Variations In Bacterial Community Of Rearing Water And Gut Of Common Dentex, Dentex dentex Linnaeus 1758, Larvae Using Three Microalgae Management Approaches

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Running title: Bacterial community in common dentex larvae rearing

Abstract

Microorganisms present in the rearing water colonize the gut of first feeding larvae and represent the first barrier against opportunistic pathogens. The aim of the experiments presented herein was to standardize a protocol for the management of rearing water and microalgae suitable for the larval rearing of common dentex. In Experiment 1, bacteria-algae interactions were tested using a monospecific microalgal community, "Tetraselmis chuii", suitable for nutritional experiments and with known antibacterial activity. In Experiment 2, the evolution of the bacterial community and larval performance (growth and survival) were monitored daily, in three conditions: 1) "Mature water"; T. chuii was added 5 days before the rearing of common dentex larvae, 2) "Green water"; T. chuii was added 1 day before, and 3) "Clear water"; no T. chuii addition. The results show the influence of the presence of T. chuii on the evolution of the bacterial communities, both in terms of bacterial density and morphology, and indicate "Green water" is the most suitable water treatment for management of larval rearing for common dentex.

Keywords

common dentex larvae, intensive rearing, Microalgae-bacteria interactions, rearing water

Abbreviations

CFU: Colony Forming Units

DAPI: 4’,6-diamidino-2-phenylindole

dph: days post-hatching

DW: dry weight

ind: individuals

MA: Marine Agar

TCBS: Thiosulfate Citrate Bile Salts Sucrose

48 Common dentex larvae, as with other marine fish larvae
49 (Chantanachookhin et al. 1991), develop immune organs during metamorphosis,
50 or onwards (Santamaría 2001). Nevertheless, the specific immune system in fish
51 larvae is usually not active until some time after the formation of the thalamus,
52 the thymus and the spleen (Manning and Tattner 1985; Magnadottir et al. 2005).
53 Microorganisms present in the rearing water colonize the gut of larvae at first
54 feeding and represent the first barrier against opportunistic pathogens. Therefore,
55 the composition of the microbial community of the rearing water affects the
56 composition of the gut microbiota, although some differences are detected
57 depending on fish species (Cahill 1990). An adequate microbial control during the
58 egg stage and early development can improve larval survival, growth, and fish
59 quality (Douillet and Pickering 1999).
60 Interactions between bacteria and microalgae are complex, multiple, and
61 have a significant impact on aquaculture production. Microalgae are able to
62 produce growth promoters and inhibitors, besides several chemical clues, that
63 affect the population density and composition of their microscopic counterpart. It
64 is hypothesized that an adequate selection of a microalgae-bacteria consortium
65 would improve aquaculture productivity, efficiency and sustainability (Natrah et al
66 2014).
67 Interactions between larvae and bacteria are complex, species-specific,
68 and not fully understood (Olafsen 2001). Negative interactions, i.e. pathologies,
69 have been the subject of most studies. In the last decades, studies on positive
70 interactions, i. e. probiotics, have become more common (Gómez-Gil et al. 2000;
71 Gatesoupe 2002). Vadstein et al. (1993) and Skjermo and Vadstein (1999)

suggest a bulk of strategies to control the microbial community and its interactions with larvae based mainly on three principles: 1) non-selective reduction of bacteria, 2) selective enhancement of bacteria, and 3) improvement of larval resistance. Enhancement of bacteria can also be made non-selectively by means of the “mature water” technique, which relies on phytoplankton-bacteria interactions (Skjermo et al. 1997).

Strict pathogens have not been identified and/or associated to high larval mortality episodes in common dentex (Company et al. 1999; Crespo et al. 2001), but well known opportunistic pathogens, mainly from the “Vibrio” genera, are present naturally in marine water, and their growth can be favoured by larval culturing conditions (Bergh 1996). Growth of these opportunistic bacteria coincides with the development of the fish immune system (Santamaría 2001), making this a period of growth for common dentex larvae where they are more vulnerable. In addition, they are very sensitive to the inherent stress of artificial rearing conditions (Rigos et al. 1998).

The aim of the following experiments was to standardize a protocol for the management of rearing water and microalgae suitable for common dentex larvae. The mature water technique used in these studies is not as described by Skjermo et al. (1997); instead, UV-filtered seawater with microalgae was maintained in tanks some days before larval stocking in order to obtain microbially mature rearing water with bacteria associated to microalgae culture stocks. “Tetraselmis chuii” was chosen mainly due to its suitability for nutritional experimental purposes and its known antibacterial activity against some species of Vibrio and “Listonella” (Riquelme and Avendaño-Herrera 2003).

Materials and Methods

Experiment 1

The data gathered in Experiment 1 (Fig. 1) was used for the design of Experiment 2. Six 500 L cylindroconical, black-bottomed tanks connected to the same IRTAmar recirculation unit (Carbó et al. 2001) were used to check the dynamics of bacterial and microalgae communities in the water during 7 days.

Four 35 L cylindrical PVC containers ("baskets") were immersed in each holding tank, in order to mimic the experimental conditions of common dentex, "Dentex dentex", larvae rearing. All the baskets had the bottom and three 10x10 cm lateral windows covered with a 150 μm diameter mesh, and were provided with an air-lift system and aeration supply. All the tanks were filled with UV-filtered seawater, and no water exchange was performed during the entire test. Tetraselmis chuii (57 x 10^3 cells/mL) was added to three of the tanks, the remaining three were used as control tanks. Temperature, salinity, oxygen, and pH were checked daily; nitrites and ammonia were checked on day 4. Irradiance was evaluated at the beginning of the experiment, measured at the water surface in the middle of the tank with a luxometer (Lutron LX-101 lux meter, Lutron Electronics Enterprise Co. Ltd., Taiwan).

Fifty mL of water were taken periodically from the middle of the rearing tanks, at 18 cm under the water surface. Water samples for T. chuii density were taken once per day. Water samples for microbial analysis were taken twice per day.

