCP reached high values on day 6 ( $90.5 \mu\text{g C } l^{-1} \text{ d}^{-1}$ ) (Fig. 2A). Bacterial growth rate varied from 0.002 to  $1.15 \text{ d}^{-1}$ .

In experiments in both years at Big Lake, bacterial abundance peaked in the first 3 days, increasing from  $3 \times 10^8$  to  $2.9 \times 10^9$  cells  $l^{-1}$  in 2008 and from  $8 \times 10^8$  to  $4.6 \times 10^9$  cells  $l^{-1}$  in 2009 (Fig. 1B). BCP showed a pattern similar to that for bacterial abundance in 2009. In contrast to the Bay of Piran experiment, BCP peaked at  $101.5 \mu\text{g C } l^{-1} \text{ d}^{-1}$  in the first 2 days in the jellyfish-inoculated bottle, but then decreased to  $8.3 \mu\text{g C } l^{-1} \text{ d}^{-1}$  at the end of the experiment (day 7) (Fig. 2B). Bacterial growth rate varied from 0.29 to  $1.14 \text{ d}^{-1}$ . Changes in bacterial abundance and BCP were much smaller in the control bottles, in both locations (Fig. 2).

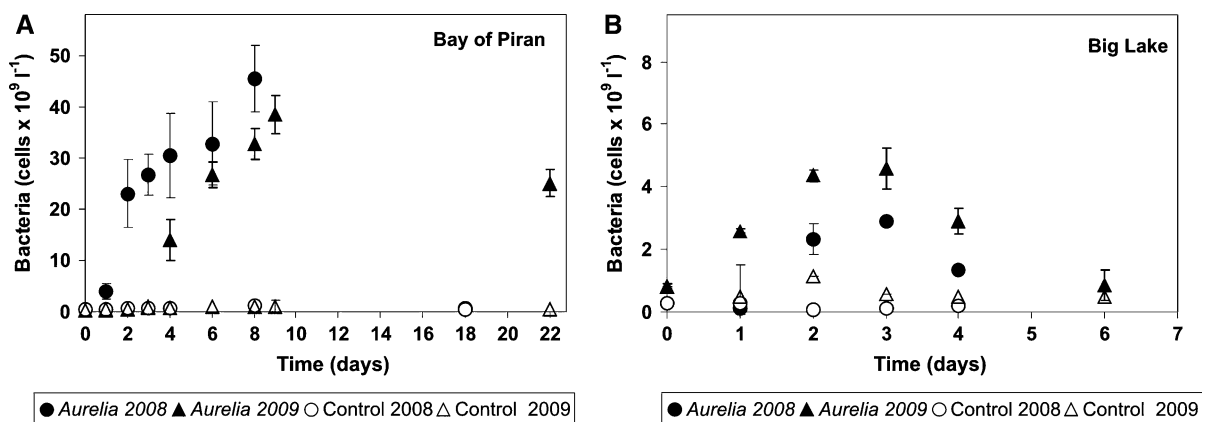
The initial dissolved protein concentration, measured immediately after addition of jellyfish homogenate, was  $2 \mu\text{g ml}^{-1}$  in 2008 and  $0.7 \mu\text{g ml}^{-1}$

in 2009 in the Bay of Piran experiment (Fig. 3A, B) and increased in the first 1–2 days of incubation. After this increase, protein concentration declined over the next 24 h by 55% in 2008 and 80% in 2009 (Fig. 3A, B).

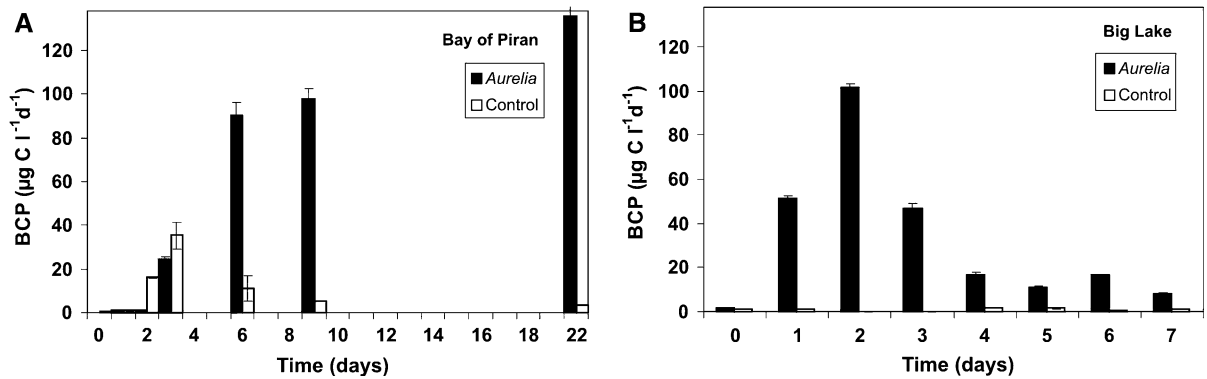
In Big Lake, the initial protein concentrations in the bottles with jellyfish was  $0.64 \mu\text{g ml}^{-1}$  in 2008 and  $1.07 \mu\text{g ml}^{-1}$  in 2009 (Fig. 3C, D). After 2–3 days of incubation, it decreased by 30 and 50% in 2008 and 2009, respectively (Fig. 3C, D). The decreases in protein concentrations corresponded to the increase in bacterial abundance and productivity. In the control bottles at both locations, the protein concentrations were consistently low (below  $0.35 \mu\text{g ml}^{-1}$ ) and did not change during the experiments.

