**Did the alien calycophoran *Muggiaea atlantica* outcompete its native congeneric *M. kochi* in the marine lakes of Mljet Island (Croatia)?**

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**Abstract**

The calycophoran siphonophore *Muggiaea atlantica* was recorded for the first time in the marine lakes of Mljet Island (South Adriatic) in 2001. The presence of *M. atlantica* in such semi-enclosed environments provided a unique opportunity to describe its life cycle based on *in situ* observations of the annual variability of its asexual nectophore stage, sexual eudoxid stage (produces gonophores) and calyconula larvae. *Muggiaea atlantica* was present the whole year in the Malo jezero (Small Lake, SL), where it was actively reproducing between May and September 2001, with a maximum density of gonophores in May (141 gonophores m⁻³) and nectophores and larvae in June (37 nectophores m⁻³ and 80 larvae m⁻³). Higher temperatures below the thermocline (average 14.5–15.6 °C), and the availability of prey (e.g. small copepods, chaetognaths and bivalve larvae) were the main environmental factors related to successful reproduction of *M. atlantica* in SL. In the Veliko jezero (Great Lake, GL) nectophores and gonophores of *M. atlantica* were found in low numbers, mainly in the upper 20 m, between September and November 2001 and no larvae were recorded. In 2001, *Muggiaea kochi*, the sole calycophoran siphonophore previously reported in the GL, was not found in the Mljet lakes. We hypothesize that the cold (<13 °C) conditions over winter of 2000/2001 were not suitable for the warm-temperate *M. kochi* and may have favoured its cold-temperate congener, *M. atlantica*, which has progressively colonized the southern Adriatic since the mid-1990s.

**Keywords**

Adriatic Sea; life cycle; marine lakes; *Muggiaea atlantica*; Zooplankton.

**Introduction**

Prior to the 1980s, *Muggiaea atlantica*, a representative of the Atlantic neritic fauna, was found most frequently in the westernmost part of the Mediterranean (Ianora & Scotto di Carlo 1981; Gamulin & Kršinić 1993). Expansion of its range to the Northwestern Mediterranean, with increased abundance, was observed from the mid-1980s (Gili *et al.* 1988; Licandro & Ibanez 2000). *Muggiaea atlantica* was first recorded in the Adriatic Sea in 1995 (Batistić 1999; Gamulin & Kršinić 2000), a year in which the advection of West Mediterranean/Atlantic water into the Adriatic Sea was related to the anticyclonic circulation in the Ionian Sea (Civitarese *et al.* 2010). A shift in dominance among Adriatic calycophorans was noted from 1996, with *Muggiaea kochi* being replaced by *M. atlantica* (Kršinić & Njire 2001; Batistić *et al.* 2007). The geographical expansion of *M. atlantica* and decrease of *M. kochi* was triggered by thermohaline circulation changes that occurred in the Mediterranean and Adriatic Sea in the last two decades, probably under the forcing of large-scale climate oscillations (Licandro & Ibanez 2000; Batistić *et al.* 2007; Licandro *et al.* 2012).

The present study is based on data collected in the two marine lakes on the island of Mljet between December 2000 and December 2001. The colonization of this
semi-enclosed marine environment by *M. atlantica* provided a good opportunity to investigate its life cycle by following the annual variability of the asexual and sexual stage and calyconula larvae. Such data are quite scarce and would be impossible to derive should *M. atlantica* co-occur with the congeneric *M. kochi*, due to morphologically indistinguishable eudoxids and larvae. Another aim of this study was to identify the main abiotic and biotic factors that influence development of *M. atlantica* in the lakes, to allow a better understanding of its recent establishment in the Adriatic and Mediterranean Sea, particularly in relation to hydroclimatic changes. A final goal was to assess the impact of this novel species on the zooplankton community of the lakes, particularly the impact on native calycophoran fauna.

