Limnocnida tanganyicae medusae (Cnidaria: Hydrozoa): a semiautonomous microcosm in the food web of Lake Tanganyika

Kalevi Salonen[1], Pia Högmander[1], Victor Langenberg[2], Hannu Mölsä[3], Jouko Sarvala[4], Anne Tarvainen[1] & Marja Tiirola[1]

[1]Department of Biological and Environmental Sciences, PO Box 35 (YAC), FI-40014 University of Jyväskylä, Finland

[2]DELTARES, Department of Water Quality and Ecology, PO Box 177, 2600 MH Delft, The Netherlands

[3]Fish Innovation Centre, Lohitie 701, FI-72210 Tervo, Finland

[4]Department of Biology, University of Turku, FI-20014 Turku, Finland

kalevi.salonen@jyu.fi, phone 358407592829

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Abstract

Medusae are important members of marine food webs, but are rare in lakes. In one of the largest lakes in the world, Lake Tanganyika, a small *Limnocnida tanganyicae* medusa is a prominent component of zooplankton, but its role in the ecosystem has remained obscure. In this study, we addressed the role of medusae in Lake Tanganyika using several approaches. These medusae occasionally reached high densities locally. They often inhabited the whole epilimnetic water column. In particular, the largest individuals showed distinct, low amplitude, diel vertical migration, which seemed to be crucial to avoid harmful UV radiation. Vertical migration and consequent adjustment to light intensity also might be important for picocyanobacteria that were regularly present in variable quantities in Tanganyika medusae. In different individuals, endosymbiotic picocyanobacteria were morphologically variable and dominated by a particular Lake Biwa type *Cyanobium* species, which typically are abundant in the Tanganyika water column. Under light, some medusae even proved to be net primary producers. Nitrogen stable isotopic ratios indicated that while the free-living cyanobacteria were nitrogen-fixers, the internal picocyanobacteria in medusae obtained their nitrogen predominantly from their host. Stable isotopic ratios of carbon and nitrogen further suggested copepod zooplankton as the most likely prey for the medusae. Lake Tanganyika medusae apparently base their metabolism both on animal and plant sources, with possible internal cycling of nutrients; however, the role of picocyanobacteria gardening for the ecosystem of Lake Tanganyika and its medusae requires quantification.

Introduction

Lake Tanganyika is the second deepest and oldest lake on the earth. Its numerous endemic biota reflect the history of about ten million years under rather stable conditions prevailing near the equator (Tiercelin & Mondeguer, 1990; Cohen et al., 1993). The total biodiversity of the lake, one of the highest in the world (Coulter, 1994), is largely confined to the littoral zone. Its pelagic biodiversity, by contrast, is low and leads to a rather simple food web. In Lake Tanganyika, a hydromedusa, *Limnocnida tanganyicae* Böhm, 1883 (Hydrozoa, Limnomedusae), is a prominent component in zooplankton (Sarvala et al., 1999; Langenberg et al., 2008). Of the two most common freshwater medusa genera, *Craspedacusta*, has colonized all continents, while *Limnocnida* is restricted to Asian and African tropics and subtropics (Dumont, 1994a; Jankowski, 2001). In Africa, *L. tanganyicae* seems to be the only species (Goy, 1977). In Lake Tanganyika, the medusa stage is predominant and in fact, the tiny (< 0.5 mm) hydroid stage of the hydromedusa was discovered later because of its small size and cryptic life style (Bouillon, 1954).

The ecology of freshwater medusae is poorly known and their taxonomy is still debated. All non-parasitic Cnidaria are predators, but due to the absence of knowledge of their food and feeding, the trophic position of freshwater medusae remains obscure (Rayner & Appleton, 1989; Dumont, 1994b). In Lake Tanganyika, medusae up to 25 mm diameter are abundant (Kurki et al., 1999), and their biomass is of the same order as that of predatory crustacean zooplankton (Sarvala et al., 1999).

The predators of freshwater medusae are unknown (Dumont, 1994a), but it has been hypothesized, albeit contradicted by the observations of Viherluoto (1999), that they might be consumed by benthic decapods. There is no evidence that pelagic fish feed on them (Coulter, 1991). Consequently, *Limnocnida* may be considered as a dead end in the food web.

Examination of *L. tanganyicae* medusae with a high resolution epifluorescence microscope during a cruise in 1996 surprisingly showed a multitude of picocyanobacteria inside them. This led us to hypothesize that these animals might be able to garden picocyanobacteria and partly base their

metabolism on that. To better understand the role of possible gardening by *L. tanganyicae* medusae in Lake Tanganyika, we studied several aspects of their ecology during several expeditions covering the whole lake, utilizing field abundance data, laboratory experiments, as well as genetic and stable isotope analyses.

Materials and methods

This study was performed during 1994-2001. To estimate medusa biomass, a regression was established between the umbrella diameter, dry mass (DM), and ash free dry mass (AFDM) of medusae. Individuals were selected over the range of sizes. After measurement of the umbrella diameters, individuals were dried on pre-weighed aluminium foil cups. These samples were taken to Finland, dried again at 60ºC, and weighed on a Cahn Electrobalance. AFDM was obtained by difference from DM after re-weighing following combustion at 500°C.

In the pelagic waters off Kigoma, Tanzania (4°51.00'S, 29°35.00'E), quantitative samples of *L. tanganyicae* medusae were taken at 10 m vertical hauls from 120 m to the surface using a 500-µm mesh closing net. In the vicinity of Mpulungu, Zambia (08°43.98'S, 31°02.43'E), medusa bloom samples were taken with a 7-liter tube sampler (Limnos Ltd, Finland) and from the immediate surface by scooping with a 10-liter PVC container. Qualitative epifluorescence microscopic observations were made of medusae collected throughout the lake. To avoid possible damage to medusae from excessive light, individuals for the experiments were collected at dusk when they ascended to the surface. Generally, single large animals were caught by hand-scooping into a 0.5 liter beaker from the surface or with a tube water sampler from slightly deeper layers. In 1998, medusae also were collected at night or in dim light by divers in $\leq$ 2 m water depth using 50-mm-diameter acrylic tubes with 300-µm-mesh plankton netting covering one end. When a medusa was captured in the tube, the open end was closed with a stopper and immediately taken to the laboratory aboard the research vessel.

In some cases, the umbrella diameters of animals were measured with the aid of a dissecting microscope. The presence of protozoans inside the animals also was recorded. The occurrence of internal algae was checked with an epifluorescence microscope (Nikon Optiphot) at 1250-power magnification. Eukaryotic algae were observed with blue excitation and prokaryotic picocyanobacteria with green excitation.

To investigate the effect of UV light on medusa survival, an experiment was conducted on board the R/V Tanganyika Explorer. Twelve or 13 animals were placed in each of three 2-liter quartz bottles, which were put in a water bath in an open, white polystyrene box. One bottle was exposed to direct sunlight, another was kept under a UV-protected polyethylene film, and one wrapped in aluminium foil was used as the dark control. Water temperature was kept similar to ambient by pumping lake water through the box. During the experiment (beginning at 11:45), spectral sunlight radiation was measured every 15 min with a Macam SR 991 spectroradiometer (planar cosine light collector). Spectral penetration of light into water of Lake Tanganyika was also measured using a 4-m quartz light cable. Without the UV-protected polyethylene film, measurable radiation was observed down to 300 nm wavelength; with the film, the limit was about 350 nm. At wavelengths longer than 400 nm, the film absorbed ca. 1/3 of the radiation. Medusae pulsing their swimming umbrella were considered alive and were counted every 10 min. In the dark bottle, animals were counted only at the end of the experiment. To avoid even short exposure to high UV radiation, the bottle under the UV-screen was counted only once before the end of the experiment; the bottle was placed in a black cotton bag and transferred to the laboratory of the ship for counting. The experiment was terminated when all animals had died in the bottle kept under direct sunlight. We tested the null hypotheses that UV radiation does not adversely affect *L. tanganyicae* medusae and that their vertical distribution shows no avoidance of surface water in bright light.