Ammonium concentration ( $\text{NH}_4^+$ ) increased with time in all of the jellyfish-inoculated bottles. At the Bay of Piran, in the first 5 days of each experiment, ammonium increased continuously from 0.5 to  $60 \mu\text{M}$  in 2008 and from 0.82 to  $100 \mu\text{M}$  in 2009. At Big Lake in the first 4 days of each experiment,  $\text{NH}_4^+$  concentration increased continuously from 0.3 to  $38.2 \mu\text{M}$  in 2008 and from 0.32 to  $17 \mu\text{M}$  in 2009. As different amounts of jellyfish homogenate were added to the bottles, the concentration of ammonium was expressed as relative accumulation per amount of added homogenate (Fig. 4).  $\text{NH}_4^+$  concentrations in all control bottles remained low and did not change throughout the experiments.

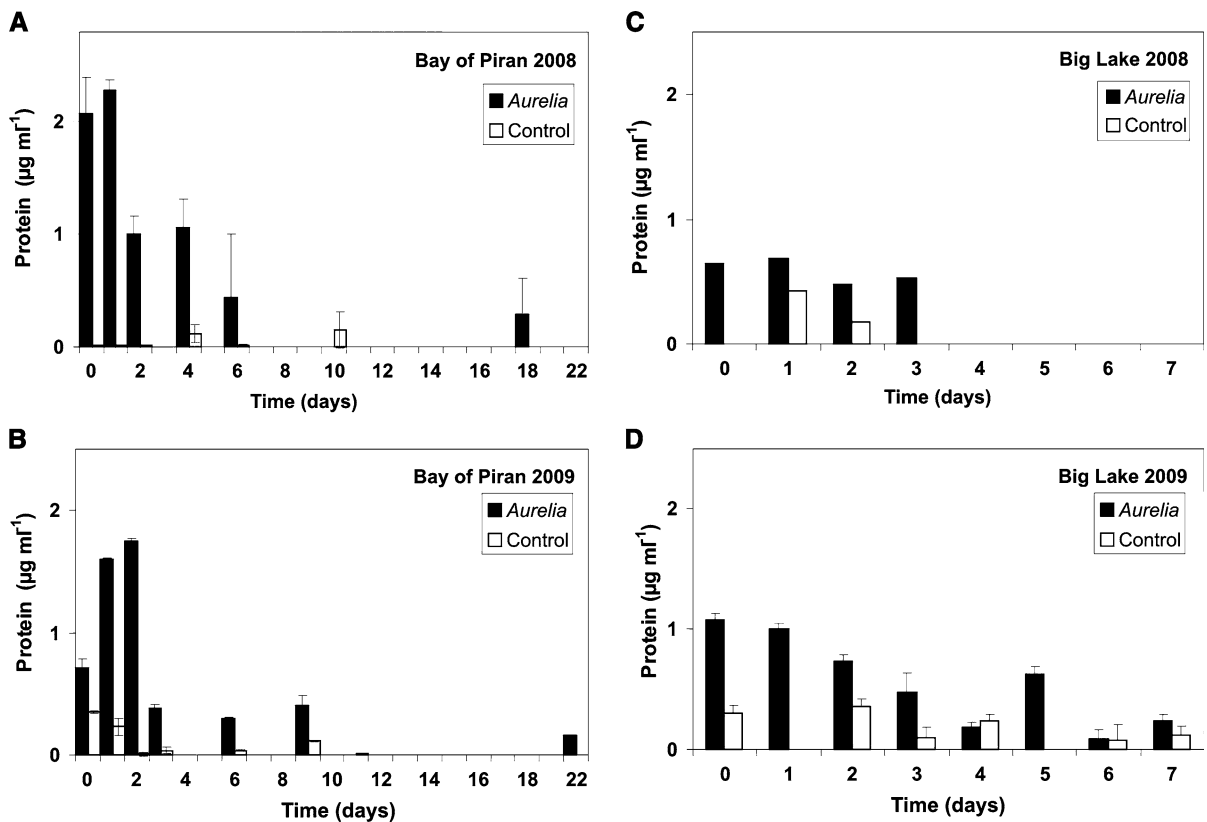
The concentrations of DOP and  $\text{PO}_4^{3-}$  were higher in all experimental bottles with jellyfish homogenate than in the control bottles (Fig. 5). In the 2008 Bay of



**Fig. 1** Bacterial abundance measured during the jellyfish enrichment experiments in the Bay of Piran (A) and Big Lake (B) in spring 2008 and 2009. Each point represents 30 replicates (mean  $\pm$  standard deviation)



**Fig. 2** Bacterial carbon production (BCP) measured during the jellyfish enrichment experiments in the Bay of Piran (A) and Big Lake (B) in spring 2009. Each point represents 3 replicates (mean  $\pm$  standard deviation)