**Study area**

The Island of Mljet, situated in the South Adriatic off Dubrovnik, is directly exposed to the incoming Ionian Sea current (Zore-Armanda et al. 1991). Two seawater lakes, Veliko jezero (Great Lake, GL) and Malo jezero (Small Lake, SL), are situated in the western part of the island (Fig. 1). Both lakes have been part of a national park for more than 40 years, thus representing an excellent study site without direct human impact. The lakes are naturally formed karstic depressions that were filled with seawater during the holocene ingression of the Adriatic Sea, about 5000–7000 years ago (Schmidt 1993). The Great Lake has a surface area of 1.45 km² and maximum depth of 46 m, and the Small Lake has a surface area of 0.25 km² and maximum depth of 29 m. Tidal currents are the main factor driving water exchange between the open sea and the lakes. This exchange is limited owing to the small tidal range and the shallow depth of the straits connecting the Soline Channel to GL (10 m width, 2.5 m depth) and GL to SL (3 m width, 0.6 m depth) (Fig. 1). The lakes are as much as 2–3 °C colder than the open sea during winter and as much as 4 °C warmer during summer, with surface temperatures as high as 28 °C and a strong thermocline (up to 4 °C m⁻¹) at 10–20 m depth (Vilibić et al. 2010). Compared with an earlier investigation that found salinity restricted to values of 35–37 (Buljan & Špan 1976), salinities greater than 37 have been found since the 1990s (Benović et al. 2000; Malej et al. 2007).

Phytoplankton abundance and structure are similar to those in the neighboring open Adriatic (Jasprica et al. 1995). The maximum microphytoplankton abundance in GL (4.0 × 10⁵ cells L⁻¹) and SL (5.9 × 10⁴ cells L⁻¹) was in summer, whereas the maximum nanophytoplankton abundance in GL (4.5 × 10⁶ cells L⁻¹) and SL (2.2 × 10⁶ cells L⁻¹) was in winter and summer, respectively (Jasprica et al. 1995).

**Material and Methods**

Monthly sampling was carried out from December 2000 to December 2001 in both Veliko jezero (GL) and Malo jezero (SL) (Fig. 1).

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**Fig. 1.** Map of the marine lakes Veliko jezero (GL) and Malo jezero (SL) on Mljet Island. Black points indicate sampling sites.
Zooplankton samples were collected using a Nansen net (40-cm diameter) fitted with a 125-μm mesh. One vertical haul was done from the bottom to the surface in SL (0–25 m) due to the relatively shallow depth; two separate layers (0–20 m and 20 m to the bottom) were sampled in GL. Samples were preserved in a buffered 2.5% formalin-seawater solution. Hydrographic parameters and chlorophyll a (Chl a) concentration were measured in water samples taken with a 5-l Niskin bottle every 5 m from the surface to the bottom. Salinity was determined using the standard titration method (Grasshoff 1983), temperature with an inverted thermometer, and dissolved oxygen using the Winkler method. Oxygen saturation (O2/O′2) was calculated from the solubility of oxygen in seawater as a function of temperature and salinity (Weiss 1970; UNESCO 1973). Chl a was determined from 0.5-l sub-samples filtered through Whatman GF/F glass-fiber filters and stored at −20°C. Filtered samples were homogenized and extracted in 90% acetone for 24 h at room temperature (Strickland & Parsons 1972). Chl a was then measured fluorometrically with a Turner TD-700 Laboratory Fluorometer calibrated with pure Chl a (Sigma-Aldrich, Taukirchen, Germany).