Fluorescent beads (Polysciences Inc.) were used to qualitatively study the ingestion of picoplankton-sized organisms. To remove possible bead aggregations, a small volume of stock

suspension of beads was filtered through a 5-µm Nuclepore filter. The final concentration of beads in lake water offered to medusae in a 50-ml water bottle was adjusted roughly to the same density as picoplankton abundance in lake water (10^5 cells ml^{-1}). We hypothesized that picocyanobacteria were taken by medusae from water.

Bacterial composition of medusae was studied from 11 large (> 10 mm) individuals sampled during December 2001 from the lake surface (0-2 m) off Kigoma harbor at dusk and then stored in 70% ethanol. Water samples (1 liter) were taken with a Limnos sampler at the same time at 10-m depth intervals from the surface to 60 m. For DNA analyses, 0.5 l of water was screened through 50-µm-mesh plankton netting and then filtered onto Filtropur acetylacetate filter units (0.2-µm pore size). Bacterial DNA extractions were performed using the combined enzymatic and bead-beating method, and the length heterogeneity-PCR (LH-PCR) targeted V1-V3 variable regions of the 16S rRNA (area 8-534, *Escherichia coli* numbering), as described by Tiirola et al. (2002a). Direct sequencing of the heterogeneous PCR products was performed bi-directionally using the ABI BigDye kit and ABI 3100 DNA sequencer (Applied Biosystems). Sequences were compared against the EMBL database using the BLAST algorithm (Altschul et al., 1997). A bootstrapped neighbor-joining tree was calculated using Jukes-Cantor correction with the MEGA 4 software. Reference sequences for inferring the tree were the following (from top to bottom in the tree): AF330249, DQ463712 (Lake Tanganyika clone), AF330250, AF448063, AF216955, AF317074, AF330247, AB015058, AF330252, AF001477, AY151249, AF098370, AF330251, AY172819, AY172811, AY172810, AY172801 AF001479, AY172833, AF245618, S000388727, AF053398, S000628344, and AY946243. We tested the null hypothesis that the picocyanobacterial assemblage in the medusae is similar with that in water.

To measure oxygen consumption, medusae were transferred individually into 50-ml bottles filled with lake water and sealed with round, glass stoppers. Bottles with the same water, but without a medusa, served as controls. After filling, oxygen concentration was measured and the

bottles were placed in an incubator. Some of the bottles were darkened with aluminium foil. In the incubator, the bottles were kept under an illumination of 511 µmol m^{-2} s^{-1} supplied by 6 daylight type fluorescent tubes. Water temperature was maintained within 1°C of the lake temperature (around 27°C) by pumping water from the lake through the incubator. Temperature was monitored with a thermometer during the oxygen measurements. Oxygen concentration was measured with an YSI BOD bottle probe with a stirrer at the upper part of the bottle. It was placed in the bottle and mixing was kept on for 30 s before reading the value. After the measurement, the bottle was re-stoppered and returned to the incubator. Oxygen consumption by medusa was calculated as the difference between the initial and final concentrations taking into account the incubation time and the bottle volume. Differences in control bottles were subtracted from the results of bottles with medusae. We hypothesized that the photosynthesis of picocyanobacteria does not affect significantly the oxygen budget of the medusa-picocyanobacteria microcosm.

In one oxygen production/consumption experiment, lake water was amended with autoclaved stock solutions of KH_2PO and NH_4Cl to final concentrations of 0.8 µmol P l^{-1} and 12.5 µmol N l^{-1}, roughly in accordance with the highest concentrations (phosphate 0.1-0.6 µmol, nitrate 1.6-3.7 µmol) reported by De Wever et al. (2008a) for the epilimnion of Lake Tanganyika. Ammonium nitrogen was used because algae preferentially take up ammonium and medusae excrete ammonium. NH_4-N is typically very low (0-0.05 g m^{-3}) down to roughly 100 m depth in the lake (Plisnier et al., 1999). If nutrients released from the digestion of invertebrate food of medusae were sufficient for endosymbiotic picocyanobacteria, then nutrient addition would not affect their photosynthesis. Therefore, wetested the null hypothesis that external nutrients do not affect the photosynthesis by the medusae.

To clarify the trophic position of the medusa, samples for stable carbon and nitrogen isotope determinations were collected off Kigoma (4°51’S, 29°35’E), in late November to early December 2001. medusae were sampled on 4-10 December 2001 either with vertical net hauls (100- or 250-

166 µm mesh) from 120 m to the surface (or from 50 m after sunset), or by scooping individual
167 medusae from surface water. In the latter case, the abundance of associated picoplankton was
168 estimated visually by color, and the medusae were sorted into five groups accordingly (colorless =
169 no or very few picoplankton; slight pink hue = few picoplankton; medium pink hue = picoplankton
170 moderately abundant; entire medusa intensely pink = picoplankton very abundant overall; and pink
171 color only around the marginal ring of the medusa). The correlation between medusa color and
172 abundance of internal picoplankton was confirmed with the epifluorescence microscope. Medusae
173 were stored in carbon- and nitrogen-free alkaline Lugol's iodine. Later, medusae were rinsed with
174 deionized water and placed in tin cups as groups of small, similar individuals or as pieces of large
175 individuals (total sample dry mass 1-4 mg). The cups were sealed, dried at 60ºC, and sent for
176 analysis by an Europa Scientific Hydra 20/20 isotope ratio mass spectrometer at the Stable Isotope
177 Facility, University of California-Davis, California, U.S.A.. The results are given using the δ
178 notation, where $\delta = [(R_{sample}/R_{reference}) - 1] \times 1000$, expressed in units per thousand (‰), and where
179 $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Reference materials were PeeDee belemnite for carbon and atmospheric N_2
180 for nitrogen. Nitrogen-fixing algae have very low $\delta^{15}N$ values (around 2‰ or less; Vuorio et al.,
181 2006). If nitrogen fixation was important for the internal picocyanobacteria, nitrogen isotope
182 signatures would be lower in the medusae that had higher abundance of picocyanobacteria.
183 Therefore, we tested the hypothesis that nitrogen isotope signatures of the medusae reflect the
184 abundance of picocyanobacteria.

185 Zooplankton, shrimps, and fish larvae from the same net hauls were fixed with carbon- and
186 nitrogen-free alkaline Lugol's iodine immediately after sampling and later sorted by species and
187 size groups in the laboratory. After rinsing with deionized water, groups of 1 to approximately 3000
188 individuals were transferred to tin cups, sealed, dried, weighed, and sent for stable isotope analysis.
189 Water was sampled from different depths (0-100 m) with a tube sampler (Limnos Ltd, Finland)
190 from 19 November-10 December 2001. The samples (4-20 l) were pre-screened through 50-µm-

mesh netting to remove zooplankton and large phytoplankton, and the filtrates were then filtered through pre-combusted (at 500ºC overnight) glass fibre filters (Whatman) using a low vacuum (< 20 kPa), first through a GF/D filter (median pore size 2.8 µm, which retained mainly eukaryotic nano- and microplankton) and then through a GF/F filter (median pore size 0.7 µm, which retained mainly picocyano- and heterotrophic bacteria). Larger phytoplankton (mainly cyanobacteria) was collected on 6-10 December 2001 in net hauls with 50-µm mesh from 5-10 m depth to the surface, which then were concentrated on GF/D filters. The filters were put on pre-combusted aluminium foil and dried at 60ºC. In Finland, the dried filters were weighed and 16-18 (GF/D) or 10 (GF/F) 3-mm-diameter subsample discs were punctured from the filters and placed in pre-weighed tin cups, weighed, and sent for stable isotope analysis.

The samples of the most important pelagic planktivore, the clupeid fish, *Stolothrissa tanganicae* Regan, were obtained on 22 and 26 November 2001 directly from the fishermen as they came ashore. The fish were measured for length and a tissue sample of the dorsal white muscle was cut from behind the dorsal fin. The tissue samples were put on aluminium foil and dried in an oven at 60ºC. In Finland, the tissue samples were ground to a fine powder and ca. 0.8 mg from each sample was transferred into pre-weighed tin cups and sent for stable isotope determinations.

Linear mixing models were applied to the isotope signatures to quantify the contributions of potential food sources to the diet of the medusae (program IsoSource; Phillips & Gregg, 2003). Isotope signatures were adjusted for the stepwise enrichment in the heavier isotopes from one trophic level to the next, using steps of 0.5 and 1‰ for ^{13}C (France & Peters, 1997), and 2 and 3.4 ‰ for ^{15}N (McCutchan et al., 2003).

