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Author(s): Ekaterini Chalkia, George Kehayias & Evangelia Doulka
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SPATIAL AND TEMPORAL VARIATION OF THE FRESHWATER MUSSEL *DREISSENA BLANCI* (WESTERLUND, 1890) LARVAE IN GREEK LAKES

Ekaterini Chalkia*, George Kehayias & Evangelia Doulka

Department of Environmental and Natural Resources Management, University of Ioannina, Seferi 2, 30100, Agrinio, Greece

ABSTRACT

Recent molecular studies on dreissenid species in southern Europe found that *Dreissena blanci* (Westerlund, 1890) resides in several lakes where previous studies identified it as *Dreissena polymorpha* (Pallas, 1771). Because *D. blanci* seems to constitute a “new record” in the Balkans, the present study provides novel information on the biology and ecology of its planktonic larvae in four natural lakes and one reservoir located in western Greece. *Dreissena blanci* larvae were present in all lakes and their abundance varied between 3.4 and 440 ind. l^{-1}. The larvae were present all year round, having greater abundance in spring and summer and lesser in winter. The species seems to have an extended reproductive period from early spring until late summer. The larvae are distributed mainly in the upper 20 m of the deep lakes Amvrakia and Trichonis, while are aggregated close to or within the thermocline layer in the latter lake. A size-specific depth distribution was observed, with larger larvae residing in deeper strata during the stratification period. Temperature appears to be the most important parameter affecting the abundance variation of the larvae in the natural lakes, while water retention time is the major parameter in Stratos reservoir. In contrast, the soft, muddy substratum of Lake Lysimachia seems to be the inhibiting factor for the existence of a viable population here. The great similarities in larval ecology between *D. blanci* and *D. polymorpha* must be taken into consideration, as *D. blanci* could become another invasive species in freshwater ecosystems. Therefore, more research is required on the life cycle of this species, and the ecological and economic consequences of its presence in lakes and reservoirs of southeastern Europe.

Key words: *Dreissena blanci,* larvae, vertical, seasonal variation, Greek lakes.

INTRODUCTION

Recent molecular studies by Albrecht et al. (2007, 2009) and Wilke et al. (2010) on the biogeographical affinities and possible origins of the dreissenid mussels of the ancient Balkan lakes revealed two distinct sister species of *Dreissena* in the Balkans, *Dreissena presbensis* (Kobelt, 1915) and *Dreissena blanci* (Westerlund, 1890). Wilke et al. (2010) found *D. blanci* to be the only species occurring in the two large lakes of western Greece (Trichonis, Amvrakia) and also in the connecting reservoirs of Acheloos River, Kremasta and Stratos. *Dreissena blanci* was originally described from the area of the ancient Lake Trichonis, leading Wilke et al. (2010) to assume that this lake is probably its ancestral region. From this area, *D. blanci* presumably extended north, and today it occurs together with *D. presbensis* in Lakes Prespa, Mikri Prespa and Pamvotis (Albrecht et al., 2007; Wilke et al., 2010).

Dreissena polymorpha was recorded in the Balkan lakes (Kinzelbach, 1992), while several other studies have also reported its presence in Greek rivers, natural lakes and reservoirs (Zarfdjian et al., 1990; Koussouris et al., 1994; Michaloudi et al., 1997; Kehayias et al., 2004; Kehayias et al., 2008; Doulka & Kehayias, 2008, 2011). Considering that *D. blanci* and *D. presbensis* are the only dreissenids present in several Balkan lakes, previous reports of *D. polymorpha* must be reconsidered. *Dreissena blanci* thus appears to constitute a “new record” for the Balkans, for which only limited information exists on its biology, ecology, and geographical distribution in the south Balkans.

Considering that *D. blanci* is closely related to the important invasive species, the zebra mussel *D. polymorpha*, and there are no previous

*Corresponding author: ahalkia@cc.uoi.gr

reports on its larval stages, the present paper adds new data on the spatial and temporal variation of its planktonic larvae and their interrelation to environmental parameters in its presumed area of origin. The investigation of elements of this taxon's larval ecology compared to those of its relative *D. polymorpha* is expected to provide interesting aspects on the ecological significance of *D. blanci* in freshwater ecosystems, regarding also the possibility that it could become another invasive species.

METHODS

Study Area

The present study was conducted in four natural lakes and one reservoir of western Greece (Fig. 1). Among these aquatic ecosystems, Lake Trichonis is the largest natural lake in Greece, having a surface area of 98.6 km^2, a maximum depth of 57 m and a catchment area of 421 km^2 (Zacharias et al., 2002); previous