Taxonomic identification and counting of net zooplankton were performed with a Zeiss stereomicroscope at 25× and 40×. The life cycle of the diphyid siphonophore *M. atlantica* was investigated by following the annual variability of the asexual and sexual stages, and the calyconula larvae. Nectophore count was used to estimate the asexual stage abundance. Gonophores, released by sexual stage eudoxids, were counted to estimate the reproductive activity. Eudoxids live autonomously, themselves budding several gonophores. The abundances of the different stages (Fig. 2) were counted in the whole sample and then converted into numbers per m³. These stages were determined according to Moser (1925), Russell (1938) and Bouillon et al. (2004). Hydromedusae, pteropoda, bivalvia larvae, cladocera, ostracoda, copepoda (divided into calanoid, plocilostomatoid, cyclopoid and harpacticoid), chaetognatha, and appendicularia were also counted as a fraction of the samples varying between 1/4 and 1/32 of the original sample volume. The abundance of those groups was then converted to number of specimens per cubic meter (ind. m⁻³).

Principal component analysis (PCA) was carried out on a matrix composed of 13 monthly abundances of nectophores and gonophores of *M. atlantica* for GL and SL. The interpretation of the first (PC1) and second (PC2) principal components, which together accounted for 91% of the total variance, involved seven environmental descriptors (water temperature, salinity, oxygen and chlorophyll a concentration integrated from the surface to bottom depth; temperature above and below the thermocline; mixed layer depth) and 11 biological descriptors (the plankton groups specified in the preceding paragraph). Only those descriptors that showed the greatest correlation to the PC1 and PC2 (i.e. Pearson correlation >0.5) are presented here.

![Fig. 2. Muggiaea atlantica: calyconula larvae (a), nectophores (b), eudoxids (c).](image-url)
Results

Hydrography

The temperature in Veliko jezero (GL) ranged from 11.1 °C near the bottom in December 2000 to 26.4 °C at the surface in August 2001 (Fig. 3). In Malo jezero (SL) temperatures ranged from the 9.1 °C near the bottom in December 2001 to 28.3 °C at the surface in July 2001 (Fig. 3). Temperature was evenly distributed throughout the water column in winter in both lakes (Fig. 3, Table 1). Inverse stratification occurred only in February in SL in the upper 5 m layer, owing to faster cooling at the surface (see also Buljan & Špan 1976). A thermocline was well established between June and September, with a maximum temperature difference of 1.4 °C m⁻¹ in August (SL) and September (GL). In June, July and August the thermocline in GL was at 15–20 m and in SL at 10–15 m. In both lakes the thermocline was deeper in September (Table 1). The temperature below the thermocline was generally 2 °C lower in GL than in SL (Table 1).

Salinity varied between 32.99 and 38.82 (yearly average = 38.00 ± 1.05) in GL, with a minimum in December at 20 m and a maximum in May at 45 m (Fig. 3). High salinity values throughout the water column were noted from December 2000 to March 2001, whereas salinity decreased slightly below 20 m from June to December 2001. Low salinity throughout the water column in GL (average 34.77) in December 2001 is consistent with data from previous years and could be caused by the flow of cold freshwater from the karstic spring 'Fontana' (Buljan & Špan 1976). Salinity in SL (Fig. 3) varied between 36.75 and 38.82 (yearly average = 38.31 ± 0.47). A pronounced halocline was found from January to May 2001, generally between 5 and 10 m, with the maximum gradient $\Delta S = 0.35$ m⁻¹ in March.

Oxygen saturation varied between 0.16 and 1.29 (0.94 ± 0.23 on average) in GL, and between 0.17 and 1.32 (0.93 ± 0.24 on average) in SL (Fig. 3). Surface
waters were generally well saturated throughout the year. Hypoxia occurred in near-bottom waters from September to November in both lakes.

**Net zooplankton**

Total net zooplankton abundance in Malo jezero (SL) was highest during the summer, with a maximum density in June and August of 14 072 and 14 118 ind. m\(^{-3}\), respectively. The lowest density of 1429 ind. m\(^{-3}\) was recorded in October. Copepods dominated, contributing 34–93% of total zooplankton abundance. Cyclopoids, with *Oithona nana* as the dominant species, contributed most to total copepod abundance (34–92%, Fig. 4). Calanoids, dominated by *Paracalanus parvus*, made up 10–68% of total copepods. Pocilopomatoids and harpacticoids made up less than 4 and 6%, respectively, of total copepod abundance (Fig. 4). Zooplankton other than copepods (ZOC), mainly bivalve larvae (>80% of the total ZOC), were abundant in July and August, with densities of 7767 and 7955 ind. m\(^{-3}\), respectively (Fig. 4). Appendicularians, chaetognaths and pteropods contributed 5–30% of total ZOC. *Muggiaea atlantica* made up less than 5% of total ZOC.