Results

Although *Limnocnida tanganyicae* are so large that they easily attract visual attention, their individual biomasses were low, with AFDM ca. 2 mg for a 12-mm-diameter medusa (Fig. 1.).

During several cruises, *L. tanganyicae* medusae were found at the surface in locally high densities. In early September 1995, we examined their detailed horizontal and vertical distributions by sampling a medusa bloom covering nine locations near Mpulungu in the southern basin of Lake Tanganyika (Fig. 2). Medusa density was roughly 3000 ind m^{-3} nearest to the coast, but it was an order of magnitude lower at more than 15 km offshore.

Vertical day and night distributions of *L. tanganyicae* were studied in April 1998 off Kigoma, when the epilimnion was less than 15 m thick (Fig. 3). Medusae were present throughout the oxygenated water column to approximately 100 m deep. Vertical distributions of medusa abundances near noon and midnight both were maximum within the 10-20 m zone; however, at night their median depth of occurrence was 13 m, while at noon it was deeper at 21 m. The difference between day and night vertical distributions was highly significant (Smirnov test, $D = 0.219$, $p < 0.001$, $n_1 = 261$, $n_2 = 367$), and we thus rejected the null hypothesis that the vertical distribution of jellyfish was even throughout the water column. Small medusae were evenly distributed but large (> 10 mm diameter) ones more frequently occurred in the upper 30 m. Visual observations also showed that, at sunset, medusae appeared near the surface when photosynthetically active radiation (PAR) above water reached ca. 200 µmol m^{-2} s^{-1} (roughly 20% of the daytime average; Sarvala et al. 1999) and large individuals arrived first. During daytime, medusae were observed only occasionally near the surface. Although they were often alive, in agreement with Dumont (1994), when used in the experiments they did not survive long, suggesting that they were somehow damaged.

Because UV radiation can be damaging in clear water lakes, such as Tanganyika, we studied whether UV might explain the absence of or damage to *L. tanganyicae*. The most harmful UV-B radiation was restricted to the top 5 m of the water column (Fig. 4). In a survival experiment performed near solar noon on the deck of the research vessel, the accumulation of UV radiation developed linearly (Fig. 4). Under a UV-screen, UV-B radiation (290-320 nm) was virtually

241 eliminated. UV-A radiation was detectable at wavelengths of 350-400 nm, but the experiment was
242 too short to cause significant mortality of medusae. By contrast, medusae exposed to natural solar
243 radiation died within one hour (Fig. 5). We rejected the null hypothesis that the UV radiation was
244 harmless for medusae.

245 Observations with an epifluorescence microscope of living *L. tanganyicae* without any
246 staining only occasionally showed cells that fluoresced under blue excitation, and only in the gut
247 region. By contrast, under green excitation, the field of view was often full of generally < 1-µm-
248 diameter orange fluorescing cells, which indicated that they contained phycoerythrine pigment of
249 picocyanobacteria. Some of the cells moved freely in the body fluid of the medusae and some were
250 stationary. Often it was difficult to judge whether stationary cells were on surface or inside the
251 medusae. Sometimes picocyanobacteria were in different types of colonies (Fig. 6). Generally, the
252 cells were globular or very short rods. When the colonies formed a felt-like structure, the
253 picocyanobacteria were rods arranged in filaments and the medusae had a pink color visible at
254 distance in the lake. Although picocyanobacteria were nearly always present in medusae caught
255 during all expeditions, their abundance differed remarkably among individuals. A sample collected
256 by net from the entire 100-m water column off Kigoma contained about 20% of 123 medusae with
257 so many picocyanobacteria that their enumeration at 1250x magnification was impossible; however,
258 56% of the medusae had only scattered single cells or small groups of picocyanobacteria.

259 In addition to picocyanobacteria, 21% of the studied 705 medusa guts carried wheel-like
260 *Trichodina* type ciliates, similar to those found for *Craspedacusta sowerbyi* (Lankester) by Green
261 (1998). When fluorescent beads 0.2-2 µm-diameter were offered, ciliates ingested large numbers of
262 all bead sizes, indicating that they can consume both bacteria and picocyanobacteria; however, we
263 found no uptake of beads by medusae. Thus we rejected the hypothesis that medusae ingested
264 picocyanobacteria from water.

Dozens of individually-caught medusae observed immediately by microscopy never had zooplankton in their guts. In contrast, medusae inspected immediately after collection by plankton net had immobilized copepods in the corners at the bottom of the gut, which we believe were caught in the concentrated sample.

LH-PCR analysis of the 16S rRNA genes showed that a single LH-PCR fragment size (470 bp) was present in all medusa samples (Fig. 7). This fragment was highly dominant, covering > 60-90% of the total fragment intensity in 7 out of the 11 studied medusae. Picocyanobacterial biomarker (470 bp) of medusae also was very abundant in the epilimnion of Lake Tanganyika, contributing up to 55% of the bacterial abundance at 0-10 m sampling depth (Fig. 8). Another fragment size (472 bp), probably belonging to another picocyanobacterial group, comprised > 15% of the bacterial abundance in the water (0-50 m), but was undetectable from medusae. This indicates that the microbial diversity of the medusae did not mirror the diversity of the environment and thus we rejected the null hypothesis that the picocyanobacterial assemblage in the medusae was similar to that in water.

Five of the 16S rRNA gene PCR products of the medusae (numbers 1-5) were subjected to bi-directional sequencing, which revealed that the consensus sequences were 100% identical (387 bp overlap). The BLAST search of all available nucleotide sequences revealed that this sequence was identical to the cyanobacterial sequence TK-SE6 (EMBL accession number DQ463712, length 1432 bp) dominating the oxic epilimnion of Lake Tanganyika (De Wever et al. 2008b) and to sequences obtained from the Chinese MiYu reservoir water (GU305743) and freshwater lakes (GU323646, GU323613, and GU323608). Similar sequences (99% identity) were obtained from *Synechococcus* sp. strains isolated from tropical and boreal freshwater environments, such as lakes Biwa (strain BS2, HM346183), Taihu (strain LBG2, AF330249), and Tuusulanjärvi (strain 0tu30s01, AM259220), as well as from marine environments (strain CCMP839, AY946244; strain KORDI-28, FJ497720). In the cluster analysis, we used the longer Lake Tanganyika sequence

(DQ463712) to reveal the detailed phylogenetic affiliation of the endosymbiont in comparison to well-established picocyanobacterial clusters (Fig. 9). Lake Tanganyika type sequence clustered with the Lake Biwa strains, which form the freshwater picocyanobacteria group E designated by Crosbie et al. (2003). This cluster contains strains recently re-classified from *Synechococcus* to *Cyanobium*, and their closest described species is *Cyanobium gracile*. One of the other medusae (#10) had a high dominance of the LH-PCR peak 522 bp. The sequence of this peak indicated the dominance of betaproteobacterial heterotroph closest to the type strain of *Vogesella indigofera* (strain ATCC 19706^{T}, AB021385, 95% identity).

The potential role of picocyanobacteria in the metabolism of the medusa-picocyanobacteria microcosm was examined in experiments. In the first light incubation experiment, medusae > 10 mm in diameter sometimes showed net oxygen production (Fig. 10). In subsequent experiments, we studied how oxygen consumption/production would develop when the same medusae were kept sequentially in light and dark. The results corroborated our initial finding, specifically, that under light, oxygen consumption decreased or even switched to production (Fig. 11). The same trend was observed in a similar experiment, although the medusae generally were net oxygen consumers (Fig. 12). When inorganic nutrients were added late in the light phase of the experiment, however, three of four individuals had remarkably increased oxygen production. Finally, after the light was switched off, no medusa produced oxygen. These results suggested high variation among individuals, both in consumption and production of oxygen by medusae. Oxygen consumption increased in relation to the diameter of medusa in the dark (Fig. 13); however, in light there was no clear trend, and oxygen production rates in the smallest size classes were surprisingly high. We rejected the hypothesis that photosynthesis of picocyanobacteria does not affect significantly the oxygen budget of medusa-picocyanobacteria microcosm. Instead, we could not reject the hypothesis that external nutrients are unimportant for the photosynthesis of the picocyanobacteria in the medusae.