Tetraselmis chuii density was determined with a coulter/particle counter (Multisizer 3, Beckman, COULTER, Miami, Florida, US). Samples for microbial

121 analysis were fixed with 25% glutaraldehyde (Sigma Aldrich, Madrid, Spain)
122 achieving 10% of the sample volume, and stored at 4ºC until analysis. Five
123 hundred µL of 4',6-diamidino-2-phenylindole (DAPI; Sigma Aldrich, Madrid,
124 Spain) were added to a subsample of 5 mL. After 10 min in darkness, the sample
125 was filtered through a 0.2 µm black polycarbonate filter (Nucleopore Track-Etch,
126 Whatman, Maidstone, England); the filter was put on a slide glass and kept at -
127 20ºC in darkness until analysis. Bacteria were counted using an epifluorescent
128 microscope (Leica Microsystems, GmbH, Wetzlar, Germany) where filters were
129 exposed to 365 nm light in order to induce DAPI's fluorescence (Kepner and
130 Pratt, 1994).

131 *Experiment 2*

132 Experiment 2 lasted for 16 days (Fig. 1). The effect of three types of rearing
133 water on common dentex larval survival and growth were tested using six 500 L
134 cylindroconical, black-bottomed tanks connected to the same recirculation unit
135 (Carbó et al. 2001); two tanks per type of rearing water. Each tank contained 4
136 baskets as described in Experiment 1. The types of water were: 1) "Mature
137 water"; tanks were filled with UV-filtered seawater and T. chuii was added 5 days
138 before the rearing of 0 days post-hatching (dph) common dentex larvae, 2)
139 "Green water"; tanks were filled with UV-filtered seawater and T. chuii was added
140 1 day before the rearing of 0 dph common dentex larvae, and 3) "Clear water";
141 two tanks were filled with UV-filtered seawater the same day of the rearing of 0
142 dph common dentex larvae, and no microalga was added. Once common dentex
143 larvae were stocked, routine management was followed, including rotifer addition
144 to the rearing tanks when larvae were 3 dph (experimental day 8), partial

145 recirculation of water through the recirculation units when larvae were 3 dph
146 (experimental day 8, 30% tank volume renewed daily), and full recirculation of
147 water when larvae were 7 dph (experimental day 14, 100% tank volume renewed
148 daily).

149 Temperature, salinity, oxygen, and pH were checked daily. Nitrites and
150 ammonia were checked on experimental days 8 and 14. Irradiance was evaluated
151 at the beginning of the experiment, measured at the water surface in the middle
152 of the tank, with a luxometer (Lutron LX-101 lux meter, Lutron Electronics
153 Enterprise Co. Ltd., Taiwan). Every day, 50 mL of water were taken from the
154 middle of the rearing tanks (n = 6) at 18 cm under the water surface for microbial
155 analysis.

156 A single batch of floating eggs was incubated in a basket immersed in a
157 500 L black-bottomed tank, at 19 ± 1 ºC, until larvae hatched 48 h later. A sub-
158 sample of eggs was incubated in 96 well EIA plates at 19 ± 1 ºC in darkness to
159 determine the batch quality, based on hatching rate and daily mortality (Giménez
160 et al. 2006a). Some freshly spawned eggs were directly plated onto Marine Agar
161 (MA) or saline Thiosulfate Citrate Bile Salts Sucrose (TCBS; Scharlau Chemie,
162 Barcelona, Spain) medium with a sterilised Pasteur pipette (Naess and Bergh
163 1994). The results were qualitative, of presence or absence of total heterotrophic
164 bacteria (growth in MA) or presumptive Vibrio spp. (growth in TCBS) on the
165 surface of the eggs.

166 Larvae at 0 dph were stocked at 40 larvae/L in each basket; larvae were
167 fed twice per day 10 individuals (ind)/mL of enriched rotifer, “Brachionus

168 rotundiformis" (2h, 20ºC seawater, 250 ind/mL, 0.1 gr/L of Easy Selco, INVE,
169 Belgium) from 3 dph until the end of the experiment (larvae of 11 dph).
170 At the end of the experiment, larvae were counted in order to determine
171 larval survival. At 0, 7 and 11 dph, 20 larvae per basket were sampled to obtain
172 dry weight (DW) data, and 20 additional larvae per tank (five per basket) were
173 sampled for quantification of gut bacteria. The protocol was a modification from
174 Muroga et al. (1987) and Bergh (1996): larvae's skin surface was sterilized with
175 0.05% iodine (Sigma Aldrich, Madrid, Spain) for 3 minutes and rinsed three times
176 with autoclaved seawater before pooling and homogenization in 10 mL of
177 autoclaved seawater, and follow with the plate count protocol.
178 Colony forming units (CFU) of total heterotrophs and presumptive Vibrio
179 spp. were determined by the plate count method: samples of water or
180 homogenates of larvae were serially diluted in autoclaved seawater and 100 µL
181 of each serial dilution were plated onto MA (for total heterotrophic bacteria), or
182 TCBS medium (for presumptive Vibrio spp.). Three plates per sample and dilution
183 were incubated for 24h at 25ºC, and the number of bacterial colonies growing in
184 the plates (CFU) were counted. Counts between 30 and 300 CFU per plate were
185 used for final calculation of bacterial load in seawater (CFU/mL) or per larva
186 (CFU/ind) using the following formulae:
187 CFU/mL = Average of plate counts x DF
188 CFU/ind = ((Average of plate counts x DF) x V) / N
189 Where DF is the dilution factor, V is the volume where D. dentex larvae
190 were homogenized (10 mL) and N is the number of D. dentex larvae (20).

191 Results of larvae DW, bacterial load in seawater and bacterial load in
192 larvae gut were analysed by One-way analysis of variance ($P < 0.05$) and a post
193 hoc pair-wise multiple comparison of the mean using Tukey's test ($P < 0.05$,
194 StatgraphicsPlus 4.1, Microsoft Inc).
195

Results and Discussion

The bacteria-algae interactions are as complex, species-specific, and partially understood, as the larvae-bacteria interactions (Olafsen 2001, Natrah et al. 2014). In addition, all variations of these algae-bacteria-larvae interactions occur at the same time and are interconnected.