Maximum zooplankton abundance in Veliko jezero (GL) was recorded in April in the 0–20 m depth layer (12 215 ind. m\(^{-3}\)) and in June at 20–40 m depth (11 617 ind. m\(^{-3}\)). Copepods were the dominant group, contributing between 20 and 96% of total zooplankton abundance (Fig. 4). Cyclopoids and calanoids made up >90% of the total copepod catch (Fig. 4). The cyclopoid *O. nana* and the calanoid *P. parvus* were the dominant species in both the 0–20 m and 20–40 m layers. Pocilopomatoids and harpacticoids contributed less than 10% to total copepod abundance (Fig. 4). In GL, the maximum density of zooplankton other than copepods occurred in April in the 0–20 m layer (2269 ind. m\(^{-3}\)), and in September in the 20–40 m layer (3971 ind. m\(^{-3}\)). Pteropods and bivalve larvae contributed 69 and 98%, respectively, to total ZOC during the April and September maxima.

**Calycophoran Muggiaea atlantica**

*Muggiaea atlantica* occurred in Malo jezero (SL) throughout 2001, with higher abundances between May and August. Gonophores reached their greatest density (141 per m\(^3\)) in May, followed by a peak of nectophores (37 per m\(^3\)) in June (Fig. 5). Calycnula larvae were present in SL from May to September, with a maximum density (80 per m\(^3\)) in June (Fig. 5).

In contrast to SL, in Veliko jezero (GL) *M. atlantica* occurred only between September and November 2001. Nectophores were mainly concentrated in the upper

| Table 1. Temperature in Veliko jezero great lake (GL) and Malo jezero small lake (SL): surface, averages above and below the thermocline and average of the whole water column from December 2000 to December 2001. |
| Surface (°C) | 15.5 | 14.5 | 12.2 | 13.6 | 14.0 | 15.3 | 15.5 | 10.6 | 20.6 | 23.0 | 22.5 | 24.3 | 26.2 | 28.3 | 23.6 | 27.6 | 22.1 | 22.7 | 20.8 | 20.7 | 17.2 | 16.7 | 11.5 | 9.1 |
| AVG above TC (°C) | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– |
| AVG below TC (°C) | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– | ––––––– |
| WC AVG (°C) | 14.2 | 14.6 | 12.3 | 11.6 | 12.1 | 11.8 | 12.7 | 13.1 | 13.8 | 14.3 | 15.7 | 16.2 | 17.0 | 19.4 | 19.9 | 19.1 | 17.5 | 17.3 | 19.4 | 19.6 | 16.7 | 11.5 | 9.1 | 9.1 | 9.1 | 9.1 | 9.1 |

AVG, average; TC, thermocline; WC, water column.
Fig. 4. Total copepod abundance (ind. m$^{-3}$) with the relative contribution of each group (%) and total abundance of other zooplankton, ZOC (ind. m$^{-3}$) in Malo jezero small lake (SL) and Veliko jezero great lake (GL) from December 2000 to December 2001.
20 m while gonophores were distributed across the whole water column (Fig. 5). A maximum of 0.64 nectophores and 10.24 gonophores per m³ were recorded in October 2001 between 0 and 20 m depth. No calyconula larvae were found in GL.