315 The stable carbon isotope signatures of *L. tanganyicae* were similar to those of crustacean
316 zooplankton and fish larvae, but lower than in big shrimps or in the planktivorous clupeid fish
317 *Stolothrissa tanganicae*, slightly higher than in pico-, nano-, and microphytoplankton, or copepod
318 nauplii, and much higher than in small shrimps or in the net phytoplankton mainly consisting of
319 cyanobacteria (Fig. 14). The nitrogen signatures of *L. tanganyicae* were similar to those of adult *S.*
320 *tanganicae*, but higher than those of all other groups except big shrimps. Neither the carbon nor
321 nitrogen isotope signatures of *L. tanganyicae* were significantly affected by the abundance of
322 internal picocyanobacteria (ANOVA, $F_{4,6} = 0.587$, $P = 0.34$ for carbon, $F_{4,6} = 0.055$, $P = 0.90$ for
323 nitrogen) and hence we rejected the hypothesis that nitrogen isotope signatures of the medusae
324 would reflect the abundance of picocyanobacteria. This implies that nitrogen fixation was not
325 important for the internal picocyanobacteria of the medusae.
326 Isotope mixing models produced broad distributions of calculated diet proportions, which
327 were sensitive to the choice and grouping of potential food sources, as well as to the trophic step
328 adjustment. The diffuse results were consistent with the fact that the isotope polygon defined by the
329 potential food sources was rather narrow (Fig. 14). No feasible solutions were obtained with a
330 nitrogen step of 3.4‰; however, use of steps of 0.5‰ for carbon and 2.0‰ for nitrogen, achieved
331 isotope mass balance for numerous combinations. When all major groups except adult and larval
332 fish were included as potential food sources for *L. tanganyicae* , the calculated contributions of all
333 sources remained variable and low (all included zero contributions); the 1-99th percentiles varied
334 from 0-18 to 0-46%, with picocyanobacteria, *Tropocyclops tenellus* (Sars), *Tropodiaptomus simplex*
335 (Sars), and copepod nauplii showing slightly higher maximum values than other groups. Net
336 phytoplankton, nano-, and microplankton, as well as small shrimps, always showed low
337 contributions. The results remained qualitatively similar if cyclopoid or all copepod adults and
338 copepodids were one group, but the importance of copepods and picocyanobacteria then became
339 more pronounced (1-99th percentiles 0-58 and 0-46%, respectively). In such cases, the big shrimps

appeared as a moderately important diet component (1-99th percentiles 14-38%), provided that phytoplankton with low carbon and nitrogen signatures was simultaneously consumed at a higher extent. Similarly, mixtures of several food sources also could include fish larvae. If copepod nauplii were grouped with adults and copepodids, no feasible solutions were found. The most clear-cut model solution was obtained when all phytoplankton groups, grouped cyclopoid adults and copepodids, *T. simplex*, copepod nauplii, and small shrimps were included as potential food sources. The highest contributions then were shown by cyclopoids, *T. simplex*, copepod nauplii, and picocyanobacteria (1-99th percentiles 2-62, 24-56, 0-40, and 0-30%, respectively).

Discussion

Our results verified that *Limnocnida tanganyicae* medusae have very low dry and ash-free biomass relative to their diameter, and the regression between diameter and dry mass was similar to that for *Craspedacusta sowerbii* (Jankowski, 2000). Medusae are an interesting product of evolution which, during the 600 million years of their existence, may have expanded their prey catching machinery as large as possible with minimum material cost.

Kurki et al. (1999) sampled monthly for two years at three sites and found typical abundances of *L. tanganyicae* between 10 and 100 ind m^{-3} in the upper 100 m, with the highest abundances (> 500 ind m^{-3}) in the northern part of Lake Tanganyika. By contrast, four lake-wide cruises indicated highest abundances in the southern basin (Kurki, 1998). In our study, the observed maximal abundances were up to 5 to 6 times higher than those observed by Kurki et al. (1999), and even higher concentrations were recorded in December 1994 in surface waters at Kigoma (personal observations). It seems clear that high abundances of medusae are temporally and spatially variable (Kurki et al., 1999; Langenberg et al., 2008). The factors behind high density blooms are poorly known. The large bloom in this study appeared in the southern basin at the end of the windy upwelling season (Coulter & Spigel, 1991), during a transition from a 3-month period of increased

365 availability of nutrients and primary production in cool, well-mixed waters to warmer water,
366 increased epilimnetic stratification, and oligotrophy (Langenberg et al., 2003). Although the blooms
367 generally seem regionally limited, this bloom covered a large area of ca. 400 km^2. The medusa
368 blooms interfere with the fishery because nearby fishing communities in Zambia and Tanzania must
369 stop fishing for the duration of the bloom (I. Kimirei, Tanzanian Fisheries Research Institute,
370 personal communication).

371 UV-radiation, particularly UV-B, is harmful to zooplankton (e.g., Williamson, 1995;
372 Hylander & Hansson, 2010). Vulnerability to radiation differs among different aquatic organisms
373 (Rhode et al., 2001) and environments, but there is no information about UV-B radiation effects on
374 freshwater medusae. Although *L. tanganyicae* was present throughout the whole water column,
375 their abundance was clearly highest in the epilimnion. Similar to marine medusae (Schuyler &
376 Sullivan, 1997), *L. tanganyicae* actively avoided water layers with high illumination. This
377 avoidance, combined with the results of our light incubation experiment (Fig. 5) and the fact that
378 animals found near the surface at daytime did not survive long, suggests that daytime UV-B
379 radiation is harmful to these highly transparent *L. tanganyicae* medusae. UV-B radiation started to
380 cause mortality of copepods at doses of ca. 0.1 W m^{-2} (Zagarese et al., 1997), which suggests that in
381 the upper 3-5 m of the water column, UV-B is excessive for zooplankton in Lake Tanganyika. In
382 fact, at high radiation levels prevailing around noon at the surface, our experimental results show
383 clear harmful effects of UV-B radiation on *L. tanganyicae* medusae (Fig. 5). For more realistic
384 estimates of the effect, lower exposures over longer periods of time are needed. It seems probable
385 that vertical migration of *L. tanganyicae* reflects avoidance of UV radiation in the upper epilimnion,
386 although other factors may be involved as well.

387 The LH-PCR results indicated that bacterial groups in medusae did not match the diversity
388 in the surrounding water. Instead, individual medusae had their own bacterial communities more or
389 less dominated by a certain biomarker size, which also was at a maximum in the water column at 10

m depth. Because several cyanobacterial and alphaproteobacterial genera have the same LH-PCR size (Tiirola et al., 2003), further sequencing was needed to identify the microbes represented by the biomarker. All the sequenced medusae carried certain *Cyanobium*-type picocyanobacteria. In other than pathogenic situations (e.g., Rantakokko-Jalava et al., 2000; Tiirola et al., 2002b), it is unusual for a PCR fragment from environmental samples amplified by universal bacterial primers to be directly sequenced without a cloning step. This is usually possible only if some rRNA gene template contributes to > 60-70% of all templates (Tiirola et al., unpublished). The prevailing 16S rRNA sequence of the medusae *Cyanobium* belonged to the same taxonomic unit that predominates in Lake Tanganyika (De Wever et al., 2008b). The characteristic *Cyanobium* sp. sequence belonged to the Lake Biwa-type freshwater cluster, but it also was closely related to sequences obtained from both freshwater and marine samples ranging from tropical to boreal environments, showing the cosmopolitan nature of the cluster (see Crosbie et al., 2003).

Among marine invertebrates, including medusae (e.g., Hofmann & Kremer, 1981; Hamner et al., 1982; Muscatine & Marian, 1982; Kremer et al., 1990), endosymbiotic algae are rather common including algae from at least five different classes and animal hosts ranging from protozoans to tunicates (e.g., Trench, 1993). By contrast, endosymbiotic cyanobacteria seem to be less common. Nevertheless, they have been found in invertebrates other than *L. tanganyicae* medusae. Erwin & Thacker (2008) found *Synechococcus*-type cyanobacteria in marine poriferans, while Lesser et al. (2004) found endosymbiotic cyanobacteria to coexist with the symbiotic dinoflagellates (zooxanthellae) in a coral and to express the nitrogen-fixing enzyme, nitrogenase. The presence of this prokaryotic symbiont in a nitrogen-limited zooxanthellate coral suggests that nitrogen fixation may be an important source of that limiting element for the symbiotic association.

