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**TITLE:** Association of bacteria with marine invertebrates: implications for Ballast Water

Management

**SUBTITLE:** Bacterial association with marine invertebrates.....

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## **Abstract**

Bacteria associated with plankton are of importance in marine bioinvasions and the implementation of ship's ballast water treatment technologies. In this study epibiotic and endobiotic bacteria associated with zooplankton including barnacle nauplii, veliger larvae and adults of the copepod *Oithona* sp., were characterized and quantified. Barnacle nauplius and veliger larva harboured  $\sim 4.4 \times 10^5$  cells  $\text{ind}^{-1}$  whereas *Oithona* sp. had  $8.8 \times 10^5$  cells  $\text{ind}^{-1}$ . Computation of bacterial contribution based on bio-volume indicated that despite being the smallest zooplankton tested, veliger larvae, harbored the highest number of bacteria, while barnacle nauplii, the largest of the zooplankton tested in terms of volume contributed the least. Pulverization of zooplankton led to an increase in bacterial numbers; for example, *Vibrio cholerae* which was initially  $3.5 \times 10^3$  increased to  $5.4 \times 10^5$  CFU  $\text{g}^{-1}$ , *Escherichia coli* increased from  $5.0 \times 10^2$  to  $1.3 \times 10^4$  CFU  $\text{g}^{-1}$  and *Streptococcus faecalis* increased from  $2.1 \times 10^2$  to  $2.5 \times 10^5$  CFU  $\text{g}^{-1}$ , respectively. Pulverized zooplankton was aged in the dark to assess the contribution of bacteria from decaying debris. Aging of pulverized zooplankton led to emergence of *Chromobacterium violaceum*, which is an opportunistic pathogen in animals and humans.

## Introduction

Shipping moves over 90% of the world's commodities and is responsible for the global transfer of approximately 2–3 billion tons of ballast water, which is routinely carried by ships to maintain their stability. A similar volume of ballast water may also be transferred domestically within countries and regions each year (GloBallast Partnerships, [www.GloBallast.IMO.org](http://www.GloBallast.IMO.org)). Ballast water has been recognized as the main source of invasive marine organisms that threaten naturally evolved biodiversity, the consequences of which are increasingly being realized (Anil et al. 2002). The unintentional introduction of disease causing pathogenic bacteria, which are transported from the place of origin or formed during transportation, can have direct impact on society and human health.

Ship's ballast tanks hold different non-indigenous vertebrates, invertebrates, plants, microscopic algae, bacteria etc. (Williams et al. 1988; Carlton and Geller 1993; Smith et al. 1996; Ruiz et al. 2000; Drake et al. 2002, 2005, 2007; Mimura et al. 2005). Microorganisms are introduced into alien environments in larger numbers than other organism owing to their high natural abundance, capability to form resting stages and capability to withstand a wide range of environmental conditions. Although all the organisms taken onboard into ballast tanks may not survive, bacteria and micro-algae are well capable of surviving prolonged periods of unfavourable conditions by forming cysts, spores or other physiological resting stages (Roszak et al. 1983; Hallegraeff and Bolch 1992; Anil et al. 2002). Once released these micro-organisms are well suited to be invasive owing to their small size which facilitates their passive dispersal and simpler requirements for survival than metazoans (Deming 1997). The concentration of cells of *Vibrio* species in ballast samples examined from ships in Singapore Harbour were in the range  $1.1\text{--}3.9 \times 10^4 \text{ ml}^{-1}$  (Joachimsthal et al. 2004). The unintentional introduction of disease-causing pathogenic bacteria can have direct societal impacts, including effects on human health. In an earlier study it was found that most of the pathogens introduced to Chesapeake Bay originated from bacteria associated with plankton rather than the water column itself (Ruiz 2000). Thus, plankton and their associated micro-organisms marine invertebrates are of major concern in ballast water treatment/management programs.

The International convention for the control and management of ship's ballast water and sediments (IMO 2004) provides ballast water performance standards for different size classes of organisms. As per the Ballast Water Performance Standard (D2- regulation of the convention), the numbers of planktonic forms need to be reduced substantially before discharge. In view of this, treatment technology developers are looking at various options. In the process of the treatment of ballast water there is a considerable likelihood of altering the microbial population. The release of

bacteria while the large planktonic organisms are killed or destroyed is an additional concern in the ballast water treatment.