Bacteria can be introduced to the rearing tanks through four main routes: 1) water, 2) microalgae, 3) eggs / larvae surfaces and 4) live prey.

The bacterial community in the water has been monitored during the present experiments. The flux of population composition of the microbiological community was observed to be dynamic. In both experiments, physico-chemical conditions did not differ between experimental groups (Table 1). In Experiment 1, bacteria-algae interactions were tested using a monospecific microalgal community; the only difference between experimental groups was the addition of microalgae at the beginning of the experiment, but this difference dramatically affected the evolution of the bacterial community (Figs. 2 and 3). Tetraselmis chuii densities were between 57 x 10^3 and 59 x 10^3 cells/mL during the experiment, while bacterial densities in tanks with T. chuii were significantly higher than those in tanks without microalgae, mainly from day 5 onwards (Fig. 2). Tetraselmis chuii was added at densities used for routine rearing of common dentex larvae in IRTA facilities; no fertilizer or nutrients were added to the rearing tanks, consequently, T. chuii growth was not promoted. Bacteria in tanks with T. chuii can grow on the organic matter produced by the microalgae, such as extracellular polymeric substances (EPS) known to be excreted by microalgae (Joyce and Utting 2015; Natrah et al. 2014), but bacteria in tanks without T. chuii do not have this

220 additional source of nutrients. Results from DAPI staining showed two different
221 morphologies: bacteria associated to microalgae were larger in size and usually
222 found linked in chains, while bacteria in seawater without microalgae addition
223 were smaller and appeared isolated (Fig. 3). These results suggest 5 days as the
224 minimum time required for detecting changes in the bacterial community in the
225 tanks with T. chuii, consequently, this time lapse was selected for the design of
226 "mature" water in Experiment 2.

227 Curves plotted using data from total heterotrophic CFU/mL were similar
228 between water types used in Experiment 2, although more similar between the
229 water types with T. chuii ("mature" and "green" water, Figs. 4 and 5) than between
230 those and clear water (Fig. 6): bacterial load increased shortly after larvae and
231 rotifers were added to the rearing tanks and it decreased to similar levels after
232 100% water renewal started 9 days after the larval addition (on experimental day
233 14). Presumptive Vibrio spp. CFU/mL were not detectable until one day after the
234 addition of common dentex larvae to the tanks in any of the water types,
235 regardless of the confinement time of this water; afterwards, they followed a trend
236 similar to that obtained from total heterotrophic bacteria in each water type (Figs.
237 4, 5 and 6).

238 Total heterotrophic bacteria reached the maximum in "clear water" (2.5×10^5
239 CFU/mL) when common dentex larvae were 3 dph and rotifers were added to the
240 tanks; in "mature" and "green" water, the maximum appeared when common
241 dentex larvae were 5 dph, and reached 10 times lower densities (5.5×10^4 and
242 6.9×10^5 CFU/mL, respectively). Presumptive Vibrio spp. also reached their
243 maximum when common dentex larvae were 5 dph in "mature" and "green"

244 waters (experimental day 10; 2.5 x 10^4 and 1.7 x 10^4 CFU/mL, respectively), while
245 in “clear” water there were two peaks, one when common dentex larvae were 4
246 dph (experimental day 9; 1.2 x 10^4 CFU/mL) and the second when they were 7
247 dph (experimental day 14; 6 x 10^3 CFU/mL), with lower presumptive Vibrio spp.
248 CFU/mL than in “green” and “mature” water.

249 The increase of CFU/mL of total heterotrophic bacteria and presumptive
250 Vibrio spp. shortly after rotifer addition to the tanks were expected based on
251 previous results of bacterial load in live prey (Giménez et al. 2006b). The present
252 data suggest a buffer effect of T. chuii, and/or the bacterial community present in
253 their culture, on the proliferation of total heterotrophic bacteria and presumptive
254 Vibrio spp. Common aquatic opportunistic pathogens can be detected, such as
255 presumptive Vibrio spp., and are the target of disinfection protocols for finfish
256 larvae (Giménez et al. 2009) and live prey (Giménez et al. 2006b). A
257 management based on the use of microalgae, combined with water exchange,
258 can be more effective than disinfecting the tanks when larvae are already
259 stocked, mainly in the case of common dentex larvae, which are very sensitive to
260 disinfectants (Giménez et al. 2009) and stress (Rigos et al. 1998).

261 There was no bacterial growth on any of the eggs directly plated onto MA
262 and TCBS; therefore, the surface of the eggs was considered virtually free of
263 bacteria and their contribution to the increase of bacterial load in the rearing water
264 is considered very low. Can et al. (2012) report the presence of total heterotrophic
265 bacteria and presumptive Vibrio spp. in common dentex eggs kept in tanks for
266 72h. It is possible that, despite the lack of detection of bacterial growth in the
267 present study, once the larvae have hatched, the high concentration of nutrients

268 from the eggs promote the increase of fast growing bacteria such as presumptive
269 Vibrio spp. It could explain the increase in bacterial load in the rearing water when
270 larvae were added, especially presumptive Vibrio spp. Common dentex eggs do
271 not survive washing several times on a mesh, nor the standard iodine disinfection
272 (unpublished data) described for "Sparus aurata", "Dicentrarchus labrax" (Moretti
273 et al. 1999) or "Hippoglossus hippoglossus" eggs (Bergh and Jelmert 1996), and
274 are more sensitive to ozone treatment than Sparus aurata, Dicentrarchus labrax
275 and "Pagrus pagrus" (Can et al. 2012), probably due to their thinner chorion
276 (Iconomidou et al. 2000). Consequently, disinfection of common dentex eggs
277 does not seem necessary, based on the results of bacterial load and their
278 sensitivity to disinfection procedures.