Medusae capture photosynthetic organisms from water (Rumpho et al., 2011) and form a symbiotic association with them. Carbohydrates and low molecular weight lipids are produced for the host, which probably return nutrients to the microorganisms. Thus, each generation has to

415 acquire its photosynthetic partner from the surrounding environment. The picocyanobacteria that
416 are predominant in *L. tanganyicae* medusae, are very abundant in Lake Tanganyika water (Vuorio
417 et al., 2003; De Wever et al., 2008b; Stenuite et al., 2009). Stenuite et al. (2009) estimated that
418 autotrophic picoplankton with '*Synechococcus*-type pigment' accounted for 41-99% of the total
419 phytoplankton biomass. Boulenger (1911) suggested that the typical function of the stomach, which
420 is reduced in *L. tanganyicae*, is accomplished by the canal system, and that the medusae live upon
421 unicellular algae and protozoa driven into the radial canals. Although we saw picocyanobacteria
422 flowing in the canals, ingestion of pico-sized particles was not confirmed by uptake of fluorescent
423 beads, which suggests an endosymbiotic association of the microbiota inside the medusae.
424 A well-known example of medusae with endosymbiotic algae is the scyphozoan*Mastigias*
425 sp., which relies up to 100% on photosynthesis of endosymbiotic algae and could contribute
426 substantially (16%) to primary production of Eil Malk Jellyfish Lake (McCloskey et al., 1994).
427 *Mastigias* sp. medusae even orient their umbrella to obtain optimum illumination (Hamner et al.,
428 1982). The vertical migration of *L. tanganyicae* medusae also could provide improved growth
429 conditions for its picocyanobacteria in Lake Tanganyika. If the medusae adjust their vertical
430 position according to the optimum illumination, they could maximize photosynthesis of their
431 endosymbionts. This possibility seems plausible because medusae arrive at the surface at dusk too
432 early when light intensity is too high to capture vertically migrating zooplankton. Previous
433 sampling was too coarse to reveal layers of medusae at 100-200 µmol m^{-2} s^{-2} PAR illumination
434 throughout the day, but the daytime vertical distribution of large individuals supports this
435 speculation.
436 In low-nutrient environments, heterotrophic-autotrophic associations of organisms offer a
437 competitive advantage through the mutual transfer of otherwise limiting resources (Yellowlees et
438 al., 2008). The results of our oxygen consumption/production experiment with medusae and
439 additional nutrients suggest that Lake Tanganyika medusae can have net nutrient uptake similar to

that of other medusae (Muscatine & Marian, 1982; Pitt et al., 2005; Todd et al., 2006). Thus, *L. tanganyicae* medusa may acquire key nutrients both from the water and from their prey; however, if they have a high phosphorus level, similar to *Craspedacusta sowerbii* (Jankowski, 2000), then while fulfilling their phosphorus requirements, the medusae obtain excess nitrogen that has to be removed.

Because many photosynthetic organisms can store nutrients, internal picocyanobacteria might be able to effectively harvest nutrients released by medusae so that the organisms form a closed semiautonomous micro-ecosystem where nutrients (at least nitrogen) are recycled internally (Pitt et al., 2009), as was supported by our stable isotope results. The nitrogen signature of the medusae was relatively high and independent of the abundance of internal picocyanobacteria. Because the concurrent free-living picocyanobacteria showed low nitrogen signatures indicative of nitrogen fixation (Vuorio et al., 2006) and thus a shortage of inorganic nitrogen in the environment, the internal picocyanobacteria most likely obtained their nitrogen from *L. tanganyicae* medusae.

The oxygen production of endosymbiotic algae may be so high that it exceeds the oxygen consumption of its host (Kremer et al., 1990). Factors affecting their oxygen production are similar to those of free-living algae (Muscatine & Marian, 1982). Contrary to our results showing high variation in *L. tanganyicae* (Fig. 13), in *Linuche uniquiculata* medusae (Kremer et al., 1990), oxygen production depended linearly on the size of the medusa, on light intensity, and on the behavior of medusae containing endosymbiotic algae (Muscatine & Marian, 1982). Although we found that *L. tanganyicae* medusae sometimes can be net producers, the high variation of the abundance of associated picocyanobacteria complicates understanding their role in medusa metabolism, as well as in the open water ecosystem. The observed picocyanobacteria abundance in the medusae may vary according to their nutritional status. When the status is poor, medusae may deplete the picocyanobacteria, while in the opposite case, picocyanobacteria flourish. Thus, both

feeding and life histories may affect the picocyanobacteria abundance. Variation in picocyanobacteria abundance was high even among large animals collected from the surface.

In Eil Malk Jellyfish Lake, *Mastigias* sp. medusae maximized their exposure to inorganic nutrients by swimming 15 m to the chemocline at night (Muscatine & Marian, 1982). It might be possible that such migration to the darker part of the water column also contributes to variation in the abundance of picocyanobacteria. Although the distance to the chemocline in Lake Tanganyika is roughly 100 m, *L. tanganyicae* may harvest nutrients there. The results of our nutrient addition experiment suggest that nutrients from digested prey are not always sufficient. If so, photosynthesis by picocyanobacteria may not be a steady source of food but photosynthesis by picocyanobacteria would extend the resources obtained from prey over a longer duration. As for algae in *Mastigias* sp. medusae (Muscatine et al., 1986), there is no direct evidence of digestion of picocyanobacteria by *L. tanganyicae* medusae. They may obtain photosynthetic products directly through the cell walls of the picocyanobacteria as in the coral-zooxanthellae relationship (Lesser et al., 2004; Woolridge, 2010).

The stable isotope results helped to clarify the trophic position of *L. tanganyicae* in the Lake Tanganyika food web. The isotope composition of an organism reflects its diet, with a stepwise enrichment in the heavier isotopes from one trophic level to the next. Enrichment in ^{13}C is small (0 –1‰), permitting identification of carbon sources (France & Peters, 1997), while the enrichment in ^{15}N is higher (2–4 ‰; empirical average 3.4‰), allowing estimation of the trophic position of consumers (McCutchan et al., 2003). In the pelagic food web of Lake Tanganyika, the ^{15}N enrichment per trophic step seems to be rather low, around 2‰ (Sarvala et al., 2003), as is common for ammonotelic freshwater organisms (Vanderklift & Ponsard, 2003). The similarity of carbon signatures and the mean difference of 1.8‰ in nitrogen signatures between the medusae and the copepodid and adult copepods are consistent with feeding on mixed copepods by the medusae, which was supported by the results of the mixing model application. The mixing model results

489 indicated that medusae also might feed on picocyanobacteria, but that feeding on small shrimps was
490 unlikely. Although feeding on big shrimps in combination with low-signature phytoplankton could
491 balance the isotope equilibrium, the big shrimps are too scarce in the lake (Bosma et al., 1998) to be
492 a realistic food source for the medusae. Thus, the mixing model can be used only to quantify the
493 contributions of known food items in the diet, but not used to identify the true diet components. In
494 general, food items with strong isotopic signatures in opposite directions appear important in the
495 calculated mixture whether or not they are actually eaten and may conceal the importance of items
496 with moderate signatures.

497 It was suggested that *L. tanganyicae* fed on fish eggs (Dumont, 1994b), but on the basis of
498 stable isotope signatures, it is unlikely that fish formed substantial portions of the diet. The nitrogen
499 signature of the medusae was only 1.3‰ higher than that of fish larvae and did not differ from the
500 signature in adult *Stolothrissa* (Fig. 14). The nitrogen signature of fish eggs is normally very close
501 to that of the female body; thus, medusae cannot have been feeding on fish eggs to any significant
502 extent. The mixing model did show that some isotopically-feasible diet combinations could include
503 fish larvae. During five lake-wide cruises in Lake Tanganyika in 1995-1998, abundances of fish
504 larvae and eggs were one or two magnitudes lower than medusae, shrimps, and copepods (Bosma et
505 al., 1998); therefore, ichthyoplankton probably would have been only occasional prey of the
506 medusae that could not be detected by the isotopic signatures.