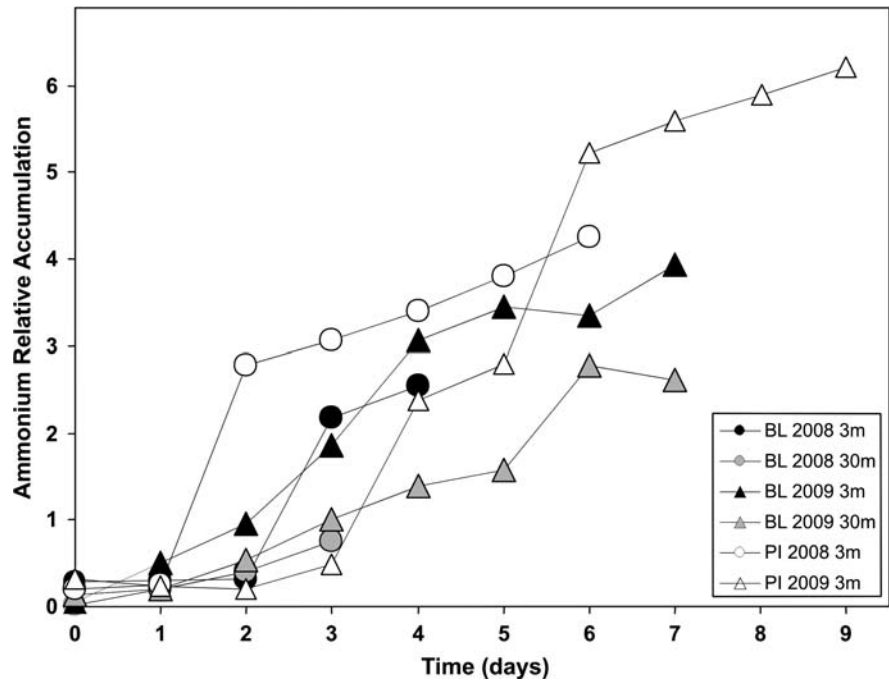


**Fig. 3** Dissolved (GF/F filtrate) protein concentration ( $\mu\text{g ml}^{-1}$ ) measured during the jellyfish enrichment experiments in the Bay of Piran (A, B) and Big Lake (C, D) during spring of 2008 and 2009. Each point represents 3 replicates (mean  $\pm$  standard deviation)

Piran experiment, the initial DOP and  $\text{PO}_4^{3-}$  concentrations were four to seven times higher than those in the controls (Fig. 5A). After the first 24 h, DOP

concentrations decreased continuously to the end of the experiment, while the  $\text{PO}_4^{3-}$  initially decreased but accumulated at the end of the experiment. A

**Fig. 4** Concentrations of ammonium, expressed as relative accumulation per amount of added jellyfish homogenate, during the jellyfish enrichment experiments conducted in the Bay of Piran (PI—3 m) and in Big Lake (BL—3 m and 30 m depth) in spring 2008 and 2009



similar pattern was observed in 2009, except that DOP increased sharply until day 2, reaching eight times the concentration in the control and then decreased to below initial level.  $\text{PO}_4^{3-}$  concentrations doubled during the first 3 days, reaching four times the concentration in the control, and then levelled off until day 9, reaching a much higher concentration on day 22 (Fig. 5B).

In Big Lake experiment in 2009, a similar pattern was observed. After an initial increase, DOP concentrations decreased slightly but steadily, while  $\text{PO}_4^{3-}$  levels decreased but then rose sharply from days 4 to 7 (Fig. 5D). The 2008 experiment lasted only 4 days, so only the initial increases of DOP and  $\text{PO}_4^{3-}$  concentrations were recorded, the latter starting to decrease slightly on day 4, which suggested a pattern similar to that observed in 2009 (Fig. 5C).

The dissolved oxygen (DO) concentration was not measured regularly, and the experimental bottles were gently aerated each sampling time; however, a decrease in DO concentration from 5.9 to 3.04  $\text{ml l}^{-1}$  was observed in Big Lake experiment in spring 2009.