Recently a mesocosm study (Rehnstam-Holm et al. 2010) investigated the interrelationship between microalgae (phytoplankton) and *Vibrio* spp.. The mesocosms were inoculated with phytoplankton (containing no larger zooplankton e.g. copepods), plankton and sediment, or sediment. The highest diatom abundances were followed by an increase in *Vibrio* numbers in all the mesocosms (Rehnstam-Holm et al. 2010). However, no similar study has been conducted for zooplankton. Zooplankton serves as microhabitat for bacteria (Tang 2005). Thus, understanding the linkages between zooplankton and bacteria is important. The recovery and survival of bacteria once separated from their zooplankton host has not been explored. Some of the bacteria which are associated with zooplankton can be potential pathogens; these species have important implications for ballast water management. In this study a qualitative and quantitative analysis of the bacteria associated with barnacle nauplii, veliger larvae of bivalves and an adult copepod (*Oithona* sp.) was taken up so that their contribution towards bacterial abundance could be accounted when ballast water zooplankton is destroyed during treatment.

## **Materials and method**

### *Collection of zooplankton*

Zooplankton was collected by horizontal hauls using a 100- $\mu$ m mesh Heron-Tranter (HT) zooplankton net with a calibrated flow meter attached. Hauls were conducted in the vicinity of Dona Paula Bay (15° 27.5' N, 73° 48' E) on the west coast of India during November 2009. The sample was equally split into four parts using a Folsom plankton splitter, and one part was used for qualitative and quantitative analysis of zooplankton groups using various identification manuals (Kasturirangan 1963; Todd et al. 1966; Conway et al. 2003). Barnacle nauplii, veliger larvae, and *Oithona* sp. were sorted from the zooplankton sample.

### *Identification of bacterial groups from zooplankton*

The remaining three parts of the zooplankton sample were diluted and maintained in 50 liter tanks (n=3). For isolation of bacteria, three 5 liter subsamples were filtered through 50 $\mu$ m mesh. The zooplankton collected on the mesh were homogenized and pulverized in a glass homogenizer under sterile conditions using 10 ml of autoclaved filtered seawater. Subsequently, the sample was diluted as required and spread plated (0.1ml) on Zobell Marine Agar 2216 (Hi-media). The pulverized zooplankton was also maintained in one liter beakers (n=3) and aged for five days in dark at 30  $\pm$

2°C in order to assess the contribution of decaying debris to the growth of the bacterial population. Untreated zooplankton was analyzed at t=0 and taken as reference for quantifying the effect of pulverization. Subsequently untreated zooplankton was aged for 5 days in dark tanks at  $30 \pm 2^\circ\text{C}$ . The sample for the analysis of total bacterial count (TBC) that includes cultivable and non-cultivable bacteria was fixed with formaldehyde (final concentration 1 to 2%; v/v). The quantification of bacteria was done using acridine orange and epifluorescence microscopy (Daley and Hobbie 1975).

Pathogenic bacteria were quantified using specific media following the manufacturer's instructions (Hi-media). Thiosulphate- Citrate-Bile Salts (TCBS) media was used for *Vibrio* spp. (sucrose fermenting, yellow, entire, raised <2 mm dia colonies were counted as *Vibrio cholerae*). The presence of sucrose in TCBS allows differentiation of *Vibrio* species (Pfeffer and Oliver 2003). Enterococcus Confirmatory Agar was used for *Streptococcus faecalis* (typically blue, <2 mm dia), Mac Conkey Agar was used for Coliforms, and HiCrome EC0157:H7 Selective Agar Base to which HiCrome EC0157:H7 Selective Supplement (FD187) was added aseptically for *Escherichia coli*. All the plates of specific media were incubated at 37°C for 24 h and colonies were counted. Although the pathogenic bacteria were counted using specific media, in order to reduce the uncertainties associated with counting of the pathogenic bacteria, *V. cholerae*, *S. faecalis* and *E. coli* were randomly picked from the selective agar, purified, and the identity of at least 150 pure isolates each of these bacteria were confirmed using a series of appropriate biochemical tests such as gram staining, string test, oxidase, catalase, motility, indole, gas from glucose, Methyl Red, Voges Proskauer and citrate utilization (Table 1). *V. cholerae* exhibited a curved or comma-like shape, *E. coli* was rod-shaped and *S. faecalis* had a coccoid shape. The abundance of pathogenic bacteria associated with the zooplankton was expressed as Colony Forming Units (CFU) g<sup>-1</sup>. These numbers are “putative” counts of specific species of bacteria (Table 2).

#### *Bacterial abundance and diversity from barnacle nauplii, veliger larvae, and Oithona sp.*

Barnacle nauplii, veliger larvae and *Oithona* sp. (~ 10 individuals each) were rinsed several times in autoclaved filtered seawater to remove the loosely adhered bacteria on the surface. Subsequently, they were transferred to flat-bottom borosilicate tubes (height = 5.6 cm, radius =1.2 cm). The sample tubes contained 5 ml of filtered (0.22 µm) sea water. Sample tubes were then sonicated at low frequency in order to separate surface-associated (epibiotic) bacteria from the zooplankton without disrupting or killing the associated zooplankton individual itself. An ultrasonic homogenizer LABSONIC U (B. Braun Biotech International GmbH) with an operating frequency of 20 kHz and a rated maximum power of 150 watts was used.