279 The study of larvae-bacteria interactions was affected by the low quality of
280 the batch of eggs used in the experiment, which possibly affected negatively the
281 results in larval survival (Table 2). Mortality of 5 dph starved larvae kept in EIA
282 plates was high (89.1%), compared to the published average mortality rate of
283 29.2% and minimum mortality rate of 4.2% obtained during a spawning season
284 from the same broodstock (Giménez et al. 2006a). There were no significant
285 differences in larval survival, i.e. larvae were at similar stocking densities. They
286 also shared the same genetic background, and were fed the same live prey.
287 Larvae reared in "green" water were significantly bigger (38.8 ± 11 μg, $P < 0.05$)
288 than larvae reared in "mature" and "clear" water (Table 2). These data suggest
289 "green" water as the most suitable technique for experimental larval rearing of
290 common dentex, although the experiment ended when larvae were 11 dph,
291 before the formation of the immunological organs was completed (Santamaría

292 2001). The beneficial effects of controlling the microbial community in the rearing
293 water during early larval rearing will probably be more evident later in
294 development, during or after metamorphosis.

295 There can be a relationship between the trends of total heterotrophic CFU
296 per larvae and in the rearing water because the larvae gut is colonized by the
297 bacteria present in the rearing water during their early development (Bergh 1996).
298 There was no bacterial colonization in the gut of 0 dph common dentex larvae
299 (Table 2)utilizing the protocol for determining gut bacteria, adapted from Muroga
300 et al. (1987) and Bergh (1996) . These results are in agreement with common
301 dentex larval development, because at 0 dph their gut is closed, neither the mouth
302 nor the anus are opened until 3 dph (Santamaría 2001). Between 7 and 11 dph,
303 a reduction of total heterotrophic CFU/ind was observed in larvae reared in
304 “green” water. Opposite results were observed in gut microbiota of larvae reared
305 in “clear” and “mature” water. When larvae were 7 dph (experimental day 12)
306 there was a higher density of total heterotrophic CFU/mL in “green” water than in
307 “mature” and “clear” water (Figs. 4, 5 and 6), when larvae were 11 dph
308 (experimental day 16) the concentrations of total heterotrophic CFU/mL were
309 similar in the three types of rearing water, lower than at experimental day 12 for
310 “green” water and higher for “mature” and “clear” water. Presumptive Vibrio spp.
311 CFU/ind were similar at 11 dph among all rearing water types, although at 7 dph
312 larvae reared in “mature” water had more presumptive Vibrio spp. CFU/ind than
313 larvae reared in “green” or “clear” water. The hypothesis of gut colonization,
314 suitable for results on total heterotrophic bacteria, is not suitable for presumptive

315 Vibrio spp. because there is no relationship with the results of presumptive Vibrio
316 spp. in the rearing tanks (Figs. 4, 5 and 6)
317 Total heterotrophic CFU/ind and presumptive Vibrio spp. CFU/ind,
318 obtained in the present experiment, are within the range found by Muroga et al.
319 (1987) for Pagrus major and "Acanthopagrus schlegeli" at similar degree days
320 but much lower than those found in "Scophthalmus maximus" (Nicolas et al. 1989;
321 Salvesen et al. 1999; Makridis et al. 2000) and in "Paralichthys dentatus" (Eddy
322 and Jones 2002). Comparisons with published results (Table 3) must take into
323 account that studies on the effects of microbiological environment on larval
324 performance have been mainly carried out with cold water species. These
325 species are cultured at a lower water temperature and undergo a longer larval
326 development than common dentex larvae.
327

Conclusions

Addition of microalgae to the rearing tanks, and the duration of their confinement before the onset of larvae rearing, affect the composition and dynamics of the microbial communities in the rearing water. The addition of larvae and rotifers resulted in an increase in the microbial load of the rearing water, both total heterotrophs and presumptive Vibrio spp., but the presence of T. chuii seems to buffer the bacteria proliferation.

Disinfection of eggs and live prey before their addition to the rearing tanks might be good practices for the reduction of bacterial load in the rearing water, but egg disinfection is not suitable for common dentex given their sensitivity to disinfectants, and not relevant taking into account the low bacterial load on their egg surface .

Results on larval growth suggest “green” water as the most suitable technique for the experimental design and for rearing of common dentex.

Acknowledgments

Funding provided by the Spanish Instituto Nacional de Investigación Agraria y Alimentaria (INIA) for GGP fellowship, and by a project from Junta Nacional Asesora de Cultivos Marinos (JACUMAR). We wish to thank M. Monllaó and J. Canoura (Centre d’Aqüicultura IRTA) for their help and advise in larval rearing, and K. B. Andree for final review of the manuscript.

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464 Table legends

465 Table 1. Rearing conditions maintained in Experiment 1 and Experiment 2.

466 Table 2. Egg quality data, survival, DW, and bacterial load results of larvae reared
467 in the different rearing water types of Experiment 2. a, b and ab superscripts
468 denote significant differences ($P < 0.05$). *counts lower than 30 CFU per plate.

469 Table 3. Summary of bibliographical data of gut-associated bacterial flora hosted
470 by fish larvae of different species. U = unspecified bactreria; H = total
471 heterotrophic bacteria; V = presumptive Vibrio spp.

472

Figure legends

Figure 1. Set up of Experiments 1 and 2. Black and white bars are the time scale of experiment, in days. Colour of the cells indicate the status of tanks: white = empty tank; dark grey = tank with water, no water exchange; grey = tank with water, 30% water exchange per day; light grey = tank with water, 100% water exchange per day. Samplings: * = microbiological sample; T = T. chuii sample; X = checking of temperature, salinity, oxygen, and pH; L = D. dentex larvae sample; N = checking of nitrites and ammonia.

Figure 2. Bacteria density (cells/mL) obtained in Experiment 1, counted at the microscope using DAPI staining. a and b superscripts denote significant differences ($P < 0.05$) between types of rearing water at the same sampling time.

Figure 3. Images showing bacterial communities in Experiment 1 at the beginning and the end of the experiment in each type of rearing water. Bacteria are stained using DAPI (cocci and rods). Images A and B: bacteria communities in the tanks with (A) and without (B) T. chuii at the beginning of the experiment, time 0h. Images C and D: bacteria communities in the tanks with (C) and without (D) T. chuii at the end of the experiment, time 146h.