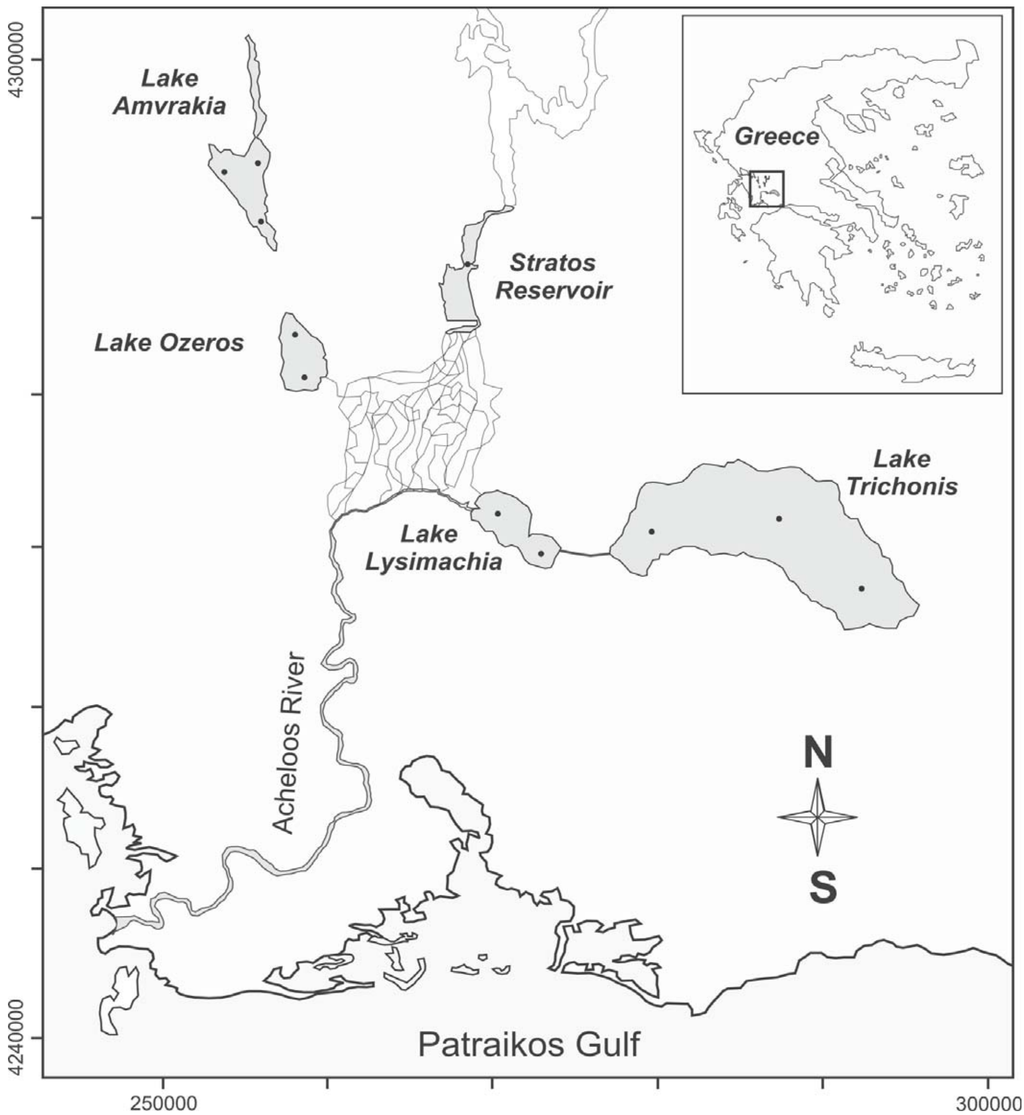


FIG. 1. Geographical location of the five lakes studied. Dots within each lake indicate the sampling sites.

TABLE 1. Sampling data for the zooplankton surveys conducted in the five water bodies.

Sampling area	Frequency/ Duration	Stations/ Depths	Net diameter/ porosity	Vertical hauls
Trichonis Lake	Monthly/24 months Sept. 2003–Aug. 2005	Three/ 48, 35, 25 m	40 cm/ 50 μm	at 10 m intervals
Amvrakia Lake	Monthly/24 months Sept. 2006–Aug. 2008	Three/ 45, 25, 23 m	20 cm/ 50 μm	at 5 m intervals
Lysimachia Lake	Monthly/12 months June 2009–May 2010	Two/ 8.1, 6.8 m	20 cm/ 50 μm	bottom to surface
Ozeros Lake	Monthly/12 months June 2009–May 2010	Two/ 5.6, 5.0 m	20 cm/ 50 μm	bottom to surface
Stratos Reservoir	Biweekly/24 months Sept. 2004–Aug. 2006	One/ 8 m	40 cm/ 50 μm	bottom to surface

studies have classified it as oligotrophic to mesotrophic (Skoulikidis et al., 1998; Doulka & Kehayias, 2008). Lake Amvrakia is a deep mesotrophic lake (maximum depth: 50 m, catchment area: 112 km^2), which belongs to the sulphate type (Overbeck et al., 1982). The lake has strong water level fluctuations due to high evaporation rates, especially during the summer, and irrigation of the surrounding agricultural area. These variations usually result in the drainage of the shallower northern part of the basin (Fig. 1) in certain periods/years and, consequently, in the fluctuation of the total surface area of the lake, which ranges between 14 and 22 km^2. Both Trichonis and Amvrakia are warm monomictic lakes, exhibiting a long period of thermal stratification from May to October.

Lakes Lysimachia and Ozeros are smaller and shallower (maximum depth 8.1 and 5.6 m, respectively), with a surface area of 13.5 and 10.1 km^2. Lake Lysimachia has positive water balance due to inflow of water from the neighbouring Trichonis Lake, to which it is connected by a 6.5 km channel. There is also an outflow of water from the lake to the Acheloos River via a channel in its western area. Until 2000, the lake received the untreated urban wastewaters of the nearby city of Agrinio, with a population of about 80,000, and thus became eutrophic. Lake Ozeros is supplied with water from torrents that occur mainly to its east-southeast, and also through a channel from the Acheloos River, when it floods. Due to their ecological importance, all the above lakes are considered as special protected areas and have been listed as NATURA 2000 Greek sites. Their major environmental disturbance is the receiving of agricultural drainage and wastes from nearby farms and villages. Finally, Stratos reservoir is the last of four reservoirs along Acheloos River and is situated between two dams, Kastraki (upstream) and Stratos (downstream). It has a surface area of 11 km^2, a maximum depth of 15.5 m and the water volume fluctuates between 60 and 70.2 X 10^6 m^3, resulting in a mean depth of between 5.5 and 6.4 m.

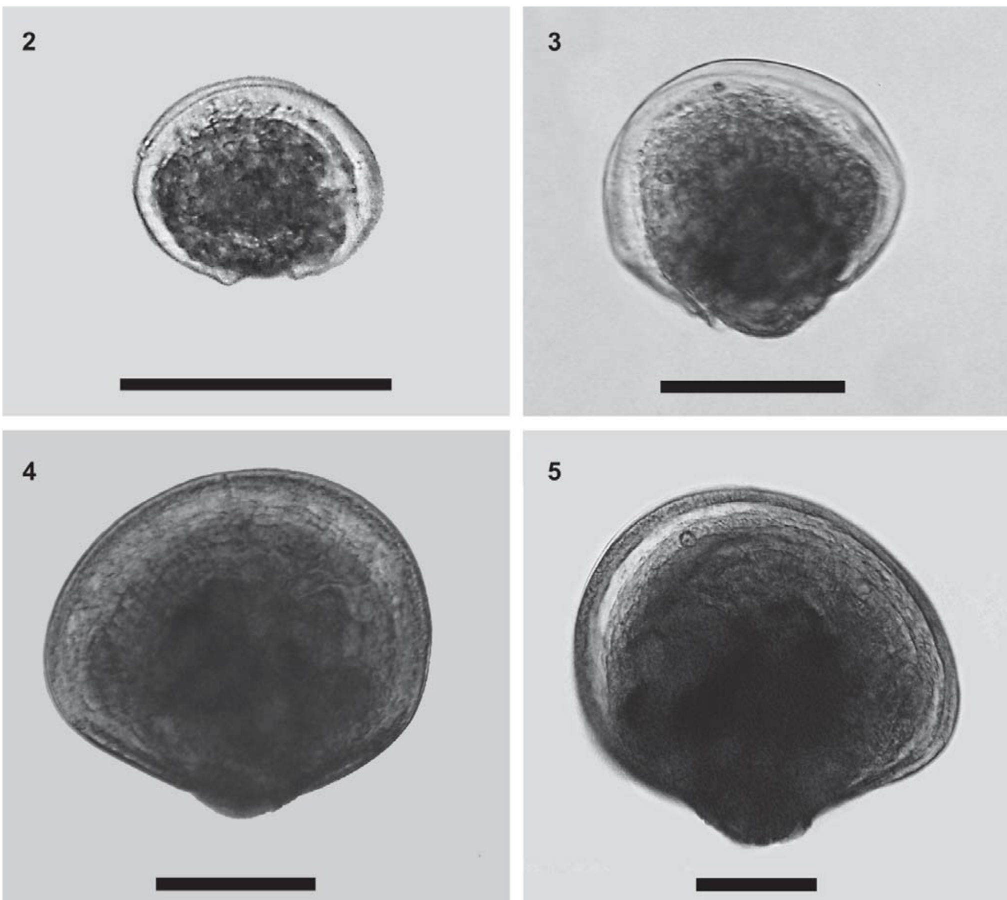
Zooplankton Surveys

Zooplankton sampling in the five lakes was conducted in various surveys of 12 and 24 months duration in different years (Table 1). The sampling was carried out using plankton nets of different dimensions but with the same porosity (50 μm), with vertical hauls from bottom to surface in the shallower lakes. Closing nets were used in the deep lakes Trichonis and Amvrakia to conduct sampling at depth intervals of 5 to 10 m (Table 1). In Stratos reservoir, the sampling was conducted from a bridge crossing the reservoir (Fig. 1). In all cases, the nets were towed at a speed of approximately 0.5 m sec^{-1} and after collection all samples were preserved in 4% neutralized formalin solution, in a final volume of 100 ml.