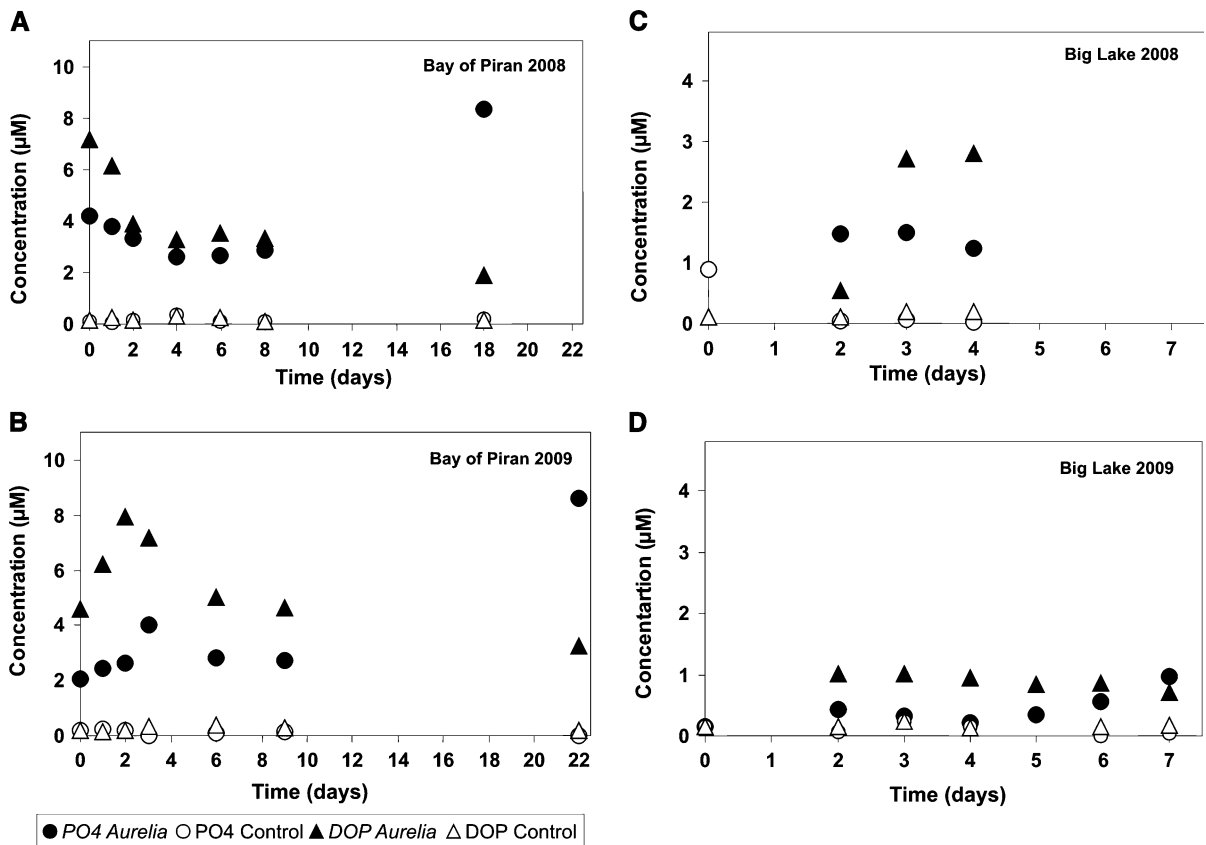
#### Bacterial community composition

PCR-DGGE analysis of 16S rDNA was used to track changes in bacterial community composition from

the beginning ( $T_0$ ) to the end of the experiments in the bottles with jellyfish homogenate ( $T_fA$ ) and controls ( $T_fC$ ) in 2008 (Fig. 6). The bacterial community composition in the jellyfish-inoculated bottles changed with time, as indicated by differences between the community fingerprints at  $T_0$  and  $T_f$ . In the Big Lake experiment, the number of bands in the fingerprints did not change between  $T_0$  and  $T_f$ , either in the jellyfish-inoculated bottles ( $T_fA$ ) or in the control ( $T_fC$ ). Comparison of the positions of bands in the fingerprints showed that some were conserved in all fingerprints ( $T_0$ ,  $T_fA$ , and  $T_fC$ ), while other bands either disappeared or appeared in the jellyfish-inoculated bottles and in the control.

In the Bay of Piran experiment, the number of bands decreased between  $T_0$  and  $T_f$ , especially in the controls ( $T_fC$ ) (Fig. 6). Although, some bands were conserved at the same position in all fingerprints, indicating that some bacterial groups were always present, both the band positions and intensities clearly showed that temporal changes in bacterial community composition occurred in the jellyfish-inoculated bottles and in the controls.

A dendrogram based on the presence and/or absence of bands showed that the bacterial communities grouped in two clusters; the jellyfish-inoculated communities (A) formed one cluster and the  $T_0$



**Fig. 5** Dissolved organic phosphorous (DOP) and orthophosphate ( $\text{PO}_4^{3-}$ ) concentrations measured during the jellyfish enrichment experiments in the Bay of Piran (A, B) and in Big

Lake (C, D) in spring 2008 and 2009 (note the difference in duration between figures)

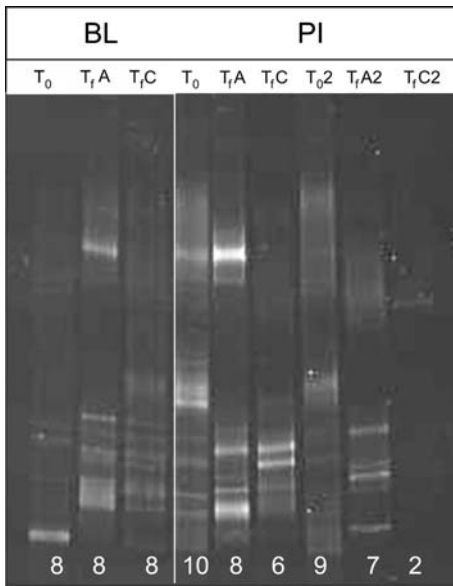
communities another (Fig. 7). The control communities fit into separate clusters.

## Discussion

It has been shown that *Aurelia* sp. exerts direct predatory pressure on mesozooplankton and microzooplankton populations (Stoecker et al., 1987; Sullivan et al., 1994; Purcell & Sturdevant, 2001; Malej et al., 2007; Lo & Chen, 2008). There is also evidence of an indirect cascading effect of *Aurelia* sp. on autotrophic and heterotrophic microbial plankton (Turk et al., 2008). Heterotrophic bacteria, as major consumers of dissolved organic matter (DOM) in marine ecosystems (Azam & Malfatti, 2007; Kirchman, 2008), may benefit most from increased DOM levels as jellyfish decompose. Decomposition of other gelatinous zooplankton, such as salps,

ctenophores, and appendicularia, contributes to the general pool of marine snow in the ocean (Caron et al., 1982). Jellyfish play an important role in providing large amounts of carbon and nutrients to the microbial loop by several possible pathways: (a) excretion, (b) mucus production and release (Schneider, 1989; Hansson & Norrman, 1995; Riemann et al., 2006), and (c) decaying biomass (Titelman et al., 2006; West et al., 2009). The decomposition of jellyfish after major bloom events releases large amounts of nutrients, and may significantly alter nutrient and oxygen dynamics in the surrounding environment (Pitt et al., 2009). Depending on the degree of mixing in the water column, decaying jellyfish may also contribute substantially to bottom water hypoxia (West et al., 2009). Moreover, the decomposition of jellyfish can lead to a large flux of particulate carbon to the sediment (Mills, 1995; Billett et al., 2006).