Figure 4. Evolution of Heterotrophic bacteria and presumptive Vibrio spp. densities (CFU/mL) in “mature water” from Experiment 2.

Figure 5. Evolution of Heterotrophic bacteria and presumptive Vibrio spp. densities (CFU/mL) in “green water” from Experiment 2.

Figure 6. Evolution of Heterotrophic bacteria and presumptive Vibrio spp. densities (CFU/mL) in “clear water” from Experiment 2.

496 TABLE 1.

497

	Experiment 1		Experiment 2		
	Control	With T. chuii	Clear water	Green water	Mature water
Initial T. chuii density (cells/mL)	0	57,425 ± 454	0	55,508 ± 378	56,251 ± 851
Temperature (ºC, mean ± SD)	17.7 ± 0.5	17.8 ± 0.6	18.8 ± 0.5	18.5 ± 0.0	18.3 ± 0.1
Salinity (g/L, mean ± SD)	34.3 ± 0.1	34.2 ± 0.1	34.8 ± 0.2	34.9 ± 0.1	35.0 ± 0.1
Oxygen (mg/L, mean ± SD)	8.7 ± 0.1	8.8 ± 0.2	9.1 ± 0.4	9.0 ± 0.3	9.5 ± 0.4
pH (mean ± SD)	8.3 ± 0.1	8.3 ± 0.1	7.9 ± 0.3	8.2 ± 0.0	8.2 ± 0.0
Nitrites (mg/L, maximum value)	0.02	0.04	0.01	0.02	0.04
Ammonia (mg/L, maximum value)	0	0.06	0	0.02	0.06
Photoperiod (hours light:hours darkness)				16:8	
Irradiance ($\mu mol/m^2$ s)				3.7	

498

499 TABLE 2.

	Clear water	Green water	Mature water
Hatching rate (EIA plates results, %)		94.8	
Mortality of starved 5 dph larvae (EIA plate results, %)		89.1	
Survival at 11 dph (%, mean ± SD)	2.8 ± 1.7	2.9 ± 1.5	1.7 ± 0.6
Initial DW (μg, mean ± SD)		29.2 ± 1.5	
7 dph DW (μg, mean ± SD)	19.3 ± 1.6[b]	22.2 ± 5[a]	21.8 ± 4.1[a]
11 dph DW (μg, mean ± SD)	31.1 ± 10.5[b]	38.8 ± 11[a]	33.1 ± 7.3[ab]
Total heterotrophic CFU/ind (mean ± SD) at 0 dph	0	0	0
Total heterotrophic CFU/ind (mean ± SD) at 7 dph	77 ± 8	301 ± 134	250 ± 143
Total heterotrophic CFU/ind (mean ± SD) at 11 dph	563 ± 105	132 ± 10	634 ± 133
Presumptive Vibrio spp.CFU/ind (mean ± SD) at 0 dph	0	0	0
Presumptive Vibrio spp. CFU/ind (mean ± SD) at 6 dph	*	*	167 ± 60
Presumptive Vibrio spp. CFU/ind (mean ± SD) at 11 dph	42 ± 22	30 ± 15	42 ± 21

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502 TABLE 3.

Species	Larval age (dph)	Water temperature (ºC)	Day degrees	Bacterial density (CFU/ind)	Reference
Pagrus major	4	18.4	92	55 (U)	Muroga et al. 1987
	18	20.6	380.4	16000 (U)	
Acanthopagrus schlegeli	4	19.2	96	1.3 (U)	Muroga et al. 1987
	18	20.0	456	4600 (U)	
Scophthalmus maximus	4	not available	not available	12000 (H)	Nicolas et al. 1989
				820 (V)	
				18000 (H)	
	6			1400 (V)	
Scophthalmus maximus	1	15	29	2500 – 21000 (U)	Salvesen et al. 1999
	5	18	98	1900 – 31000 (U)	
	12		224	53200 - 868000 (U)	
Scophthalmus maximus	1	16	30	74000 – 34000 (U)	Makridis et al. 2000
	2	18	48	*Bioencapsulation	
	3		66		
	6		120		
	9		174		
	12		228		
Paralichthys dentatus	1 – 90	not available	-	10^3 - 10^4 (U)	Eddy and Jones 2002
Hippoglossus hippoglossus	5	not available	-	<5 (U)	Verner-Jeffreys et al. 2003
Dentex dentex	0	19	19	0 (H)	Present experiments
				0 (V)	

6	133	77 – 301 (H) 167 (V)
11	228	132 – 634 (H) 30 – 42 (V)

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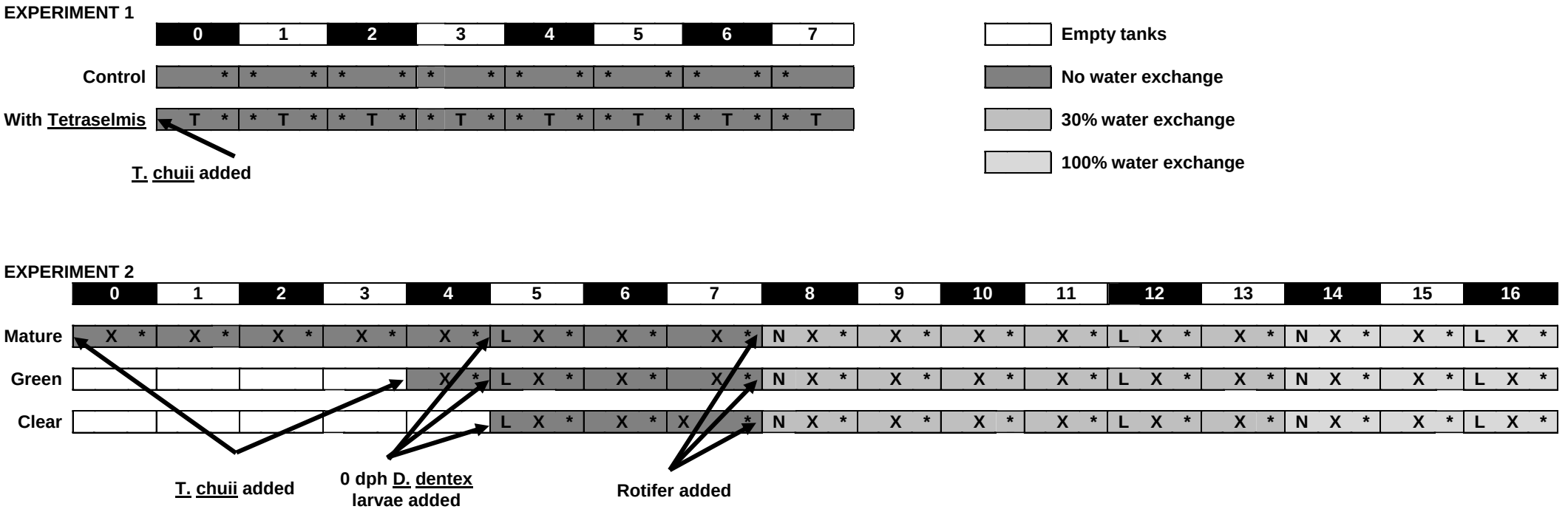
504 FIGURE 1.