During each zooplankton survey vertical profiles of temperature, oxygen concentration, pH and conductivity were taken from the surface down to 40 m, using portable WTW instruments in Lake Trichonis and Stratos reservoir, and a Troll 9500 (In-Situ Inc.) water quality multi-parameter instrument in lakes Amvrakia, Lysimachia and Ozeros. Water transparency was measured in all cases with a Secchi disc. To estimate total phosphorus (TP), phosphates

(PO_4), nitrates (NO_3), nitrites (NO_2), ammonia (NH_4) and silicates (SiO_2), water samples were collected at the deepest station from various depth intervals with a 5 L water sampler (Hydro-Bios free flow water sampler). Analyses of all chemical parameters were performed in the laboratory according to A.P.H.A et al., (1998). To determine chlorophyll-a concentration (chl-a), 1500 ml of the water samples taken from the above depths were filtered through a Whatman GF/A CatNo 1820-047 (1.6 μm) glass fiber filter shortly after collection. Pigment extraction was made in 90% acetone and concentrations were determined spectrophotometrically using a BOECO S-20 spectrophotometer (A.P.H.A. et al., 1998).

For the abundance analysis of zooplankton, three counts of 1.5 ml subsamples from each sample were made on a Sedwick-Rafter cell having a total volume of 100 ml (Doulka & Kehayias, 2008). Length measurements were taken from all specimens of *Dreissena blanci* larvae in each sample using an optical micrometer fitted in a microscope. Four size classes (S1, S2, S3 and S4) were established for specimens having a body length less than 100 μm (S1), for specimens of 100–200 μm (S2), of 200–300 μm (S3) and for specimens longer than 300 μm (S4). Photographs of specimens of each of the four size classes are presented in Figures 2–5. Larvae smaller than 100 μm are probably at the stage of entering the plank-



FIGS. 2–5. Photographs of *D. blanci* specimens. Scale bars = 100 μm. FIG. 2: Size class S1 (< 100 μm); FIG. 3: Size class S2 (100–200 μm); FIG. 4: Size class S3 (200–300 μm); FIG. 5: Size class S4 (> 300 μm).

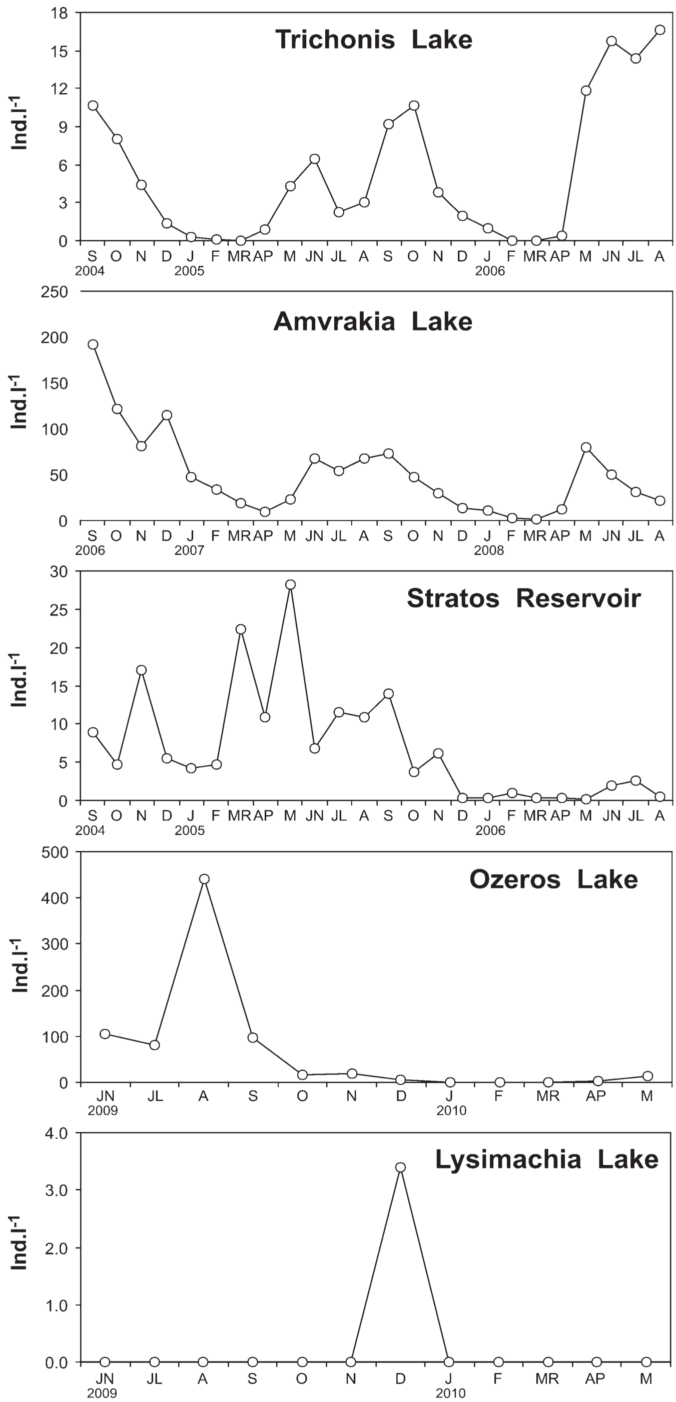


FIG. 6. Seasonal variation of the abundance (ind. l^{-1}) of *D. blanci* larvae in the five lakes studied. (In lakes Trichonis and Amvrakia the values correspond to the average abundance in the 0–20 m depth layer).

totrophic period, as reported by Sprung (1993) for *Dreissena polymorpha*. Similarly, larvae of S2 are considered in a pre-settling stage, while S3 and S4 larvae are at the settling stage.