In order to obtain endobiotic bacteria the sonicated zooplankton were then homogenized in 0.22 µm filtered sea water with a Teflon pestle. In all the treatments, the cultivable as well as the total bacteria were quantified. The total bacterial abundance (epibiotic and endobiotic) from barnacle nauplii, veliger larvae, and *Oithona* sp. was quantified using acridine orange and epifluorescence microscopy (Daley and Hobbie 1975). The total cultivable bacterial abundance from barnacle nauplii, veliger larvae, and *Oithona* sp. (epibiotic and endobiotic) was quantified as CFU ind<sup>-1</sup>. The sample was diluted as required and subsequently spread plated (0.1ml) on Zobell Marine Agar 2216 (Hi-media). The cultivable bacteria isolated from the plates were maintained at room temperature (30 ± 2 °C) on Zobell Marine Agar 2216 slants for further identification. The isolated bacteria (epibiotic and endobiotic) were identified following Bergey's manual of systematic bacteriology (Krieg 1984; Table 3). Those species that were more than 50% of the total species were considered as dominant or major cultivable forms. The biovolume of barnacle nauplii, veliger larvae, and *Oithona* sp., was calculated by following the method described by Viñas et al. (2010).

## Results

The sample comprised several forms of zooplankton, and there was variation in the abundance of these different forms (Fig. 1). The total number of individuals in the zooplankton sample larger than 50µm was 3675 m<sup>-3</sup>. Total bacterial abundance in the water sample containing the zooplankton was estimated to be 1.6 x 10<sup>9</sup> l<sup>-1</sup>. The major cultivable bacteria associated with the zooplankton were *Chromobacterium lividum*, *Photobacterium angustum*, *Aeromonas schubertii*, *Bacteroides corrodens*, *Acetovibrio cellulolyticus*, *Aeromonas salmonicida salmonicida*, *Chlorobium phaeobacterioides*, *Thiocapsa pfennigioides*, *Alcaligenes faecalis*, *Enhydrobacter aerosaccus*. *Chromobacterium violaceum*, violet pigment producing bacteria, was observed only on aging of the pulverized zooplankton. The other dominant culturable bacteria after aging were *A. schubertii*, *Bacteroides corrodens* and *Alcaligenes faecalis*.

Untreated zooplankton showed an 87% increase in bacterial abundance when aged in the dark for a period of 5 days (Fig. 2 a). However, when the zooplankton was pulverized, the bacterial numbers increased 3.5 fold and subsequently on ageing the bacterial abundance increased further by 44% (Fig. 2b). The number of potential pathogenic bacteria such as *S. faecalis* and *V. cholerae* increased subsequent to pulverization of the zooplankton. *S. faecalis* increased from 2.1 x 10<sup>2</sup>±4.9 x 10<sup>1</sup> CFU g<sup>-1</sup> to 2.5 x 10<sup>5</sup>±3.8 x 10<sup>4</sup> CFU g<sup>-1</sup>, and *V. cholerae* from 3.5 x 10<sup>3</sup>±1.4 x 10<sup>3</sup> CFU g<sup>-1</sup> to 5.4 x 10<sup>5</sup>±3.1 x 10<sup>5</sup> CFU g<sup>-1</sup>, respectively. The initial count of *E. coli* was 5.0 x 10<sup>2</sup>±2.8 x 10<sup>2</sup> CFU g<sup>-1</sup>, and after pulverization of zooplankton this increased to 1.3 x 10<sup>4</sup>±7.6 x 10<sup>3</sup> CFU g<sup>-1</sup> (Table 2).

The epibiotic and endobiotic bacteria (total number of bacteria and number of CFU) associated with barnacle nauplii, veliger larvae, and *Oithona* sp. that were segregated from the zooplankton sample were quantified. The abundance of epibiotic bacteria was higher in the case of copepods and was significantly different from that observed in the case of veliger larvae ( $p=0.041$ , Tukey's test) and barnacle nauplii ( $p=0.029$ , Tukey's test). However, the abundance of epibiotic bacteria was not significantly different between the barnacle nauplii and veliger larvae (Fig. 3). The abundance of endobiotic bacteria showed a similar trend. The abundance of cultivable epibiotic bacteria was highest for barnacle nauplii, whereas *Oithona* sp. showed significantly higher abundances of cultivable endobiotic bacteria when compared to barnacle nauplii or veliger larvae (Fig. 4).