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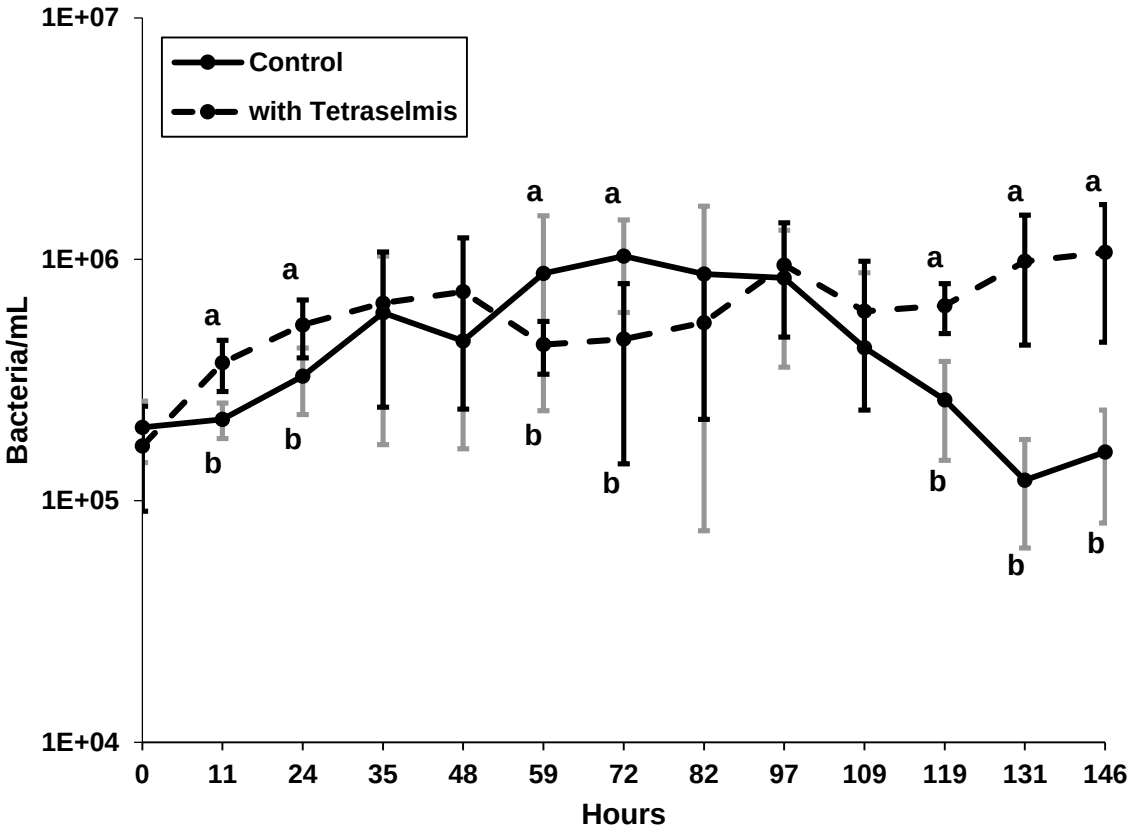
508 FIGURE 2.



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510 FIGURE 3.

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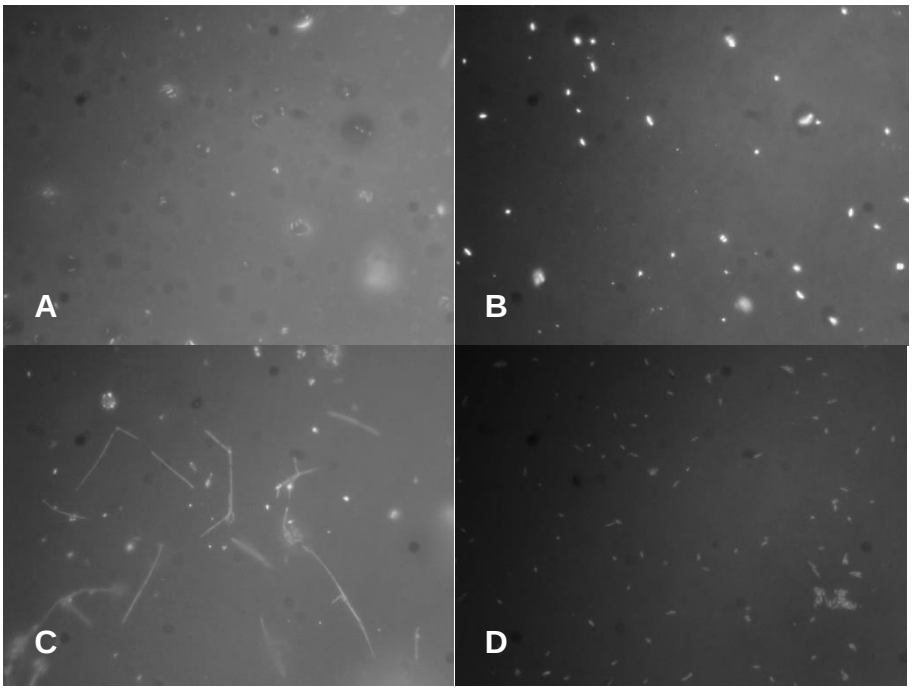