To obtain comparable data of the vertical distributions of the *D. blanci* larvae, the weighted mean depth (WMD) was calculated for each species as follows:

$$WMD = \frac{\sum (NTi \times Ti)}{\sum NTi}$$

where WMD = weighted mean depth, NT*i* = abundance in depth *i*, and T_i = depth (m). Although the weighted mean depth cannot represent the actual vertical distribution of a species, it is a good numerical base for the application of statistics. Thus, differences between the WMDs of *D. blanci* larvae were tested using the non-parametric Kruskal-Wallis and Mann-Whitney (U) tests. The same tests were also applied to compare abundance and size distribution results. Multiple regression analysis was used to clarify the influence of the environmental factors on *D. blanci* larvae for each of the zooplankton surveys. The analysis was performed using forward stepwise selection to identify the significant factors. These factors were then correlated with each other (Pearson's *r*) to eliminate those having strong correlation. Finally, the explanatory factors were standardized so as to compare their relative importance. All data analyses were performed using the SPSS 17.0 (SPSS, Inc. 2008) statistical package.

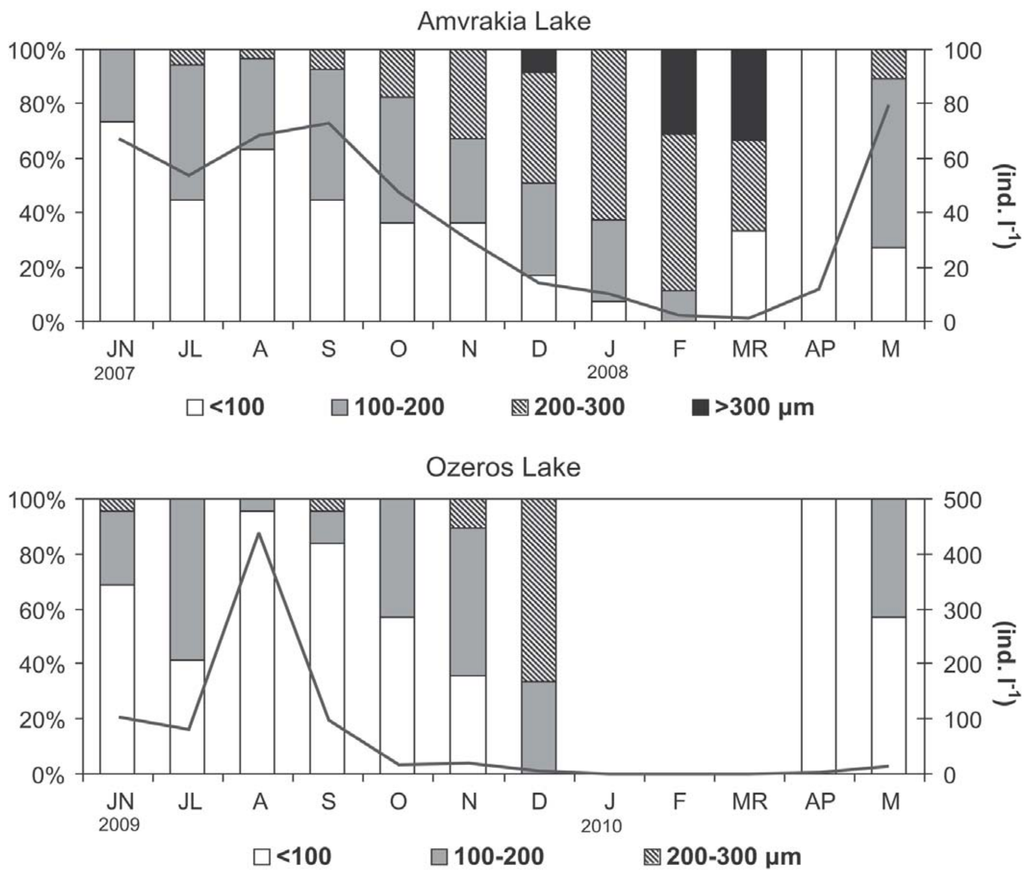


FIG. 7. Size distribution of *D. blanci* larvae in lakes Amvrakia and Ozeros expressed as percentages (%) of the four size classes S1 (< 100 μm), S2 (100–200 μm), S3 (200–300 μm) and S4 (> 300 μm) in the total population. Solid lines indicate the seasonal variation of the total abundance (ind. l^{-1}) of the larvae in each lake.

RESULTS

Geographic Distribution and Seasonal Variation

Dreissena blanci larvae were present in all the sampling sites, although there were considerable differences in their abundance between the five lakes. The highest abundance was recorded in Lake Ozeros (439.9 ind. l^{-1} in August 2009) followed by Lake Amvrakia (194.2 ind. l^{-1} in the 0–20 m layer in September 2006). In contrast, the lowest value was recorded in Lake Lysimachia, where a few specimens were found in only one occasion (December 2009). Concerning seasonal variation, *D. blanci* larvae had an almost continuous presence in the deep lakes Amvrakia and Trichonis and Stratos reservoir, while they were absent from Ozeros Lake samples from winter to early spring (Fig. 6). Generally, higher abundances were recorded during the warmer months of the year. The abundance variation pattern was similar for the deep lakes Amvrakia and Trichonis, where a decrease of abundance was recorded during autumn and winter, while greater abundances were found in spring and summer. Similarly, the highest abundance in Lake Ozeros was recorded in late summer, while in Stratos reservoir the highest abundance was recorded in May 2005 and the lowest in March 2006 (18.0 and 0.28 ind l^{-1}, respectively).

Differences in the abundance of the larvae between the two sampling years were recorded in Amvrakia Lake and Stratos reservoir. In Stratos, the larvae were significantly more abundant during the first sampling period (September 2004 to August 2005), than the second (September 2005 to August 2006) when their abundance was nearly four times lower (U-test, $p = 0.0001$). Similarly, in Amvrakia Lake, the larvae were more abundant from September 2006 to August 2007 than September 2007 to August 2008 (U-test, $p = 0.004$). In contrast, no such difference was detected in Lake Trichonis (U-test, $p = 0.773$).

Length measurements of the larvae found in Lake Amvrakia showed that the smallest S1 larvae were present almost all year round, and only in February 2008 were they absent from the samples (Fig. 7). S2 larvae were also present in most months except March and April 2008, while S3 were not present in the population in spring and early summer (Fig. 7). Finally, the larger S4 class larvae were found only from winter 2007 to early spring of 2008. The percentage contribution of S1 to the total larvae abundance was greater in spring while the larger specimens of S3 and S4 had greater contribution in winter. The findings were similar in Lake Ozeros, except that no *D. blanci* larvae were found from January to March 2010. Moreover, the maximum length of the larvae in Lake Ozeros was never greater than 300 μm, while in Lake Amvrakia there were several occasions of specimens having lengths greater than 300 μm (reaching up to 350 μm).