The epibiotic and endobiotic bacterial abundance from a single barnacle nauplius and veliger larva was estimated to be  $4.4 \times 10^5 \text{ ind}^{-1}$  and  $4.4 \times 10^5 \text{ ind}^{-1}$ , respectively and  $8.8 \times 10^5 \text{ ind}^{-1}$  for *Oithona* sp. The biovolume of a barnacle nauplius was greatest ( $1.41 \times 10^7 \mu\text{m}^3$ ), followed by *Oithona* sp. and the veliger larva ( $7.1 \times 10^6 \mu\text{m}^3$  and  $6 \times 10^5 \mu\text{m}^3$ , respectively). Bacterial contribution when computed based on bio-volume translated to 3, 12 and 72 cells per  $100 \mu\text{m}^{-3}$  in the cases of barnacle nauplius, *Oithona* sp. and veliger larva, respectively. The ratio of endobiotic to epibiotic bacteria was highest for veliger larvae (2.3) followed by *Oithona* sp. and the barnacle nauplius (1.5 and 1.4, respectively). Thus, the smallest zooplankton form examined, the veliger larva harbored the highest numbers of bacteria while the barnacle nauplius with largest bio-volume harbored the lowest.

A certain degree of specificity with each of the zooplankton forms tested was observed for endobiotic bacteria (Table 3). *Aeromonas salmonicida salmonicida* and *Photobacterium angustum* were the major cultivable forms associated with barnacle nauplii. In the case of veliger larvae, *P. angustum* and *Vibrio fischeri* were dominant, while in the case of *Oithona* sp. the dominant species was *Aeromonas schubertii*. *A. schubertii* was associated with all the three zooplankton forms.

## Discussion

Studies on the bacterial abundance in ballast water of vessels arriving to Chesapeake Bay from foreign ports (Ruiz et al. 2000) reported that ballast water of vessels arriving at the bay contained an average of  $8.3 \times 10^8$  bacteria per litre. We observed a one fold higher bacterial abundance (total bacteria  $3.2 \times 10^9 \text{ l}^{-1}$ ) in our sample of coastal ocean water, with a zooplankton abundance of  $3675 \text{ m}^{-3}$ . Using the abundance of the constituent forms in the zooplankton sample, the bacterial inputs (epibiotic and endobiotic) from barnacle nauplii, veliger larvae, and *Oithona* sp. were

estimated to be  $3.8 \times 10^8$ ,  $8.7 \times 10^7$ ,  $7.9 \times 10^8 \text{ m}^{-3}$ , respectively. Marine copepods and invertebrate larval forms have complex body structures and extensive surface area for the attachment of bacteria. The exoskeleton and gut lining of copepods provide favorable surfaces for the bacterial attachment (Carman and Dobbs 1997). Bacterial abundance associated with copepods can be orders of magnitude higher than that in ambient water (Tang 2005). A recent study (Tang et al. 2009) has shown that the estuarine copepod *Acartia tonsa* harbors diverse bacterial strains on its body. Algal species composition and copepod life history serve to regulate the bacterial community composition associated with copepods, and spatial and temporal variations in composition of algal communities and copepod origin could alter bacteria-copepod interactions (Tang et al. 2009). Although conventional ecological research views meso-zooplankton and bacteria as indirectly connected functional groups (Azam and Malfatti 2007), they are closely related in occurrence and ecological functions (Harris 1993; Tang et al. 2009).

Microbial decomposition of zooplankton carcasses provides an alternative pathway for nutrient regeneration, elemental recycling and microbial production in many aquatic ecosystems (Lee and Fisher 1992). When the carcasses of marine zooplankton are exposed to natural seawater, they are rapidly colonized and decomposed by ambient bacteria (Tang et al. 2006). During this process a large number of bacteria emerge from the decomposing carcasses and contribute to the bacterial production in the surrounding water column. In the present study, untreated zooplankton showed an increase in bacterial abundance by 87% when aged in dark for a period of 5 days. Thus, zooplankton in ballast water represent a major concern for treatment/management initiatives as they offer extensive body surfaces for attachment of bacteria. Decomposition of zooplankton would contribute to increased bacterial production within the ballast tank environment. When the zooplankton was pulverized, as might occur during treatment of ballast water, the bacterial numbers increased 3.5 times and subsequently when this residue was aged the bacterial abundance further increased by 44%. Also, after aging emergence of *C. violaceum* in this study is a cause of concern as this bacterium is an opportunistic pathogen in animals and humans and cause fatal septicemia accompanied with liver and lung abscesses (Richard 1993). A recent study (Carney et al. 2011) addressed the effect of prolonged darkness on marine phytoplankton and reported that plankton water samples showed high nutrient levels after the dark period. Bacteria release while large planktonic organisms are killed or destroyed by rupturing is an additional concern in the ballast water treatment. Since the destruction of bacteria is facilitated only after they are separated from their associated organism, it is important to account for bacterial contribution from the plankton harboring them. This has implications in ballast water management as treatment technologies are primarily developed to destroy the larger

plankton; contribution of plankton-associated bacteria after plankton debris degenerates is rarely explored.