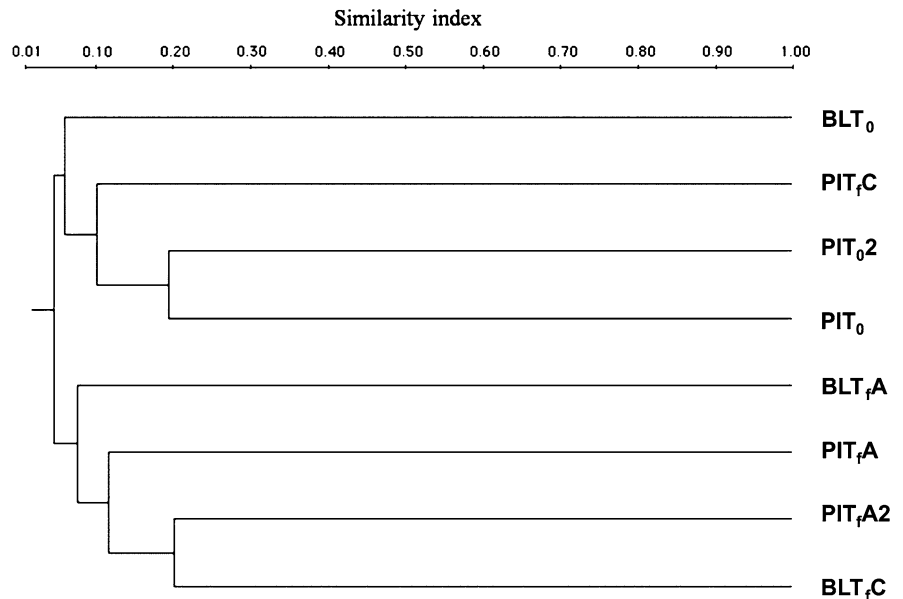


**Fig. 6** Temporal changes in bacterial community composition fingerprints analyzed by DGGE during the jellyfish enrichment experiments conducted in the Bay of Piran and Big Lake in spring 2008. First three lanes represent the results of the community fingerprints from the experiment in the Big Lake (BL) and next six lanes community fingerprints determined during the jellyfish enrichment experiments in the Bay of Piran (PI, two replicates).  $T_0$ —at the beginning of the experiments,  $T_t$ —at the end of the experiments, A—jellyfish-inoculated bottle, C—control. The numbers below each lane are the numbers of bands detected

We have evaluated the response of native bacterial communities to a large influx of particulate organic matter in marine ecosystems—the Bay of Piran (northern Adriatic Sea) and Big Lake (Mljet Island, southern Adriatic Sea). The important difference between these ecosystems is the year-round presence of *Aurelia* sp. in Big Lake (Benović et al., 2000) as compared with their periodic occurrence in great abundance in the Gulf of Trieste (Purcell et al., 1999; Kogovšek et al., this volume). Furthermore, Big Lake is an enclosed marine lake, connected to the Adriatic Sea by a long, narrow, shallow channel, but the Bay of Piran is part of the regularly flushed northern Adriatic Sea. The experiments were conducted in both locations during the spring, and ambient temperature, salinity, chlorophyll *a*, nutrient levels, and bacterial abundance were similar, though temperatures were slightly lower and phytoplankton biomass slightly higher in the Bay of Piran experiments (data not shown).

The addition of homogenized jellyfish to sea water triggered considerable changes in bacterial community dynamics and in the nutrient regime at both locations (Figs. 1, 2, 3, 4, 5). Although different concentrations of inoculate were added, similar patterns of bacterial and nutrient dynamics were observed in different years at each location (Table 1). The initially high

**Fig. 7** Dendrogram constructed from DGGE banding patterns using the cluster analysis by the unweighted-pair group method using arithmetic average. The number of discernible bands used in the construction of the dendrogram is indicated below each lane in Fig. 6. BL—Big Lake, PI—Bay of Piran,  $T_0$ —at the beginning of the experiment,  $T_t$ —at the end of the experiment, A—jellyfish-inoculated bottle, C—control bottle



concentrations of protein, DOP, and  $\text{PO}_4^{3-}$  in the inoculated bottles maintained an increase in bacterial abundance and productivity. In the Bay of Piran experiment, the protein concentration increased in the first 24–48 h due to rapid jellyfish degradation, as also observed by West et al. (2009). This was followed by a steady decrease, presumably due to proteolysis and uptake by bacteria; however, in Big Lake, protein concentration peaked upon addition of jellyfish homogenate to the bottles and decreased rapidly thereafter. The addition of jellyfish homogenate to water from Big Lake triggered a rapid bacterial community response, with increased bacterial abundance and production in 24–48 h (Fig. 1B, 2B). In contrast, the bacterial response lagged in the Bay of Piran experiment, with the greatest increase in bacterial abundance and production after 6 days (Fig. 1A, 2A). One explanation for the difference in the bacterial community response times could be the higher seawater temperature in Big Lake in both years (Table 1). Another explanation is that the native bacterial community in Big Lake may be predisposed toward decomposition of jellyfish tissue because *Aurelia* sp. occur and die in the lake year-round, whereas the bacterial community from the Bay of Piran may need to adapt to the new jellyfish substrate.

Utilization of substrates is believed to be driven by the extent of nutrient or energy limitation in the system (Thingstad et al., 1997; Caron et al., 2000). Given that the two ecosystems have low ambient concentrations of  $\text{NH}_4^+$ , rapid depletion of proteins followed by an accumulation of ammonium in the incubation bottles is not surprising (Figs. 3, 4). Although  $\text{PO}_4^{3-}$  increased steadily, the DOP concentration did not change dramatically in Big Lake, suggesting that other sources of phosphorous may have been available to the microbial community (Fig. 5D). Because inorganic and organic phosphorous compounds are the limiting elements for microbial growth in both ecosystems, those available from the degrading jellyfish were rapidly consumed by bacteria, and  $\text{PO}_4^{3-}$  accumulation was observed only in the experiments with prolonged incubation (>6 days) (Fig. 5A, B).