Vertical Distribution

The vertical distribution of *D. blanci* larvae was studied along with the spatial distribution in each of the two deeper lakes. In both lakes, greater abundance values of *D. blanci* larvae were recorded in the upper 20 m (Figs. 8, 9) and diminished with depth, especially during the stratification period (May to October). However, the vertical distributions of *D. blanci* larvae differed significantly between the two lakes considering their mean depths (U-test, $p = 0.0001$), with a deeper distribution in Lake Amvrakia. During the stratification period in Amvrakia, the larvae aggregated close to or within the thermocline layer, where chlorophyll-a and oxygen concentrations were higher, while their vertical distribution during the overturn (November to April) was rather uniform (Fig. 9). This pattern was not so obvious in Lake Trichonis, where the larvae mainly seemed to follow the pattern of temperature variation with depth (Fig. 8). It is notable that, despite the general decrease of abundance with increasing depth in Lake Amvrakia, specimens of *D. blanci* larvae were also present in the hypoxic (and sometimes anoxic) deepest layers.

A size-specific depth distribution was noticed in Lake Amvrakia (Figs. 10, 11), with larger larvae residing in deeper strata during the stratification period (Kruskal-Wallis test, $p = 0.0001$). Indeed, the larval size increased with depth until the lower limit of the thermocline (25 m), while below this depth there was no significant difference in larval size (Kruskal-Wallis test, $p = 0.102$). In contrast, during the overturn there was no difference in the size of the larvae under 5 m (Kruskal-Wallis test, $p = 0.273$), whereas the smaller larvae resided in the upper 0–5 m depth (Figs. 10, 11).

Effects of Abiotic Elements

Multiple regression analysis revealed that of the environmental parameters, temperature was the most important influence on the distribution of *D. blanci* larvae in lakes Amvrakia,

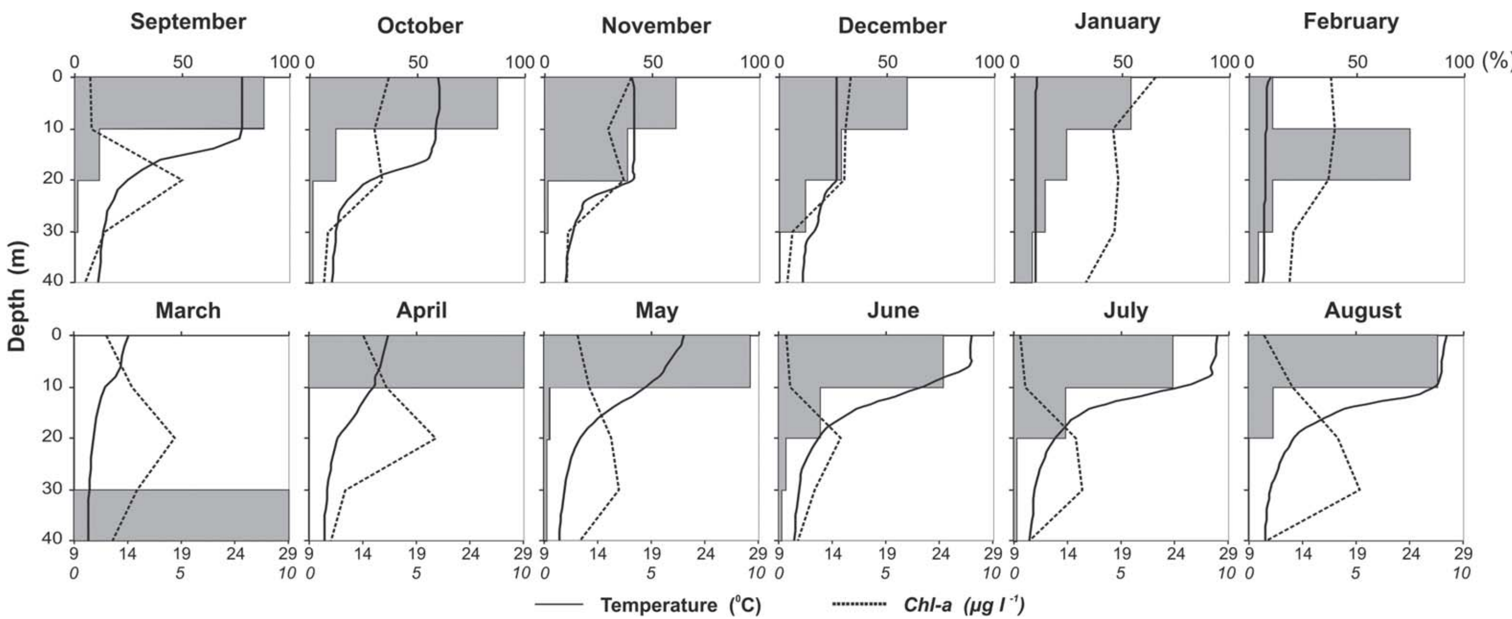


FIG. 8. Vertical profiles of the average temperature (°C) and chlorophyll-a (μg l^{-1}) in Lake Trichonis. The shaded area represents the vertical distribution of *D. blanci* larvae as the average percentage (%) of the total caught during the two-year sampling period. Scales on the x-axis represent temperature (normal) and chlorophyll-a (italics).