Temperature and *M. atlantica* abundance co-varied in both GL and SL (PCA analyses, Fig. 6). In SL, high densities of *M. atlantica* nectophores and gonophores were recorded when the temperature below the thermocline and the oxygen concentration were relatively high and when copepods (in particular calanoids and cyclopoids) and bivalves were abundant (Fig. 6). In GL, *M. atlantica* was negatively correlated with chlorophyll a, O₂ concentration and with poecilostomatoid copepods (Fig. 6).

**Discussion**

Since 1995 *Muggiaea atlantica* has progressively established itself in the Southern Adriatic Sea, becoming an abundant calycophoran in coastal waters (Batistić 1999; Krsinić & Njire 2001; Lučić et al. 2005; Batistić et al. 2007). *Muggiaea atlantica* was found for the first time in the marine lakes of Mljet in 2001, at high abundance throughout the year in Malo jezero (SL). *Muggiaea kochi* was previously reported as the sole calycophoran in GL (Vučetić 1957, 1966, 1995; Lučić & Bender-Pojatina 1995) but during 2001 it was not found in the Mljet lakes.

The temporal sequence of occurrence of *M. atlantica* nectophores, gonophores and calyconula larvae suggests
that this species underwent a complete life cycle in SL, actively reproducing from May to September when the water column was well stratified. The PCA analysis indicated that high abundance of *M. atlantica* in SL was mainly related to higher temperatures, in particular values below the thermocline (average 14.5–15.6 °C). It seems that high temperatures above the thermocline (>24 °C) in SL during summer depress the presence of *M. atlantica* in this layer. Marques et al. (2008) found a steep decline in the abundance of *M. atlantica* in waters warmer than 24 °C. Records of *M. atlantica* nectophores and gonophores during the winter of 2001 indicate that this species was able to successfully generate sexual eudoxids in SL at temperatures <13 °C. However, calyconulae larvae did not appear before May. This suggests that gonophores matured more slowly at lower winter temperatures than in spring–summer. Higher temperatures in the spring–summer period trigger the fast release of eudoxids and maturation of gonophores, and as a consequence there is a rapid growth in abundance of the population of *M. atlantica*.

The PCA analysis also indicated that high abundance of *M. atlantica* in SL was related to high abundance of planktonic prey. According to Purcell (1982) the number of eudoxids produced by *M. atlantica* and their growth rate tend to increase with the density of prey, mainly nauplii and the juvenile stages of small copepods. The positive correlation between *M. atlantica* density in SL and the abundance of the small copepods, in particular the cyclopoid *Oithona nana* and the calanoid *Paracalanus parvus*, seems to support this hypothesis. *Muggiaea atlantica*, known to be a voracious predator, needs an average of 2000 copepods per day to sustain a population of 100 colonies per m³ (Purcell 1982). The abundance of copepods (cyclopoids and calanoids) decreased in May, which is in accordance with high densities of *Muggiaea atlantica* nectophores and gonophores.

*Muggiaea atlantica* nectophores and gonophores were found in low numbers in GL, mainly in the upper 20 m, between September and November 2001. There were no larvae. This suggests that *M. atlantica* was not efficiently reproducing in GL and that its presence there likely was the result of transport through the shallow (2.5 m) and narrow Soline Channel. This transport is limited by this area’s small tidal range, but the inflow from the open sea tends to increase during the warmer seasons (Buljan & Špan 1976). In addition, an upwelling recorded at the open sea near the Soline Channel at the end of the summer (Buljan & Špan 1976) brings species that usually occur below the thermocline to the surface; these species may then be advected into GL. Different factors could cause the low productivity of *M. atlantica* in GL. For example, temperatures below the thermocline in GL during the period of stratification were always lower than in SL (on average 2 °C). This is consistent with previous investigations that reported differences of up to 6 °C between the two lakes (Buljan & Špan 1976; Jasprica et al. 1995; Benović et al. 2000). Temperatures below the thermocline in GL, therefore, may have been too low to allow an efficient growth of the population of *M. atlantica*.
during the warmer seasons. High temperatures above the thermocline in GL also may have been limiting. The swarms of the scyphomedusa *Aurelia* sp. regularly reported in GL suggest that *M. atlantica* was exposed to higher predation pressure than in SL, where *Aurelia* was only found occasionally (Benović et al. 2000; Malej et al. 2007; Turk et al. 2008; Alvarez Colombo et al. 2009).