Protein consumption coupled with  $\text{NH}_4^+$  accumulation and oxygen consumption in the jellyfish-inoculated bottles suggests that the proteins were catabolized for energy rather than assimilated in salvage pathways (Cherrier & Bauer, 2004; West

et al., 2009). Because dissolved oxygen levels in the incubation bottles decreased with time, the hypoxic conditions may have affected bacterial community composition as well as their metabolic processes. Information from several recently completed bacterial genome sequences shows substantial cellular allocations to amino acid and peptide transport, which suggests that these substrates are important for meeting bacterial nitrogen demands (Giovannoni et al., 2005). Among the nitrogen-containing reduced carbon substrates, dissolved-free amino acids and protein (or dissolved combined amino acids) appear to support the greatest fraction of bacterial growth (Kirchman, 2008).

In addition to the different times of the bacterial response to the addition of jellyfish homogenate, the bacterial community structure dynamics diverged in the two ecosystems. The number of bands in the community fingerprints did not change between  $T_0$  and  $T_f$  in the Big Lake experiment. Although, the final bacterial community fingerprint ( $T_f$ ) appears to differ from that of the  $T_0$  community, both  $T_fA$  and  $T_fC$  groups appear in the same cluster, which indicates a similar community composition. The similarities between the  $T_f$  community fingerprints suggest that the native Big Lake bacterial community is adapted to decomposing jellyfish biomass, which occurs throughout the year in this environment. The rapid response of the bacterial community to the addition of jellyfish homogenate in Big Lake also supports this conclusion. The relative intensities of bands from the  $T_f$  communities in Big Lake indicate that certain bacterial groups in the  $T_fA$  community proliferate in the presence of jellyfish homogenate, suggesting that some specific groups are better adapted than others to utilize this substrate for their growth.

In contrast, in the Bay of Piran where jellyfish are not always present, the number of bands decreased from  $T_0$  to  $T_f$  bacterial community fingerprints ( $T_fA$  and  $T_fC$ ). This decrease in bacterial diversity could be due to the lack of certain nutrients in the bottles, as compared to ambient concentrations, because no supplements other than jellyfish homogenate were added to the enclosed communities. This could explain the large decrease in the number of bands in the  $T_fC$  community fingerprints. Surprisingly, the number of bands in  $T_fA$  was considerably greater than that in the  $T_fC$  fingerprints, and the high intensity of

some bands indicates that certain bacterial groups in the  $T_rA$  communities were dominant. These results, together with high and constantly increasing productivity of the bacterial community exposed to decaying jellyfish tissue, suggest that jellyfish detritus supports bacterial growth, although only part of the bacterial community was able to utilize this substrate. Changes in bacterial community composition resulting from introduction of a new substrate are not surprising because ectohydrolytic enzyme profiles and activities are highly variable among different bacterial groups (Martinez et al., 1996). Adaptation of the bacterial community to the new substrate apparently took time because the greatest increase in bacterial abundance and productivity was recorded after 6 days (as compared to the rapid response in Big Lake). Another explanation for this result is the difference in the ambient seawater temperature (Table 1). The dendrogram (Fig. 7) shows that jellyfish-inoculated bacterial communities ( $T_rA$ ) clustered together, indicating similarities in species composition, which may be due to stimulation or inhibition of specific bacterial groups (Titelman et al., 2006).

## Conclusions

Addition of jellyfish homogenate to ambient (GF/F filtered) seawater triggered responses in the bacterial communities from two different Adriatic Sea marine ecosystems. The initially high concentrations of protein, DOP, and  $PO_4^{3-}$  in the inoculated bottles, resulting from addition of jellyfish homogenate, supported increased bacterial abundance and production, coupled with  $NH_4^+$  accumulation and oxygen consumption in both ecosystems. The response of the bacterial community to the added substrate was more rapid in Big Lake, where jellyfish occur all year, than in the Bay of Piran where jellyfish are not always present. In addition, the bacterial community composition diverged in the two ecosystems. In Big Lake, the final and control community fingerprints were not much different; however, the addition of jellyfish homogenate to Bay of Piran seawater resulted in dramatic changes in bacterial community composition. Our results suggest significant effects of decaying jellyfish blooms on bacterial and nutrient dynamics. Despite divergence in bacterial community responses to jellyfish homogenate, increased bacterial

biomass and growth rates in both distinctive marine systems indicate potentially significant effects of decaying jellyfish blooms on microbial plankton.

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## References

- Allredge, A. L., 1972. Abandoned larvacean houses: a unique food source in the pelagic environment. *Science* 177: 885–887.
- Allredge, A. L., 1976. Discarded appendicularian houses as sources of food, surface habitats and particulate organic matter in planktonic environments. *Limnology and Oceanography* 21: 14–23.
- Allredge, A. L., 2005. The contribution of discarded appendicularian houses to the flux of particulate organic carbon from oceanic surface waters. In Gorsky, G., M. J. Youngbluth & D. Deibel (eds), *Response of Marine Ecosystems to Global Change*. Contemporary Publishing International, Paris, France: 309–326.
- Allredge, A. L. & M. J. Youngbluth, 1985. The significance of macroscopic aggregates (marine snow) as sites of heterotrophic bacterial production in the mesopelagic zone of the subtropical Atlantic. *Deep Sea Research* 32: 1445–1456.
- Allredge, A. L., J. Cole & D. A. Caron, 1986. Production of heterotrophic bacteria inhabiting organic aggregates (marine snow) from surface waters. *Limnology and Oceanography* 31: 68–78.
- Alvarez-Colombo, G., A. Benović, A. Malej, D. Lučić, T. Makovec, V. Onofri, M. Acha, A. Madriolas & H. Minzan, 2008. Acoustic survey of a jellyfish-dominated ecosystem (Mljet Island, Croatia). *Hydrobiologia* 616: 99–111.
- Arai, M. N., 1997. *A Functional Biology of Scyphozoa*. Chapman & Hall, London.
- Arai, M. N., 2005. Predation on pelagic coelenterates: a review. *Journal of the Marine Biological Association of the United Kingdom* 85: 523–536.
- Azam, F. & F. Malfatti, 2007. Microbial structuring of marine ecosystems. *Nature Reviews Microbiology* 5: 782–791.
- Båmstedt, U. & H. R. Skjoldal, 1980. RNA concentration of zooplankton: relationship with size and growth. *Limnology and Oceanography* 25: 304–316.
- Benović, A., D. Lučić, V. Onofri, M. Peharda, M. Carić, N. Jasprica & S. Bobanović-Čoli, 2000. Ecological characteristics of the Mljet Island seawater lakes (Southern Adriatic Sea) with special reference to their resident populations of medusae. *Scientia Marina* 64: 197–206.