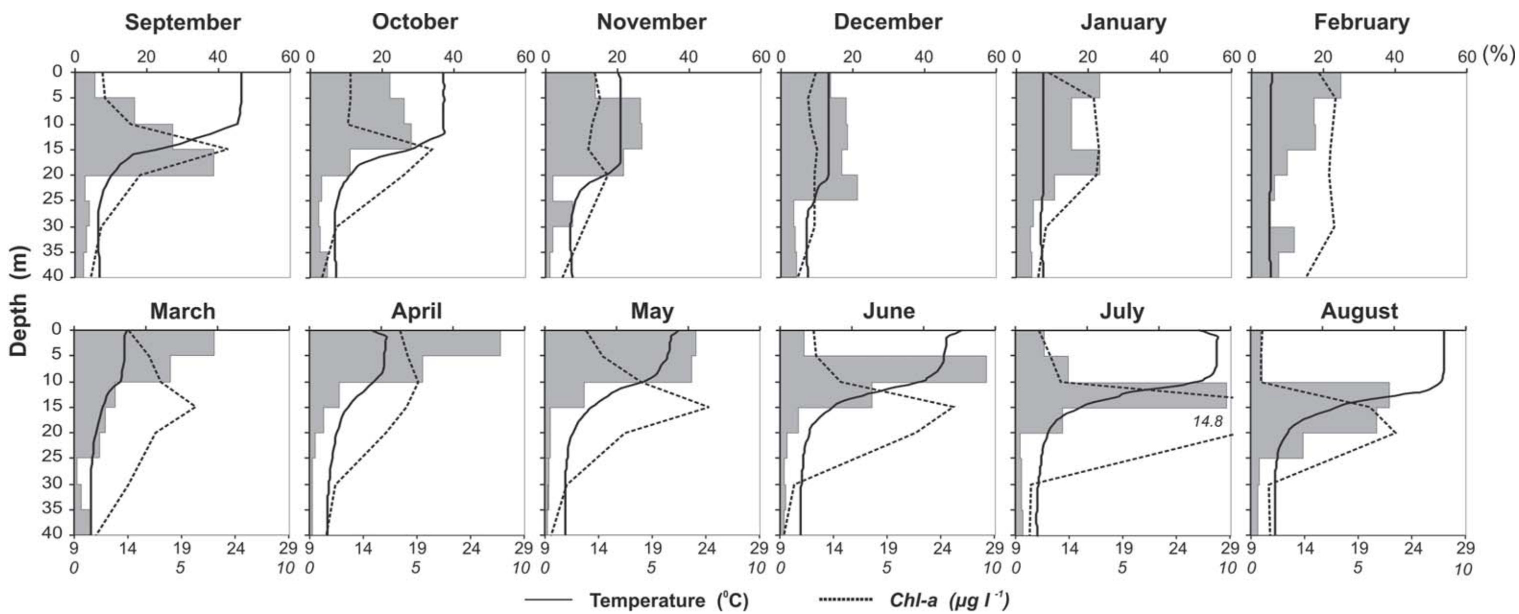


FIG. 9. Vertical profiles of the average temperature (°C) and chlorophyll-a (μg l^{-1}) in Lake Amvrakia. The shaded area represents the vertical distribution of *D. blanci* larvae as the average percentage (%) of the total caught during the two-year sampling period. Scales on the x-axis represent temperature (normal) and chlorophyll-a (italics).

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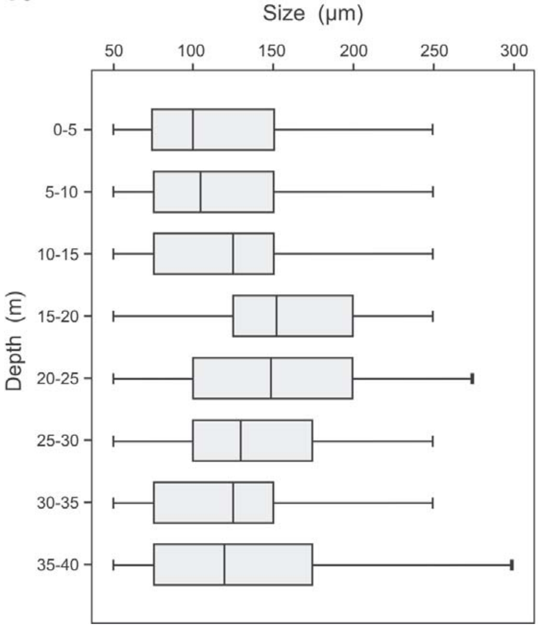

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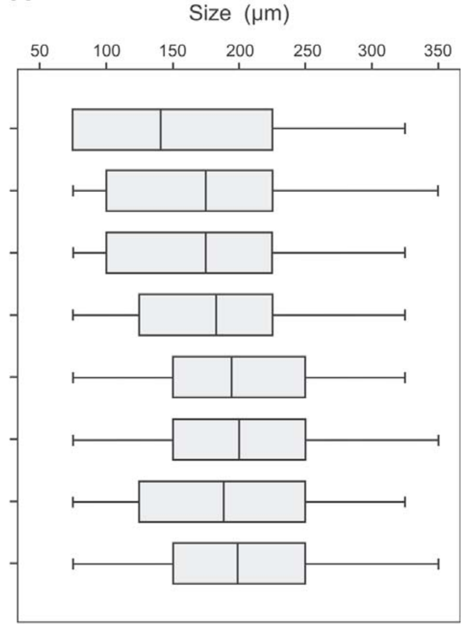

FIGS. 10, 11. Size variation of *D. blanci* larvae in the depth intervals sampled. Each boxplot shows the interquartile range of size distribution (box), median (vertical line within the box), and the size range (horizontal lines outside bars). FIG. 10: Size variation during the stratification period (May to October); FIG. 11: Size variation during the turnover period (November to April).

Trichonis and Ozeros. In Stratos reservoir, their distribution was mainly influenced by the water retention time (Table 2). Moreover, the abundance of *D. blanci* larvae was enhanced by conductivity and chlorophyll-a, while transparency seems also to be a significant factor influencing their abundance and distribution. Finally, a significant correlation was found between the larvae abundance variation and water level (WL) in Lake Amvrakia, and with pH in Lake Ozeros.

DISCUSSION

Geographic Distribution

Previous studies on the zooplankton of Lake Trichonis and Stratos reservoir reported the existence of *Dreissena polymorpha* larvae in both freshwater ecosystems (Kehayias et al., 2004; Kehayias et al., 2008; Doulka & Kehayias, 2008, 2011). However, after the recent study of Wilke et al. (2010), who reported the ab-

TABLE 2. Results of the multiple regression analysis between environmental factors like temperature (Temp.), pH, conductivity (Cond.), chlorophyll-a (Chl-a), transparency (Trans.), water retention time (WRT), water level (WR), and the larvae of *Dreissena blanci* in the studied lakes; ** $p < 0.01$.

Lakes	Temp.	pH	Cond.	Chl-a	Trans.	WRT	WL	r^2	df
Amvrakia	0.633**			0.261**	0.245**	-	0.483**	0.565	143
Trichonis	0.989**					-	-	0.762	51
Ozeros	1.004**	-0.404**				-	-	0.906	11
Stratos						0.707**	-	0.499	15
Total area	0.507**		0.581**	0.139**	-0.200**	-	-	0.550	224

sence of *D. polymorpha* from the area and the presence of *Dreissena blanci* using molecular techniques, the previous findings of the above studies must be reconsidered. According to the present results, *D. blanci* was found in all the five lakes studied, including lakes Ozeros and Lysimachia, for which there were no records in the earlier studies of Albrecht et al. (2007) and Wilke et al. (2010). Consequently, the present investigation supplements the data provided by Albrecht et al. (2007) and Wilke et al. (2010) relative to the geographical distribution of *Dreissena* spp. in the Balkans.

Dreissena blanci is resident in all the lakes of western Greece, including the reservoirs of Acheloos River (Kastraki and Stratos). Apart from Lake Amvrakia, all the other natural lakes (Trichonis, Lysimachia and Ozeros) are connected by natural or man-made channels to the Acheloos River. Its presence in the "isolated" Amvrakia Lake is difficult to explain, unless an accidental transfer of this species from another lake of the area has occurred, e.g. adult mussels attached to boat hulls (Carlton, 1993; Johnson & Carlton, 1996). Another possible explanation for this geographical distribution comes from the geological history of the area. At the end of Pliocene, all the natural lakes of this area (Trichonis, Amvrakia, Lysimachia and Ozeros) formed one large lake (the "Aetoloakarnanian Basin") that had been created by the Acheloos River outflow. With the gradual lowering of the water level, individual basins became isolated, creating separate lakes (Verginis & Leontaris, 1978). Thus, it may be that *D. blanci* was not transported passively or actively out of Lake Trichonis, but remained as relict populations in these newly isolated lakes.