According to Rutherford & Thuesen (2005), *M. atlantica* is an oxyconformer and shows good tolerance to hypoxic conditions. When it was most abundant in SL, the water column was well aerated both above and below the thermocline. Hypoxic conditions were observed only near the bottom, from September to November in both lakes.

It therefore seems reasonable to conclude that, of the two marine lakes, Malo jezero (SL) presented a more suitable habitat for *M. atlantica*, mainly because of favorable temperatures below the thermocline, more abundant prey and possibly lower predation pressure.

Previous investigations reported no calycophorans in SL and *M. kochi* as the only calycophoran in GL (Vučetić 1957, 1966; Lučić & Bender-Pojatina 1995). *Muggiaeae kochi* typically was present throughout the year in GL, with higher numbers in August and September (Vučetić 1961, 1966). This species, which is a neritic warm-water species with a preference for relatively high temperatures (Alvarino 1974), was concentrated mainly in the upper 20 m (above the thermocline) in summer (Vučetić 1961). A complete life cycle of *M. kochi*, including release of eudoxids by the pylargastic colony, has been described for temperatures between 18 and 24 °C, while at a temperature of 13 °C only the pylargastic phase (nectophores) was observed (Carré & Carré 1991). We therefore hypothetize that the winter temperatures below 13 °C recorded in GL during 2000/2001 limited the occurrence of *M. kochi* there. Some nectophores of *M. kochi* still may have survived below 13 °C, but without production of eudoxids it would have been impossible to start a new generation the following spring. Therefore, the autochthonous *M. kochi* might not have been competitively displaced by the allochthonous *M. atlantica*. This is also supported by the preference of *M. atlantica* for SL, contrary to *M. kochi*. Further, in general, during the warmest period, *M. kochi* (Vučetić 1961) and *M. atlantica* seek different temperature niches (i.e. above and below the thermocline, respectively). According to the above hypothesis, the occurrence of *M. kochi* in GL depends on constant repopulation from the open sea.

Based on earlier reports, these two congeneric species rarely occur in the same ecosystem (Russell 1934; Alvarino 1974; Mackie et al. 1987). After 1980, however, they did co-exist in the Ligurian Sea (Licandro & Ibanez 2000), and after 1995 in the Adriatic Sea (Batistić et al. 2007). Suitable hydrographic conditions and the stronger inflow of less saline, colder Atlantic water (Civitarese et al. 2010) likely enabled the imigration of *M. atlantica* from the Western Mediterranean to the Adriatic Sea. During 1996, *M. kochi*, the most numerous Adriatic calycophore (Gamulin & Kršinić 1993), decreased in abundance, whereas *M. atlantica* constituted more than 30% of total calycophoran nectophores during winter and late summer (Batistić et al. 2007). It thus seems that the occurrence of these two congeners is driven by hydroclimatic changes, in particular the temperature regime, as temperature is the key determinant of their reproductive success. In view of ongoing climatic changes, it seems paradoxical that cold-temperate species such as *M. atlantica* would expand their geographical range within an already subtropical sea such as the Mediterranean. The arrival of *M. atlantica* into the Adriatic was caused by circulation shifts, which in turn depend on larger scale processes. This is a likely scenario for future colonisations in which alien species arrive due to climate-induced disruption of circulation barriers.

Overall, the results of this study demonstrate that *M. atlantica* has the potential to increase in abundance very rapidly when environmental conditions are favourable. It has, indeed, adapted to the shallow closed ecosystem of the Mljet lakes more successfully than its congener *M. kochi* has.

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**Conflicts of Interest**

None of the authors have any potential conflicts of interest.

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