The abundance of epi- and endobiotic bacteria was inversely related to the volume of the zooplankton form. This observation emphasizes that it is not the size but the type of organism that determines the bacterial contribution. Host specificity was also observed in the case of endobiotic bacteria of each of the tested forms. An earlier study has reported that bacteria associated with plankton are the principal contributors of pathogenic bacteria introduced to Chesapeake Bay (Ruiz et al. 2000). Zooplankton contained high numbers of pathogenic bacteria such as *V. cholerae* followed by *E. coli* and *S. faecalis*. The genus *Vibrio* and closely-associated genera within the Vibrionaceae now comprise a group of over 80 easily cultured, Gram negative, heterotrophic bacteria (Thompson et al. 2004) commonly isolated from marine and freshwater environments, members of which are fast growing opportunists those typically dominate the bacterial flora of plankton and the intestinal microbiota of fish. Members of the bacterial genus *Vibrio* spp. seem to have a strong affinity for surfaces, especially under nutrient limiting conditions (Dawson et al. 1981). Hood et al. (1984) also observed that *V. cholerae* appeared to prefer epibiotic habitat.

A number of approaches or technologies (physical and chemical) for the treatment of ballast water have been evaluated (Jyoti and Pandit 2004; Sawant et al. 2008; Lloyd's Register 2010). Foladori et al. (2007) assessed the effects of sonication on bacterial viability in wastewater treatment plants. Recently, ultrasonic treatment was explored to quantify the energy required for the destruction of larvae of barnacle *Balanus amphitrite* (Seth et al. 2010). Following pulverization of the barnacle larvae, abundance of bacteria increased and the rate of release of bacteria was dependent on power level and treatment time (Seth et al. 2010). The current study indicates that numbers of *E. coli* increased following pulverization. Potential pathogenic forms such as *S. faecalis* and *V. cholerae* increased in large numbers subsequent to pulverization of zooplankton. *V. cholerae* is often found in close association with copepods (Heidelberg et al. 2002, Belkin and Colwell 2005). *V. cholerae* outcompetes other bacterial taxa associated with zooplankton and is commensal with copepods. Copepods thus serve as a reservoir of *V. cholerae* (Colwell 1996). In a recent review, Pruzzo et al. (2008) emphasized the importance of *V. cholerae* interactions with chitin, a major component of the exoskeleton of crustaceans, as a factor mediating virulence. Huq et al. (1984) reported that live copepods stimulated *V. cholerae* to grow from densities of  $10^4$  ml<sup>-1</sup> to  $10^8$  ml<sup>-1</sup>. When bacteria were incubated with dead instead of live copepods bacterial abundance increased to only  $10^6$  ml<sup>-1</sup>. Live or dead copepods did not stimulate similar growth by other bacterial species tested (*V. parahaemolyticus* or *Pseudomonas* sp.). *V. cholerae* readily attached to copepods and the attached



cells were particularly concentrated in the oral region and on egg sacs of copepods. Huq et al. (1984) also suggested that seasonal variability in abundance of *Vibrio* may be closely linked with the variability in the abundance of copepods.

According to IMO guidelines for performance of ballast water treatment technologies (2004), treatment should reduce the toxicogenic *V. cholerae* (O1 and O139) to less than 1 colony forming unit (CFU) per 100 mL or less than 1 CFU g<sup>-1</sup> (wet weight) zooplankton in the sample. *E. coli* numbers should be less than 250 cfu per 100 mL and intestinal Enterococci should be less than 100 cfu per 100 mL. While designing the treatment technology the inputs of bacteria from both the water column and the plankton, and their contribution subsequent to treatment, must be taken into account. *V. cholerae* can remain in the cultivable state in seawater for a relatively long time sufficient to be carried by ocean currents to distant geographical locations (Munro and Colwell 1996). Thus, release of pathogenic bacteria in vast numbers with the destruction of zooplankton is a cause of concern.

The present study accounts for the inputs of pathogenic bacteria associated with zooplankton and changes in their abundance upon destruction or aging and highlights the existence of host specificity in the case of endobiotic bacteria. Associations between bacteria and animals are widely reported, and many involve a mutualistic symbiosis between a single bacterial strain and its animal host (McFall-Ngai and Ruby, 1991; Polz et al., 1994). Although modern molecular techniques allow detailed phylogenetic investigation of bacterial communities (Peter and Sommaruga 2008), understanding of the physiology and ecology of host-specific bacteria, and elucidating their significance demands their survival in the cultivable form close to their natural environment. Future studies should focus on isolating the bacteria using media and substrates simulating the conditions in nature. These studies will pave the way to exploiting host specificity and understanding bacterial physiology in the free-living state.