507 Medusae can be powerful modifiers of the zooplankton community. In River Yamuna, India
508 *Limnocnida indica* medusae removed cladocerans (*Moina* sp.), as well as the rotifer *Keratella* sp.
509 from zooplankton; however, some rotifers (*Asplanchna* sp. and *Brachionus* sp.) and some copepods
510 were not affected (Sharma & Chakrabarti, 2000). *Craspedacusta sowerbii* medusae consume
511 zooplankton up to 2 mm (Dodson & Cooper, 1983; Jankowski et al., 2005; Smith & Alexander Jr.,
512 2008; Stefani et al., 2010). Thus, *L. tanganyicae* medusae also may exert strong influence on
513 zooplankton particularly during blooms in Lake Tanganyika. More information on the feeding of *L.*

514 *tanganyicae* on different components of zooplankton is needed to better understand its trophic role
515 in Lake Tanganyika.

516 *Limnocnida tanganyicae* medusae are a prominent component in the open water ecosystem
517 of Lake Tanganyika with many metabolic and behavioral adaptations. Primarily, the medusae are
518 likely predators of zooplankton and probably also can garden photosynthetic picocyanobacteria,
519 which enables their survival in the oligotrophic conditions of Lake Tanganyika. This study provides
520 novel insights into the ecology of freshwater medusae.

521

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698

699 **Figure Captions**

700 **Fig. 1** Correlation of dry and ash free dry mass of *Limnocnida tanganyicae* medusae with umbrella
701 diameter. Dotted line for dry mass of *Craspedacusta sowerbii* medusae (Jankowski, 2000) is shown
702 for comparison

703 **Fig. 2** Abundance of *Limnocnida tanganyicae* medusae in the southern basin of Lake Tanganyika
704 near the town Mpulungu, Zambia 2-4 September 1995. Numbers 1-9 represent sampling stations

705 **Fig. 3** Abundances (left side lines; day = grey line, night = black line) and mean umbrella diameters
706 (day = white bars; night = grey bars) with ranges (horizontal lines) of *Limnocnida tanganyicae*
707 medusae in the epi- and metalimnion of Lake Tanganyika off Kigoma Harbour during 7 April 1998.
708 The temperature vs. depth curve is shown at the right

709 **Fig. 4** Penetration of UV-B radiation into water of Lake Tanganyika (April 1998) and cumulative
710 UV-B radiation during the UV-exposure experiment (Fig. 5) on ship board in January 2001

711 **Fig. 5** Survival of >10-mm-diameter *Limnocnida tanganyicae* medusae collected off Kigoma
712 Harbor and kept in darkened, UV-film-covered, and uncovered (sunlight) quartz bottles in a water
713 bath on ship board in December 2001

714 **Fig. 6** A trichocyst battery (A), filamentous (B) and globular colonies (C) as well as
715 picocyanobacteria in a tentacle (D) of a *Limnocnida tanganyicae* medusa

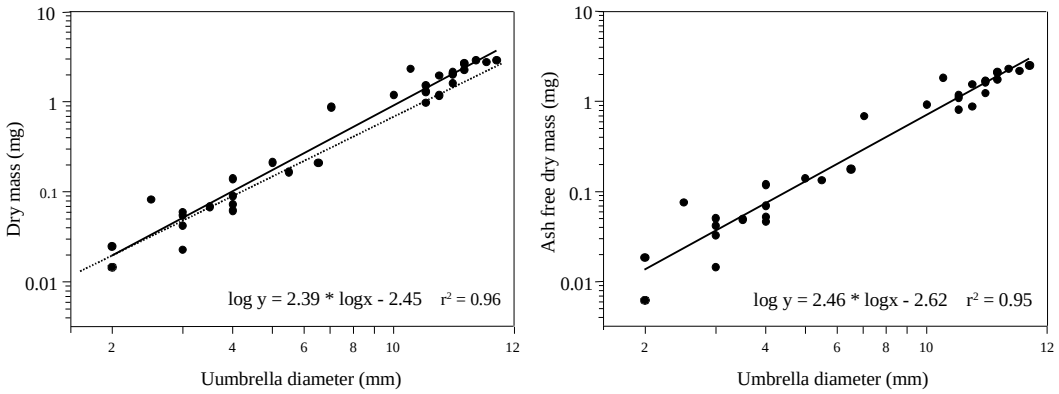
716 **Fig. 7** Diversity of bacteria in 11 *Limnocnida tanganyicae* medusae according to LH-PCR analysis
717 of the 16S rRNA gene. The biomarker size 470 bp was affiliated to the *Cyanobium* -type
718 picocyanobacteria

719 **Fig. 8** Depth distributions of bacteria in Lake Tanganyika water column according to LH-PCR
720 analysis of the 16S rRNA gene. The gray line shows that the biomarker 470 bp had its relative
721 maximum at 10 m depth

722 **Fig. 9** A neighbor-joining tree of the 16S rRNA gene sequence of the *Cyanobium* (represented by
723 clone TK-SE6) dominating in *Limnocnida tanganyicae* medusae, with reference sequences of non-

724 marine and marine picocyanobacteria. Terminal branches display strain code and place of isolation
725 (for Lake Biwa cluster), or the affiliated picocyanobacteria cluster (for strains of other non-marine
726 clusters), based on the clusters designated by Ernst et al. (2003) and Crosbie et al. (2003). Numbers
727 at nodes indicate the percent frequency (if >50%) obtained from the bootstrap analysis of 1273 nt
728 positions of the tree
729 **Fig. 10** Oxygen consumption or production by individual *Limnocnida tanganyicae* medusae kept
730 for two hours in light and dark bottles at ~27°C during November 1996 off Mpulungu, Zambia.
731 Medusa diameters ranged from 10 to 20 mm
732 **Fig. 11** Oxygen consumption or production by 7 *Limnocnida tanganyicae* medusae kept first in
733 darkness and then in light at ~27°C during November 1996. The bell diameters of medusae ranged
734 from 10 to 20 mm. Each individual is represented by a uniquely shaded bar
735 **Fig. 12** Oxygen consumption or production at ~27°C of 4 *Limnocnida tanganyicae* medusae kept
736 first in darkness, then in light, and finally with additional phosphorus and nitrogen during
737 November 1996. The bell diameter of medusae ranged from 10 to 20 mm. Different individuals are
738 represented by differently-shaded bars
739 **Fig. 13** Oxygen consumption or production of *Limnocnida tanganyicae* medusae in relation to their
740 size (umbrella diameter) in light (white boxes) and darkness (grey boxes) at ~28°C during March
741 1998. The box represents the middle interquartile range of 50% of the observed values. The
742 whiskers show the highest and lowest values, excluding outliers (black dots 1.5-3 box heights;
743 circles > 3 box heights from the edge of the box). Horizontal lines across the boxes indicate
744 medians. The numbers denote the number of animals in each size class
745 **Fig. 14** Mean stable isotopic composition (with standard deviations) of *Limnocnida tanganyicae*
746 medusae and other major groups of organisms in the plankton of Lake Tanganyika. Numbers of
747 replicate determinations are in parentheses
748

749 Fig. 1



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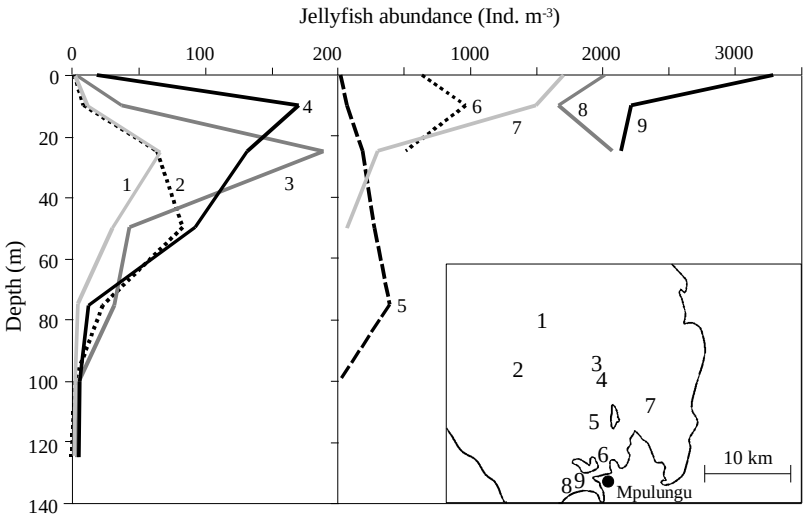
752 Fig. 2