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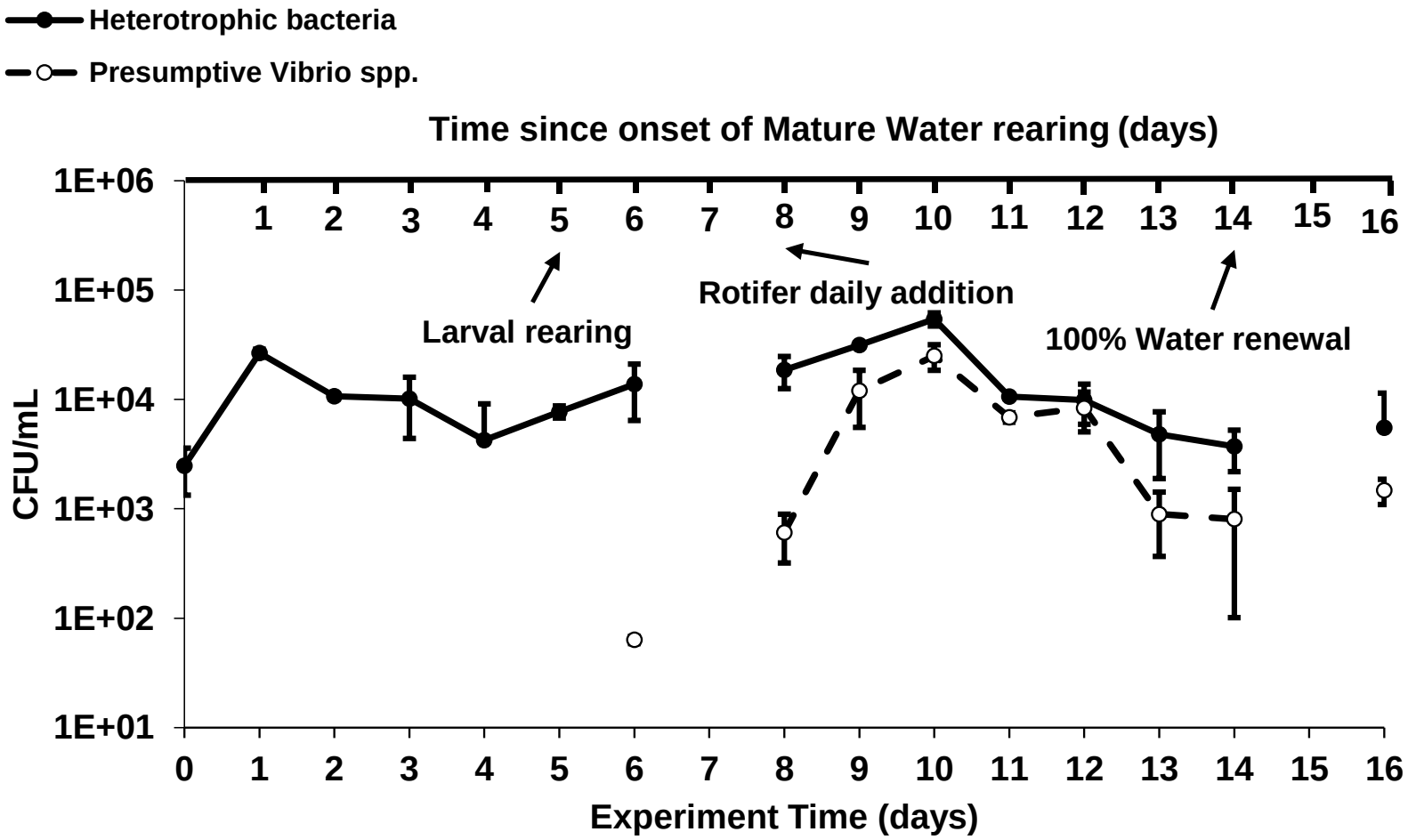
514 FIGURE 4.