Although *D. blanci* exists in all sampling sites, there were certain differences in the abundance of its larvae between the five lakes studied. Although these differences might have been due to annual fluctuations, as the samplings were conducted in different years, there could be also other explanations. Surprisingly, the larvae were nearly absent from the zooplankton in Lake Lysimachia, where only a few specimens were found in just a single sampling occasion. Since Lake Lysimachia is connected to and receives water from Lake Trichonis, where a population of *D. blanci* is well established, one would expect a continuous presence of this species in this lake too. Moreover, there are great similarities in the physicochemical characteristics of the water between these two lakes. However, the substantial difference of Lake Lysimachia, not only with Lake Trichonis but also with the other lakes studied, is its muddy bottom substrate, which is in contrast to the rocky structure of the bottom of the other lakes. Lewandowski (1982b) found that mud and sand are unsuitable substrata for the settlement of the larvae of the relative species *D. polymorpha*. Similarly, it is assumed that the muddy bottom of Lake Lysimachia could be the restricting factor for the existence of *D. blanci* larvae in this area. In addition, several samplings conducted in the canal connecting Lysimachia and Trichonis lakes always revealed specimens of *D. blanci* larvae, meaning that there was a constant "inflow" of larvae from Trichonis. Consequently, it is suggested that most, or probably all, the larvae that enter Lysimachia from Trichonis fail to settle in the former because due to the unsuitable substratum, they are not able to form a viable population. In fact, it is possible that the few larvae found in Lake Lysimachia on only one occasion, could have been specimens just recently transferred from Trichonis Lake. On the other hand, differences in the abundance of *D. blanci* larvae between the two deep lakes Trichonis and Amvrakia could be due to their trophic status, as Trichonis is considered oligo- to mesotrophic (Doulka & Kehayias, 2008), whereas Amvrakia is mesotrophic (Overbeck et al., 1982; Chalkia et al., 2012). Thus, the higher abundances of *D. blanci* larvae in Lake Amvrakia may have been due to the higher presence of food here.

Seasonal Variation

The seasonal variation of *D. blanci* larvae was characterized by their presence in the zooplankton for most of, or for the entire year. This is also noticed for *D. polymorpha* according to Lewandowski (1982a), who stated that the duration of the planktonic phase of its larvae differs among lakes and, generally, increases from north to south in the Balkan lakes as a result of temperature. However, there have been some cases where over-wintering larvae of *D. polymorpha* have been found in the plankton even at temperatures as low as 7.2°C (Lucy, 2006). This could also explain the presence of *D. blanci* larvae in Stratos reservoir during February 2005 and 2006, when temperatures were lower than 9°C. Increasing water temperature during spring has been widely reported as the primer environmental trigger for the reproduction of *D. polymorpha* when surface temperatures rise above 12°C (Sprung, 1989; Borcherding, 1991), or above 15°C (Einsle, 1973; Stańczykowska, 1977; Karatayev et al., 1998). Similarly, the first appearance of the smallest (S1) larvae of *D. blanci* after the winter period was in March (Lake Amvrakia), or April (Lake Ozeros), when the surface temperature reached 13.5°C and 19°C, respectively. Moreover, the influence of temperature on the seasonal variation of *D. blanci* larvae was reflected by the characteristic increase of abundance during the warmer period of the year, starting in spring, while there was a decrease in autumn and winter. This is in agreement with reports on the seasonal abundance variation of *D. polymorpha* from other lakes of Europe (Borcherding, 1991; Sprung, 1993), the northern Balkans (Stanković, 1960; Lewandowski, 1982a) and northern Greece (Michaloudi et al.,1997; Zarfdjian et al.,1990).

Considering the seasonal size distribution of *D. blanci* larvae with their abundance variation in lakes Amvrakia and Ozeros, it is suggested that the reproductive period of this species starts in spring and continues until late autumn. Based on reports on the life cycle of the related *D. polymorpha*, we assumed that larvae smaller than 100 μm (S1) were probably at the stage of entering the planktotrophic period (Sprung, 1993). These larvae must have been the offspring of an earlier reproduction and, consequently, their presence in almost all sampling months indicates the existence of several reproductive incidents, or continuous reproduction. S2 larvae (100–200 μm in length) could correspond to those in a pre-settling stage, while S3 larvae (200–300 μm) are probably at the settling stage. The maximum length of the larvae in Lake Ozeros was lower compared to Lake Amvrakia, where several specimens reached lengths up to 350 μm. According to Sprung (1993), larger larvae may be the result of unsuitable substrata, causing the larvae to delay their metamorphosis. Conditions with negative impacts on settling success of *D. polymorpha* larvae include unfavorable oxygen concentrations in the hypolimnion and unsuitable bottom structures (Stańczykowska, 1977), while the depth at which the larvae generally settle is usually not greater than 10 m (Sprung, 1993). The oxygen content in Ozeros Lake was sufficient during the entire sampling period, while there were no hypoxic or anoxic conditions within the water column in any case. In contrast, hypoxic (dissolved oxygen < 2 mg l^{-1}) or even anoxic (dissolved oxygen < 0.2 mg l^{-1}) conditions were recorded in Lake Amvrakia in the hypolimnetic layer and especially under 30 m during the stratification period. In addition, the greater depth of the sampling sites of Lake Amvrakia (over 20 m) could have been responsible for a lower settling success and consequently to the occurrence of larger larvae in the zooplankton samples.

Vertical Distribution

Investigation of the vertical distribution of *D. blanci* larvae in the two deeper lakes of western Greece (Trichonis and Amvrakia) showed that most of the specimens inhabited the epilimnetic layer. This general trend was especially recorded during the stratification period (May–October), in contrast to their nearly uniform distribution during the overturn period (November–April). Similarly, studies on the vertical distribution of the relative species *D. polymorpha*, reported maximum abundance within the epilimnion during the stratification period, and a uniform vertical dispersion along the water column during the unstratified period (Sprung, 1993; Lewandowski & Eijsmont-Karabin, 1983).

Although there were certain similarities in the vertical distribution patterns of the larvae between the lakes Trichonis and Amvrakia, differences were also noticed resulting in a general trend of deeper distribution in Amvrakia. On several occasions, mainly during the stratification period, the larvae in Lake Amvrakia were aggregated to the metalimnion where maximum chlorophyll-a was observed. Indeed, this trend was verified by the significant correlation between chlorophyll-a and larval abundance in the vertical axis. As chlorophyll-a was used to estimate primary production in Amvrakia Lake, there seems to be an interrelation of the larvae to the phytoplankton concentration, which, in turn, could suggest a kind of dependence on specific food elements. The verification of this hypothesis would require larvae diet analysis, as well as data on the abundance, distribution and composition of phytoplankton in Lake Amvrakia.

Thermal stratification in both lakes Trichonis and Amvrakia played a crucial role on the vertical distribution of the larvae. It has been well documented that stratification results in the existence of optimum epilimnetic environmental factors (temperature, pH and dissolved oxygen) for *D. polymorpha* compared to those prevailing in the hypolimnion (Sprung, 1987; Hincks & Mackie, 1997; Karatayev et al., 1998; Gelda et al., 2001). In Lake Trichonis there was little variation in pH values with increasing depth, with an average value of 8.11. In Lake Amvrakia, however, the pH value for the entire water column was lower than Lake Trichonis (7.62) and there was a sharp decrease in the hypolimnion (7.31), especially during stratification (7.23). In addition, a strong positive correlation (Pearson's $r = 0.40$, $p < 0.001$) was found between pH values and larval abundance in the vertical axis. Consequently, this parameter could have significant influence on the vertical distribution of *D. blanci* larvae in Lake Amvrakia.