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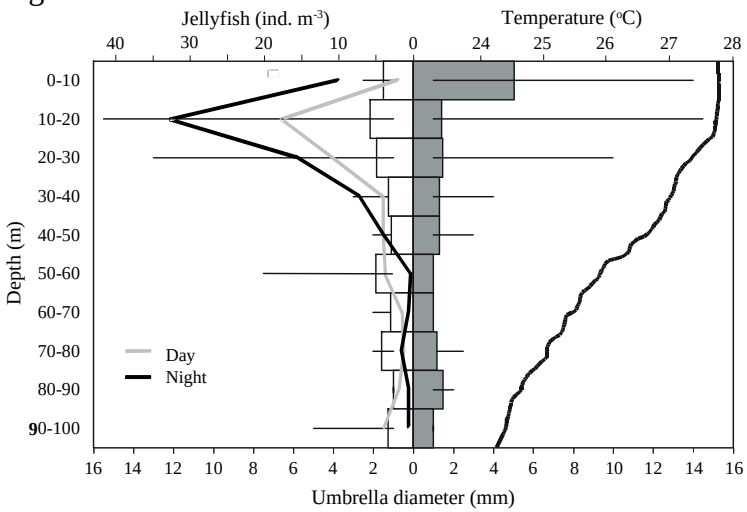
755 Fig. 3



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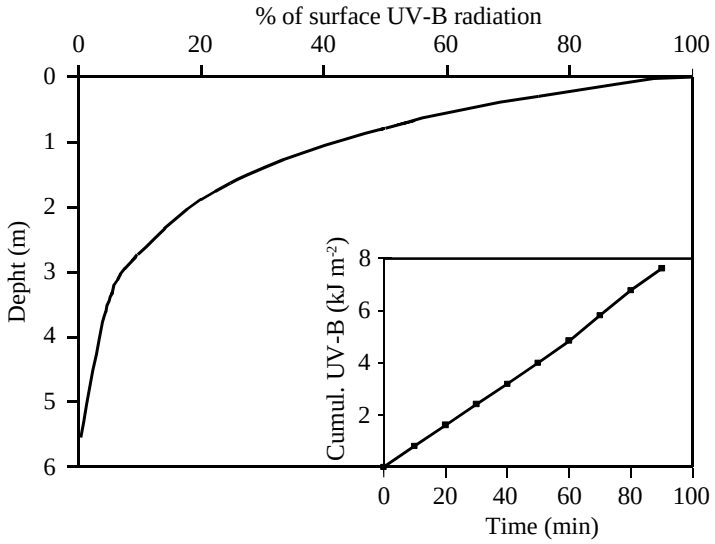
758 Fig. 4



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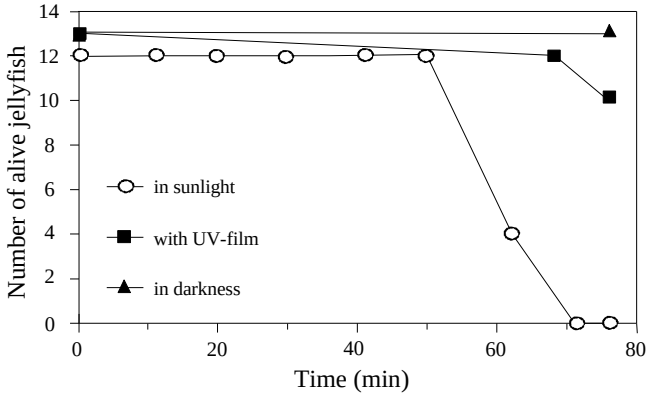
761 Fig. 5



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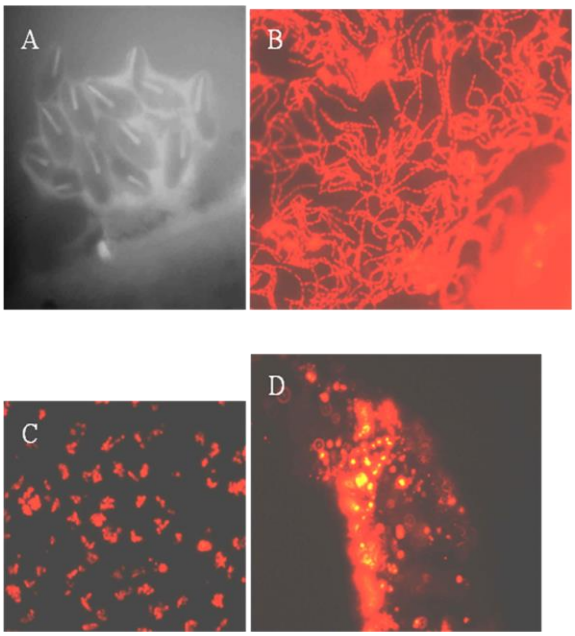
764 Fig. 6



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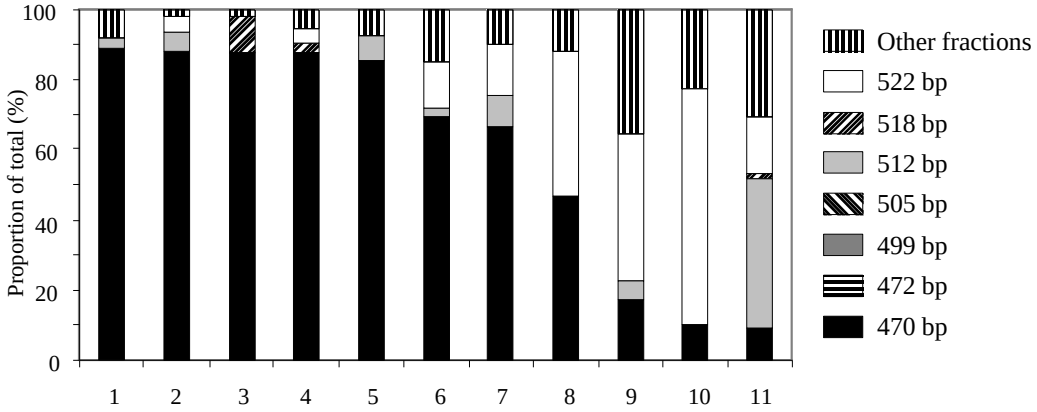
767 Fig. 7



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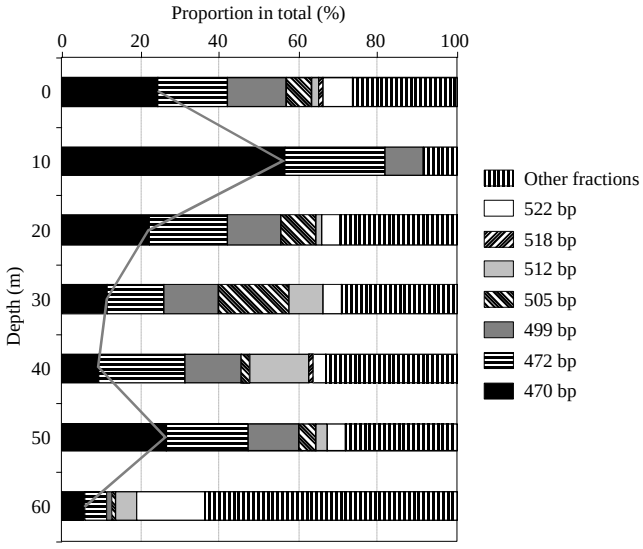
770 Fig. 8