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**Table 1 Biochemical tests performed on pathogenic bacteria isolated using selective media to confirm their identity.**

ND-Not Done

Biochemical tests	<i>V. cholerae</i>	<i>S. faecalis</i>	<i>E. coli</i>
Gram staining	-	+	-
String test	+	-	+
Motility	+	-	+
Catalase	+	-	+
Oxidase	+	-	+
Indole	+	-	+
Growth at 37 °C	+	+	+
H <sub>2</sub> S	-	ND	-
Methyl Red	+	ND	+
Voges Proskauer	-	ND	-
Gas from glucose	-	-	+
Citrate utilization	+	+	-

**Table 2 Pathogenic bacteria associated with the zooplankton. The values are presented as mean numbers  $\pm$  S.E. using replicates (n=6) and repeated twice.**

Pathogens	Initial (CFU g <sup>-1</sup> )	After pulverization (CFU g <sup>-1</sup> )
<i>Streptococcus faecalis</i>	$2.1 \times 10^2 \pm 4.9 \times 10^1$	$2.5 \times 10^5 \pm 3.8 \times 10^4$
<i>Vibrio cholerae</i>	$3.5 \times 10^3 \pm 1.4 \times 10^3$	$5.4 \times 10^5 \pm 3.1 \times 10^5$
<i>Escherichia coli</i>	$5.0 \times 10^2 \pm 2.8 \times 10^2$	$1.3 \times 10^4 \pm 7.6 \times 10^3$

**Table 3 Major cultivable bacteria associated with barnacle nauplii, *Oithona* sp. and Veliger larvae**

<b>Type of organisms</b>	<b>Epibiotic bacteria</b>	<b>Endobiotic bacteria</b>
Barnacle nauplii	<i>Aeromonas salmonicida salmonicida</i> <i>Aeromonas schubertii</i> <i>Photobacterium angustum</i>	<i>Bacillus brevis</i>
<i>Oithona</i> sp.	<i>Aeromonas schubertii</i>	<i>Actinobacillus capsulatus</i> <i>Bacillus brevis</i>
Veliger larvae	<i>Vibrio fischeri</i> <i>Photobacterium angustum</i> <i>Aeromonas schubertii</i>	<i>Aeromonas schubertii</i>

### Legends to Figures

Figure 1 Abundance of different larval forms (ind m<sup>-3</sup>) in the zooplankton sample. Vertical lines indicate standard deviation from the mean and are shown as positive.

Figure 2 Total Bacterial Abundance expressed as log (no. g<sup>-1</sup>) that includes cultivable and non-cultivable bacteria from a) untreated zooplankton and b) pulverized zooplankton on aging for five days in dark tanks at 30 ± 2°C . Vertical lines indicate standard deviation from the mean and are shown as positive.

Figure 3 Total epibiotic and endobiotic bacterial abundance expressed as (log no. ind<sup>-1</sup>) from barnacle nauplius, veliger larva and *Oithona* sp. Vertical lines indicate standard deviation from the mean and are shown as positive.

Figure 4 Total cultivable epibiotic and endobiotic bacterial abundance expressed as (log CFU ind<sup>-1</sup>) from barnacle nauplius, veliger larva, and *Oithona* sp. Vertical lines indicate standard deviation from the mean and are shown as positive.