- Billett, D. S. M., B. J. Bett, C. L. Jacobs, I. P. Rouse & B. D. Wigham, 2006. Mass deposition of jellyfish in the deep Arabian Sea. *Limnology and Oceanography* 51: 2077–2083.
- Boström, K. H., K. Simu, A. Hagström & L. Riemann, 2004. Optimization of DNA extraction for quantitative marine bacterioplankton community analysis. *Limnology and Oceanography Methods* 2: 365–373.
- Bradford, M. M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248–254.
- Carlson, C. A., S. J. Giovannoni, D. A. Hansell, S. J. Goldberg, R. Parsons, M. P. Otero, K. Vergin & B. R. Wheeler, 2002. Effect of nutrient amendments on bacterioplankton production, community structure and DOC utilization in the northwestern Sargasso Sea. *Aquatic Microbial Ecology* 30: 19–36.
- Caron, D. A., P. G. Davis, L. P. Madin & J. Mc N. Sieburth, 1982. Heterotrophic bacteria and bacterivorous protozoa in oceanic macroaggregates. *Science* 218: 795–797.
- Caron, D. A., E. L. Lim, R. W. Sanders, M. R. Dennett & U. G. Berninger, 2000. Responses of bacterioplankton and phytoplankton to organic carbon and inorganic nutrient additions in contrasting oceanic ecosystems. *Aquatic Microbial Ecology* 22: 175–184.
- Cherrier, J. & J. E. Bauer, 2004. Bacterial utilization of transient plankton-derived dissolved organic carbon and nitrogen inputs in surface ocean waters. *Aquatic Microbial Ecology* 35: 229–241.
- Clarke, A., L. J. Holmes & D. J. Gore, 1992. Proximate and elemental composition of gelatinous zooplankton from the Southern Ocean. *Journal of Experimental Marine Biology and Ecology* 155: 55–68.
- Don, R. H., P. T. Cox, B. J. Wainwright, K. Baker & J. S. Mattic, 1991. ‘Touchdown’ PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research* 19: 4008.
- Giovannoni, S. J., H. J. Tripp, S. Givan, et al., 2005. Genome streamlining in a cosmopolitan oceanic bacterium. *Science* 309: 1242–1245.
- Grasshoff, K., M. Ehrhardt & K. Kremling, 1983. *Methods of Seawater Analysis*. Verlag Chemie, Weinheim.
- Hamner, W. M. & M. N. Dawson, 2009. A review and synthesis on the systematic and evolution of jellyfish blooms: advantageous aggregations and adaptive assemblages. *Hydrobiologia* 616: 161–191.
- Hansson, L. J. & B. Norrman, 1995. Release of dissolved organic carbon (DOC) by scyphozoan jellyfish *Aurelia aurita* and its potential influence on the production of planktonic bacteria. *Marine Biology* 121: 527–532.
- Hollibaugh, J. T. & F. Azam, 1983. Microbial degradation of dissolved proteins in seawater. *Limnology and Oceanography* 28: 1104–1116.
- Hoppe, H. G., S. J. Kim & K. Gocke, 1988. Microbial decomposition in aquatic environments: combined process of extracellular enzyme activity and substrate uptake. *Applied and Environmental Microbiology* 54: 784–790.
- Houghton, J. D. R., T. K. Doyle, M. W. Wilson, J. Davenport & G. C. Hays, 2006. Jellyfish aggregations and leatherback turtle foraging patterns in a temperate environment. *Ecology* 87: 1967–1972.
- Keil, R. G. & D. L. Kirchman, 1993. Dissolved combined amino acids: chemical form and utilization by marine bacteria. *Limnology and Oceanography* 38: 1256–1270.
- Kirchman, D. L., 2008. Introduction and overview. In Kirchman, D. L. (ed.), *Microbial Ecology of the Ocean*, 2nd edn. Wiley-Blackwell, New Jersey.
- Kirchman, D. L., E. K’Neas & R. Hodson, 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Applied and Environmental Microbiology* 49: 599–607.
- Kremer, P., 1975. Excretion and body composition of the ctenophore *Mnemiopsis leidyi* (A. Agassiz): comparison and consequences. In 10th European Symposium on Marine Biology, Ostend, Belgium: 351–362.
- Kremer, P., 1977. Respiration and excretion by the ctenophore *Mnemiopsis leidyi*. *Marine Biology* 71: 149–156.
- Larson, R. J., 1986. Water content, organic content, and carbon and nitrogen composition of medusa from the northeast Pacific. *Journal of Experimental Marine Biology and Ecology* 99: 107–120.
- Lee, S. H. & J. A. Fuhrman, 1987. Relationships between biovolume and biomass of naturally derived bacterioplankton. *Applied and Environmental Microbiology* 53: 1298–1303.
- Lo, W. T. & I. L. Chen, 2008. Population succession and feeding of scyphomedusae, *Aurelia aurita*, in a eutrophic tropical lagoon in Taiwan. *Estuarine Coastal and Shelf Science* 76: 227–238.
- Lucas, C. H., 2001. Reproduction and life history strategies of the common jellyfish *Aurelia aurita*, in relation to its ambient environment. *Hydrobiologia* 451: 229–246.
- Malej, A., V. Turk, D. Lučić & A. Benović, 2007. Direct and indirect trophic interactions of *Aurelia* sp. (Scyphozoa) in a stratified marine environment (Mljet Lakes, Adriatic Sea). *Marine Biology* 151: 827–841.
- Martinez, L., D. C. Smith, G. F. Steward & F. Azam, 1996. Variability in the ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. *Aquatic Microbial Ecology* 10: 223–230.
- Mills, C. E., 1995. Medusae, siphonophores, and ctenophores as planktivorous predators in changing global ecosystem. *ICES Journal of Marine Science* 52: 575–581.
- Muyzer, G. & K. Smalla, 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Van Leeuwenhoek* 73: 127–141.
- Muyzer, G., E. D. Waal & A. G. Uitterlinden, 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* 59: 695–700.
- Passow, U. & A. L. Alldredge, 1994. Distribution, size, and bacterial colonization of transparent copolymer particles (TEP) in the ocean. *Marine Ecology Progress Series* 113: 185–198.
- Passow, U., A. L. Alldredge & B. E. Logan, 1994. The role of particulate carbohydrate exudates in the flocculation of diatom blooms. *Deep Sea Research* 41: 335–357.