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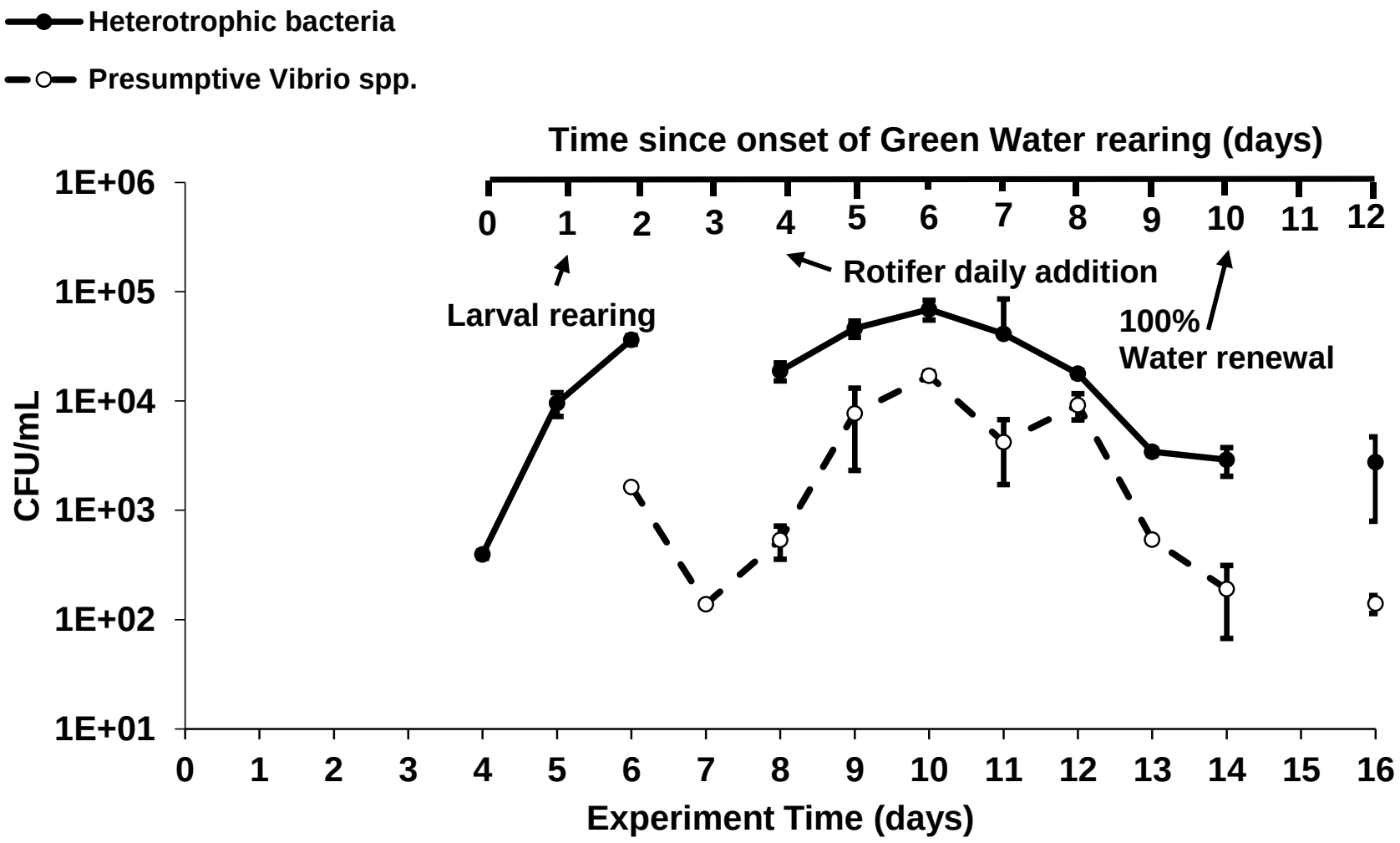
517 FIGURE 5.



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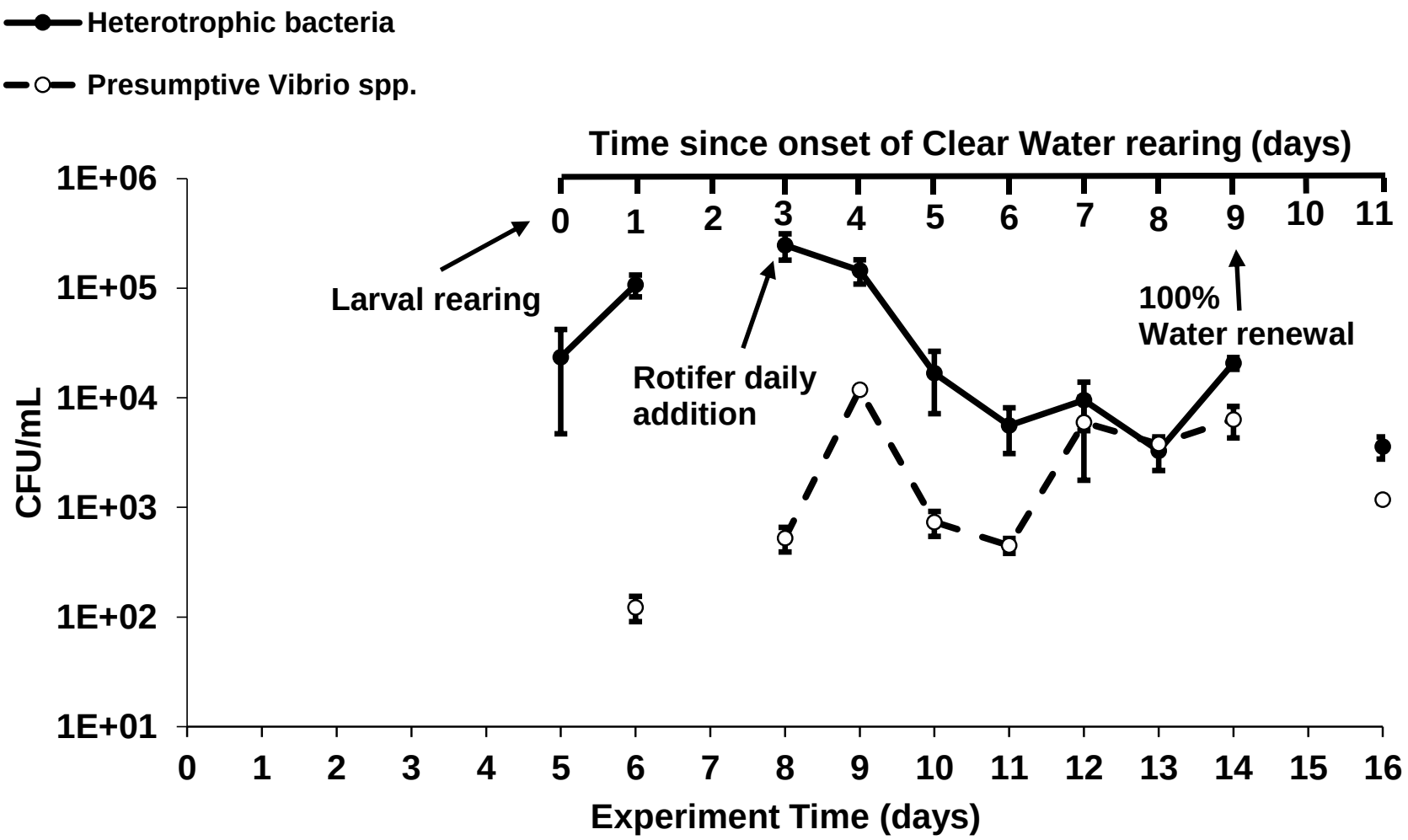
520 FIGURE 6.



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