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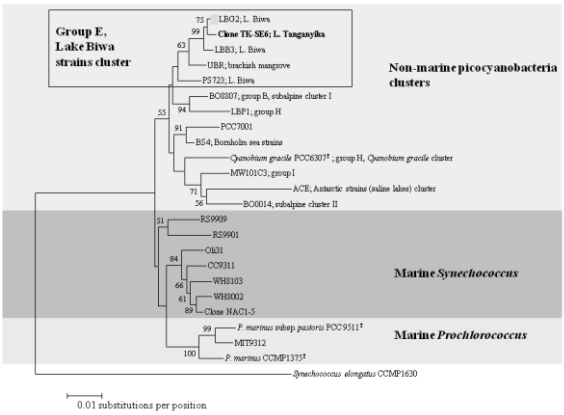
773 Fig. 9



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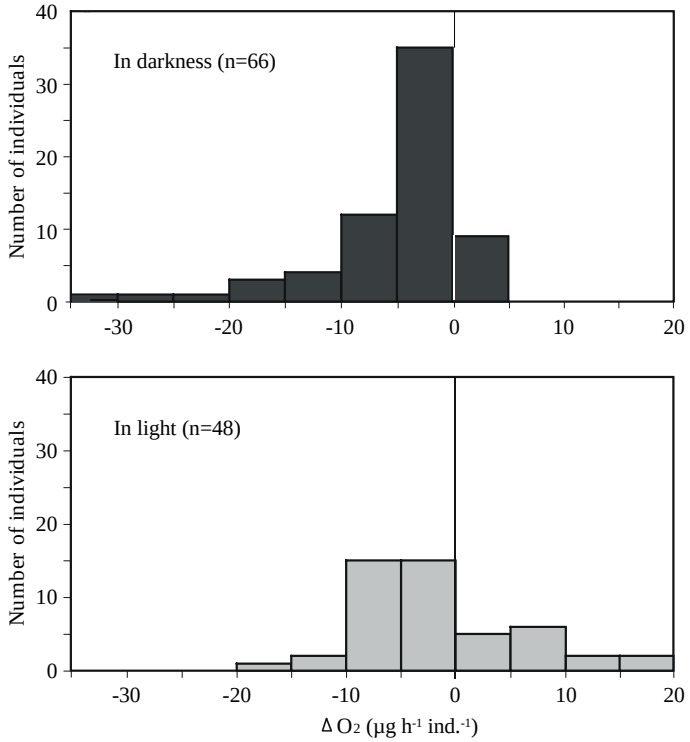
776 Fig. 10



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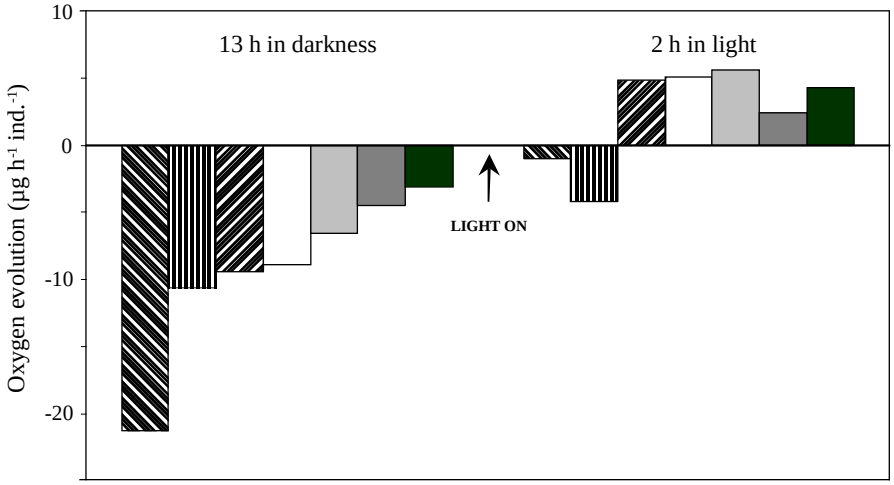

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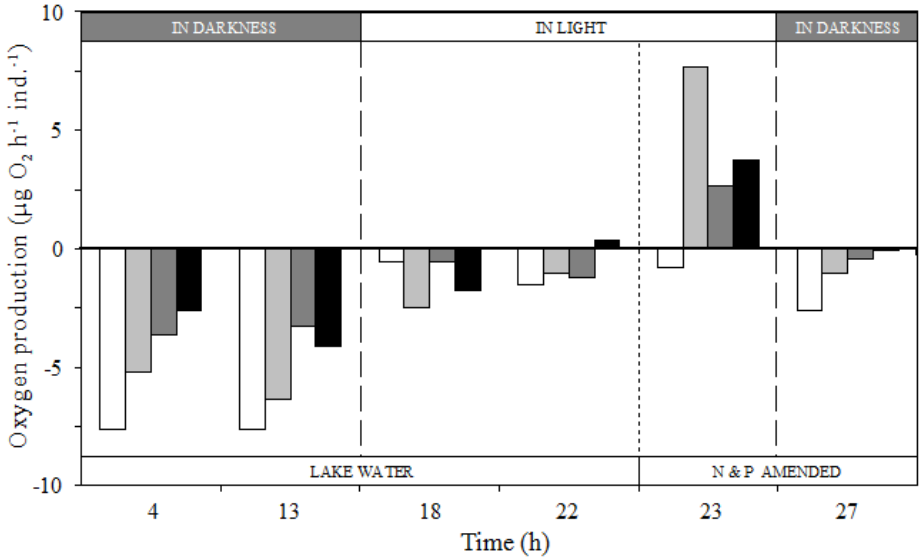
782 Fig. 12



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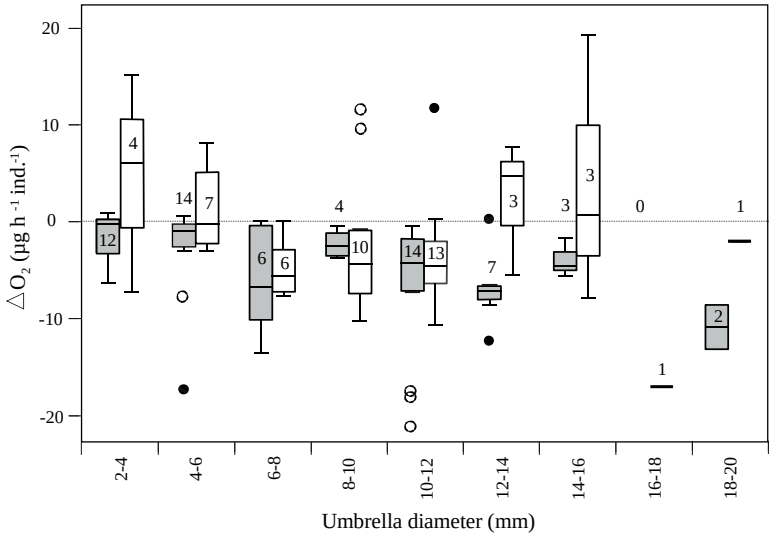
785 Fig. 13



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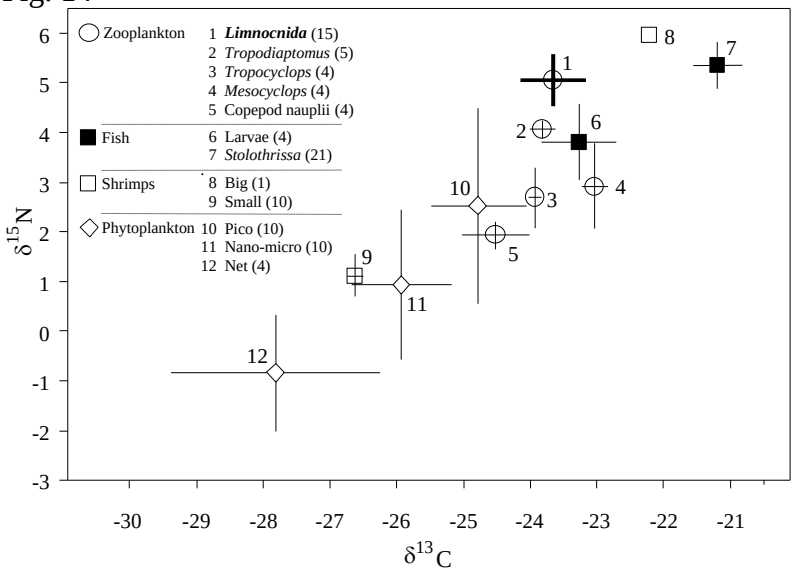
788 Fig. 14



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