- Pinhassi, J., F. Azam, J. Hemphala, R. A. Long, J. Martinez, U. L. Zweifel & A. Hagstrom, 1999. Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. *Aquatic Microbial Ecology* 17: 13–26.
- Pitt, K. A., D. T. Welsh & R. H. Condon, 2009. Influence of jellyfish blooms on carbon, nitrogen and phosphorous cycling and plankton production. *Hydrobiologia* 616: 133–149.
- Porter, K. G. & Y. S. Feig, 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnology and Oceanography* 25: 943–948.
- Purcell, J. E. & M. V. Sturdevant, 2001. Prey selection and dietary overlap among zooplanktivorous jellyfish and juvenile fishes in Prince William Sound, Alaska. *Marine Ecology Progress Series* 210: 67–83.
- Purcell, J. E., A. Malej & A. Benović, 1999. Potential links of jellyfish to eutrophication and fisheries. In Malone, T. C., A. Malej, L. W. Harding, N. Smolaka & R. E. Turner (eds), *Ecosystems at the Land-Sea Margin: Drainage Basin to Coastal Sea* (Coastal and Estuarine Studies, 55). American Geophysical Union, Washington, DC: 241–263.
- Riemann, L., J. Titelman & U. Båmstedt, 2006. Links between jellyfish and microbes in a jellyfish dominated fjord. *Marine Ecology Progress Series* 325: 29–42.
- Schafer, H., L. Bernard, C. Courties, et al., 2001. Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in genetic diversity of bacterial populations. *FEMS Microbiology Ecology* 34: 243–253.
- Schneider, G., 1989. The common jellyfish *Aurelia aurita*: standing stock, excretion and nutrient generation in the Kiel Bight, western Baltic. *Marine Biology* 100: 507–514.
- Simon, M. & F. Azam, 1989. Protein content and protein synthesis rates of planktonic marine bacteria. *Marine Ecology Progress Series* 51: 201–213.
- Simon, M., A. L. Alldredge & F. Azam, 1990. Bacterial carbon dynamics on marine snow. *Marine Ecology Progress Series* 65: 205–211.
- Smith, D. C. & F. Azam, 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using  $^3\text{H}$ -leucine. *Marine Microbial Food Webs* 6: 107–114.
- Stoecker, D. K., A. E. Michaels & L. H. Davis, 1987. Grazing by the jellyfish, *Aurelia aurita*, on microplankton. *Journal of Plankton Research* 9: 901–915.
- Sullivan, B. K., J. R. Garcia & G. Klein-MacPhee, 1994. Prey selection by the scyphomedusan predator *Aurelia aurita*. *Marine Biology* 121: 335–341.
- Thingstad, T. F., A. Hagström & F. Rassoulzadegan, 1997. Accumulation of degradable DOC in surface waters: it is caused by malfunctioning microbial loop? *Limnology and Oceanography* 42: 398–404.
- Titelman, J., L. Riemann, T. A. Sørnes, T. Nilsen, B. Griekspoor & U. Båmstedt, 2006. Turnover of dead jellyfish: stimulation and retardation on microbial activity. *Marine Ecology Progress Series* 325: 43–58.
- Turk, V., D. Lučić, V. Flander-Putrlje & A. Malej, 2008. Feeding of *Aurelia* sp. (Scyphozoa) and links to the microbial food web. *Marine Ecology* 29: 495–505.
- West, E. J., D. T. Welsh & K. A. Pitt, 2009. Influence of decomposing jellyfish on the sediment oxygen demand and nutrient dynamics. *Hydrobiologia* 616: 151–160.
- Yamamoto, J., M. Hirose, T. Ohtani, K. Sugimoto, K. Hirase, N. Shimamoto, T. Shimura, N. Honda, Y. Fujimori & T. Mukai, 2008. Transportation of organic matter to the sea floor by carrion falls of the giant jellyfish *Nemopilema nomurai* in the Sea of Japan. *Marine Biology* 153: 311–317.