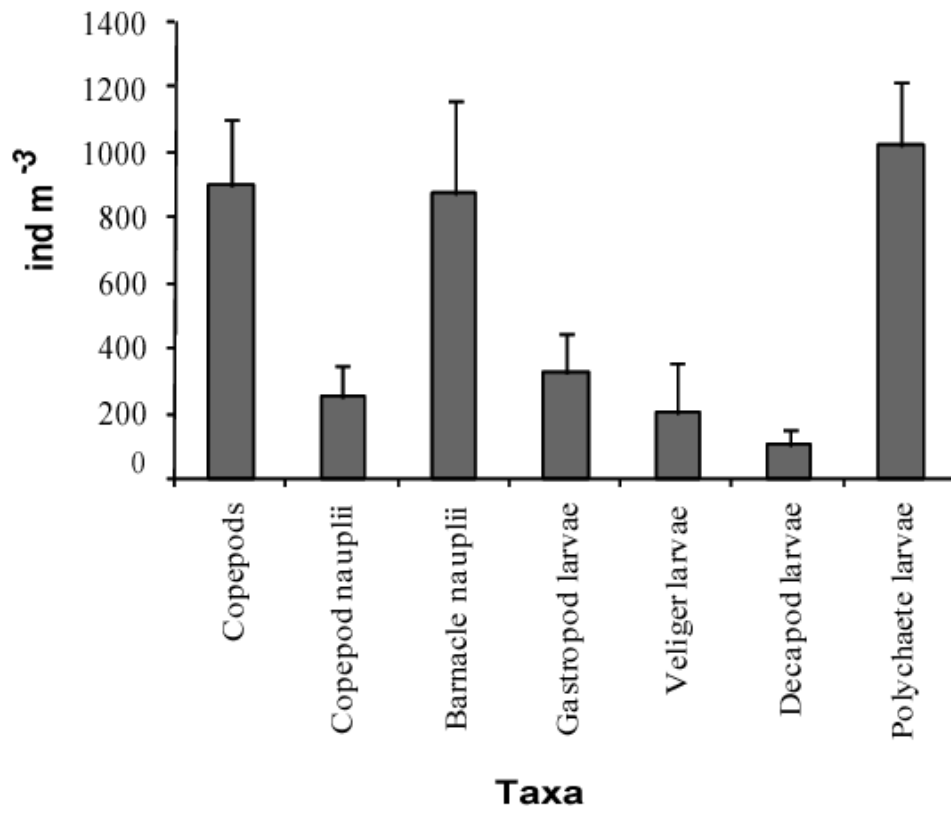


Figure 1



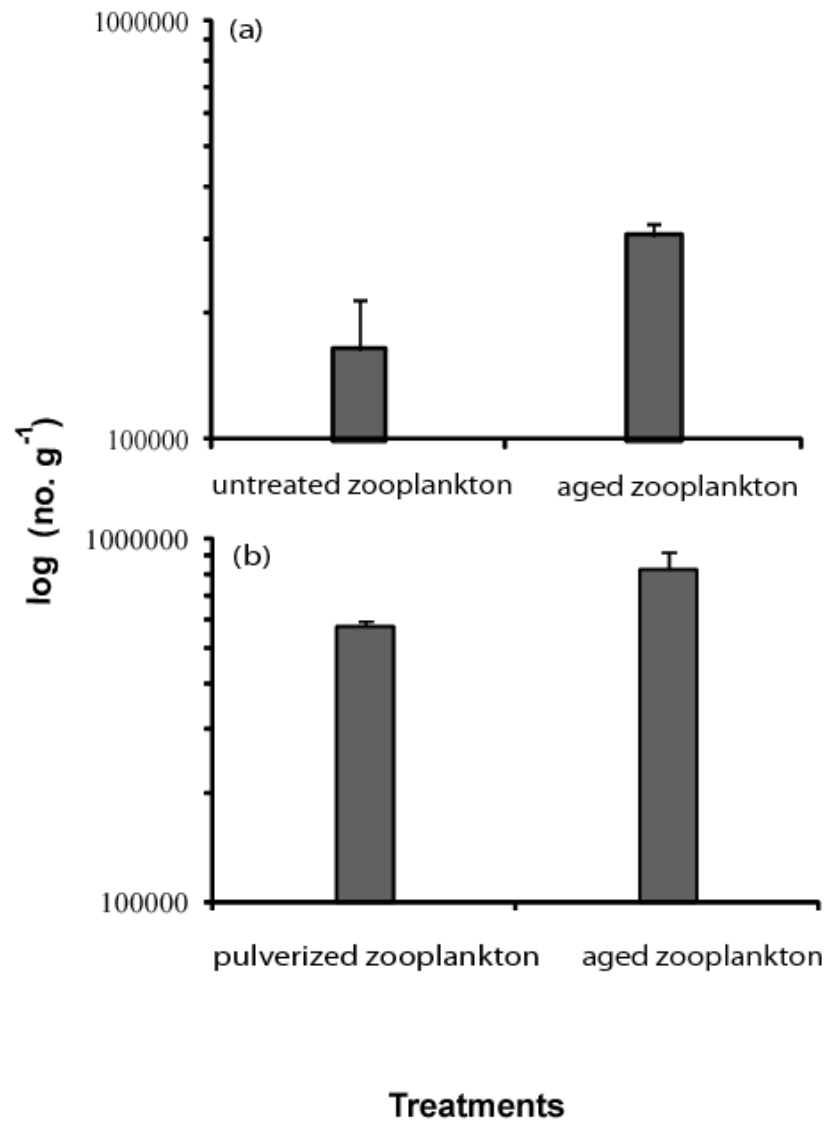


Figure 2