Oxygen concentration is considered among the most important abiotic parameters affecting zooplankton along the vertical axis of aquatic ecosystems, since most of these organisms tend to avoid living in hypoxic or anoxic conditions and remain restricted to the well oxygenated parts of the water column (Żurek, 2006; Vanderploeg et al., 2009, and references therein). The oxygen concentrations in the epilimnion and especially in the metalimnion of Lake Amvrakia were always high and, thus, sufficient for larvae growth and survival. However, in the hypolimnion the oxygen concentrations in certain periods were below 2 mg l^{-1}, which is a known critical level for *D. polymorpha* growth (Mihuc et al., 1999). Surprisingly, a considerable number of specimens of *D. blanci* larvae were recorded in these hypoxic parts of the lake and even in the deeper layer (35–40 m), which was anoxic at the end of stratification period. It has been documented that freshwater mussels such as *D. polymorpha*, are able to survive short-term exposure (< 5 days) to anoxic or hypoxic conditions in their habitat (Johnson & McMahon, 1998). In addition, some crustacean species can survive in hypoxic and even anoxic conditions for a small period of time using hypoxic zones as a potential refuge against fish predation during daylight, while they migrate to shallower depths at night (Vanderploeg et al., 2009). Doulka & Kehayias (2011), investigating the diel vertical migration (DVM) of zooplankton taxa in Lake Trichonis, found that *D. blanci* larvae (misidentified as *D. polymorpha*) were able to perform vertical migrations and ascend to shallower depths during the night. This ethological adaptation could be a possible explanation for the presence of larvae in such unfavorable conditions during the day, although there is lack of evidence for DVM in Lake Amvrakia as samplings were conducted only in morning hours.

An interesting feature of *D. blanci* larvae was their size-specific depth distribution. During stratification, an increase of their size up to the lower limit of the metalimnion was observed, while this phenomenon was not recorded during the unstratified period. This means that stratification was probably responsible for or enhanced this particular pattern of vertical distribution. There could be several explanations for this. One could assume a passive sinking of the larger larvae, which probably have higher specific weight, until they reach the metalimnion, where the increased water density balances their descent. Also, considering an energetic movement of the larvae, the presence of larger specimens in the metalimnetic layer could be associated with the higher phytoplankton concentration, taking into account the high chlorophyll-a concentrations recorded in this layer. The reduction of intraspecific food competition may also be another mechanism of size separation of zooplankton in the vertical axis (Doulka & Kehayias, 2008, 2011). Finally, the selection of greater depth by the larger larvae could reduce their predation by planktivorous fish foraging in the illuminated epilimnetic waters (De Meester et al., 1999).

Effects of Abiotic Elements

As previously discussed, temperature is clearly the decisive factor in the life cycle of other dreissenids. Thus, the present results on *D. blanci* larvae are in accordance with reports about the positive relation of temperature on the abundance variations of *D. polymorpha* larvae in lakes of Poland (Stańczykowska & Lewandowski, 1993) and Mikri Prespa Lake (Michaloudi et al., 1997). The influence of temperature may be considered as direct, controlling the length of time that the larvae are found in the plankton and affecting the growth of adult specimens (Sprung, 1993; Karatayev et al., 2006), or indirect due to increased food availability (Mantecca et al., 2003).

The correlation between phytoplankton biomass (as reflected by the chlorophyll-a concentration) and the abundance of *D. blanci* larvae in Lake Amvrakia, was due mainly to similarities in their vertical distribution than to their seasonal variation. In any case, it is expected that food quality rather than quantity is most important for growth and reproduction of *D. blanci* larvae, as found also for the related *D. polymorpha* (Stoeckmann & Garton, 2001).

The negative interrelation of pH with larvae variation in Lake Ozeros is contradictory to the positive interrelation of this element in Lake Amvrakia, as previously discussed. To our knowledge, there is not much data on this issue, except the reference of Hincks & Mackie (1997), who reported that positive growth of *D. polymorpha* larvae only occurred at pH levels greater than 8.3.

Water level (WL) measurements, which were conducted only in Lake Amvrakia, showed differences between the two years of study, while this variation turned to have some considerable effects in the abundance of *D. blanci* larvae. Water level fluctuation is expected to exercise several indirect influences affecting the total nutrient load, phytoplankton production and consequently the chlorophyll-a concentration within the lake. On the other hand, the decrease of larval abundance in the second year could have been a direct effect of water level reduction, which uncovered an extensive area of the bottom of the lake, being the habitat for the adult forms of this mollusk. Therefore, it can be assumed that this habitat reduction probably resulted in the reduced reproductive capacity of the population.

Water retention time (WRT) was estimated only in Stratos reservoir for a two-year period and varied between 2.3 to 10.8 days (Kehayias et al., 2008), and presented significant differences throughout the two-year study. WRT proved to be the most significant parameter in this reservoir, exercising a positive effect on the abundance variation of *D. blanci* larvae. Stratos reservoir receives water from the upstream Kremasta reservoir, which is also inhabited by *D. blanci* larvae (reported as *D. polymorpha* by Koussouris et al., 1994). In the Kremasta reservoir, however, this species was by far dominant in the zooplankton, in contrast to Stratos. The main difference in these two reservoirs lies in their WRT, which for Kremasta is nearly a year, in contrast to a few days for Stratos. Thus, it could be assumed that *D. blanci* is probably better adapted to the higher stability of the lacustrine zone in Kremasta reservoir, where it can form larger populations. This could also explain the decrease of its abundance during the second sampling year, when the water outflow into Stratos reservoir was greater, resulting in even lower WRT values. However, several reports point out that the attachment of *D. polymorpha* larvae can be facilitated by high water flow, which probably increases the frequency of contact between organisms and substrate (Navarro et al., 2006).

In conclusion, the present results reveal that there are great biological and ecological similarities between *D. blanci* and *D. polymorpha* larvae. This could be of great importance when taking into account the notorious invasive character of the sister species *D. polymorpha* and its various impacts on freshwater ecosystems (Minchin et al., 2002). In this sense, there is a potential threat that *D. blanci* could become another invasive species in freshwater ecosystems and therefore appropriate management measures would be required also for this species. Moreover, similar to its sister species (*D. polymorpha*), *D. blanci* could cause certain economic effects, such as the fouling of intake pipes and drinking water pipelines, as in the case of Kremasta and Stratos reservoirs (unpublished data). Consequently, the present study can be considered as a first step in the investigation of the biology and ecology of *D. blanci*, however considerably more research is needed on the life cycle of this species, and the ecological and economic consequences of its inhabitance in lakes and reservoirs of this area of Europe.

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