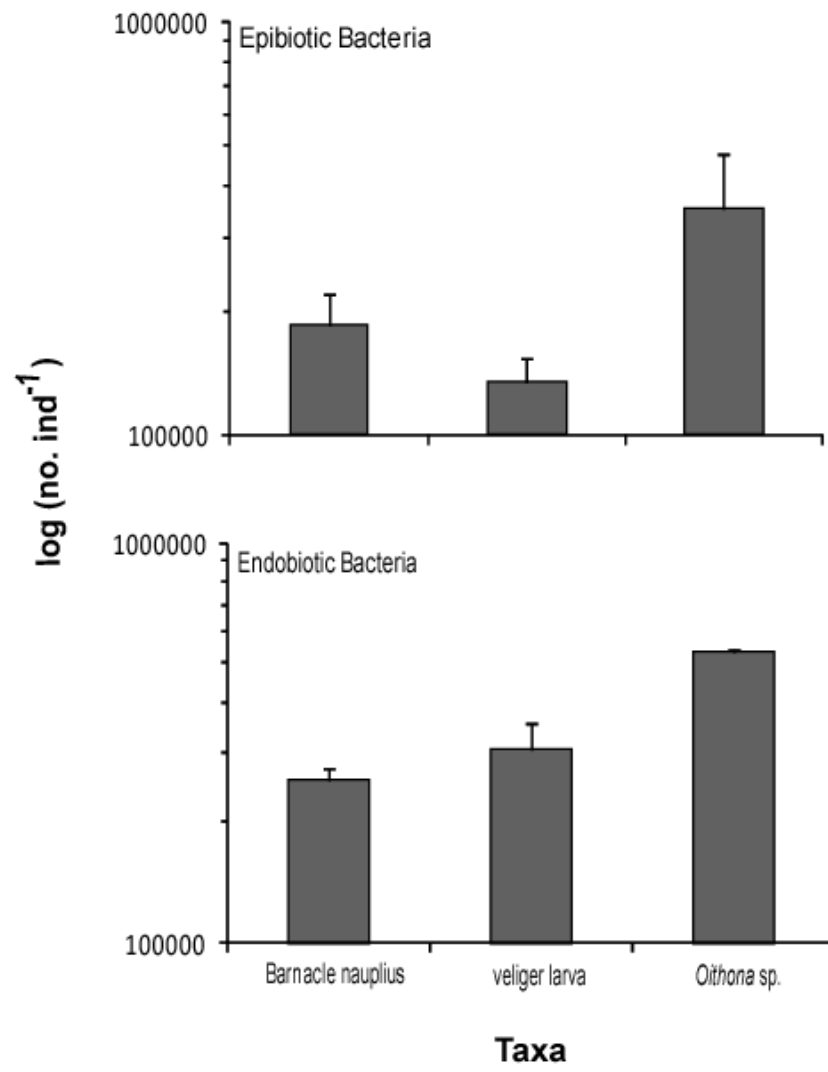


Figure 3

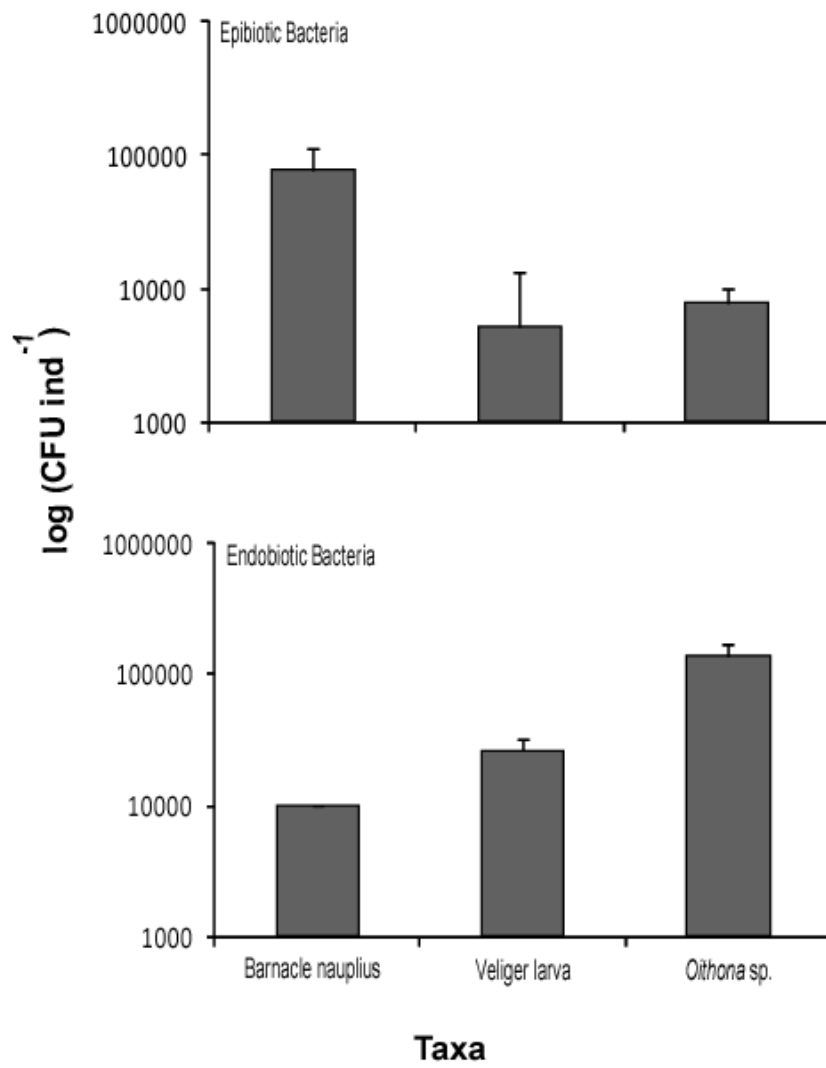


Figure 4