How does interannual trophic variability caused by vertical water mixing affect reproduction and population density of *Daphnia longispina* group in Lake Iseo, a deep stratifying lake in Italy?

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Abstract

Lake Iseo is a deep meromictic lake in Italy. During the last 20 years (1993-2013), the lake experienced complete mixing of the water column only in spring 2005 and 2006. The full overturn episodes in these two years resulted in an increase of nutrients in both years, but an increase in phytoplankton biovolume occurred in the surface layers only in 2005. Our study examined if the magnitude of the vertical mixing and P increase in surface waters can help predict reproduction and abundance of the main primary consumers, the cladocerans. We investigated the lake for nine years (2001-2009) and compared annual changes in the Cladocera community and reproduction of the *Daphnia longispina* group between years of full and partial mixing. During the complete vertical mixing years (2005 and 2006), the taxonomical composition of Cladocera did not change, and density relationships among taxa shifted slightly towards an increase in the population density of *Daphnia* spp. Phytoplankton biovolume was significantly related to *Daphnia* mean clutch size. The increase TP, subsequent to the complete vertical mixing during late-winter/early spring, also seem to predict reproduction and population density of the *Daphnia* species better.

Introduction

Planktonic crustaceans, particularly the cladocerans, are highly sensitive to environmental changes. Abiotic factors, i.e. temperature, light (Straile and Geller 1998, Alekseev and Lajus 2009), pH, and other water quality parameters (Krause-Dellin and Steinberg 1986), may affect the structure of the *Daphnia* populations. These factors modify the rates of survival, growth, and reproduction in daphnids (Hall and Burns 2002). There is also evidence that biological processes as well as the structure and seasonal dynamics of planktonic communities may be influenced by qualitative and quantitative changes in food conditions (Moore 1980, Rohrlack et al. 1999), comprising nutritional elements and compounds (e.g. Müller-Navarra 1995), and predation by fish or invertebrates (e.g. Lampert 1993, Leoni and Garibaldi 2009). Among the Cladocera, *Daphnia* is a keystone taxon with several species encountered in lentic environments. It's because of their pivotal position in the foodweb, the presence of *Daphnia* spp. is widely utilized as an indicator taxon to assess the ecosystem's response to a trophic degree and improved food conditions (Bērzinš and Bertilsson 1989, Visconti et al. 2008).

The extent of the spring lake overturn has significant effects on the annual development of the phytoplankton structure and biovolume in large and deep stratifying lakes, such as those located south of the Alps in Italy from the east to the west: Garda, Iseo, Como, Lugano, and Maggiore (Salmaso 2005). The year-to-year variations in the degree of the vertical mixing, the nutrient replenishment, and the phytoplankton development in these lakes are very similar, because of the similarity of the winter climate in this subalpine region. The nutrient replenishment and phytoplankton development are proportionally more evident in the oligomictic lakes, especially those that tend to be meromictic (Wetzel 2001, Garibaldi et al. 2003, Salmaso 2011), and in relatively more eutrophic lakes, i.e. Iseo and Lugano, because of their nutrient-rich hypolimnia (Salmaso 2005). In Lake Iseo the spring mixing events have been investigated over the last 20 years (Salmaso et al. 2007, Mosello et al. 2010).

Many lab-cum-field experiments have provided evidence that P availability, i.e. absence of P limitation, improves quality of the zooplankton food directly, especially for *Daphnia* species (Gulati and DeMott 1997, DeMott et al. 2001). *Daphnia* spp. has the highest P requirements among all of the crustacean zooplankters (Sterner and Hessen 1994; see review in Gulati and DeMott, 1997). Moreover, the stoichiometric theory predicts that the daphnids are more sensitive to P limitation than other common cladoceran taxa (e.g. Bosminidae, Schulz and Sterner 1999).

The present study was aimed at investigating: the magnitude of spring vertical mixing, the accompanying nutrient replenishment from the deeper layers, and the following increased development of phytoplankton, and if they can help predict changes in foodweb involving the main primary consumers, e.g. *Daphnia* species. In order to assess the effects on the Cladocera seasonal dynamics and on the reproduction of the *Daphnia longispina* group, we analysed abiotic and biotic parameters of Lake Iseo monthly for nine years between 2001 and 2009. We hypothesized that if during the years when mixing was complete (2005 and 2006), compared with rest of the years when mixing was incomplete, than *Daphnia* would increase both in abundance and clutch size.

Methods

Study site

Lake Iseo (also known as Lake Sebino) is the fourth largest lake in the sudalpine district (Italy), with a surface of 61.8 km^2, average water volume of 7.6 km^3, maximum depth of 285 m and average depth of 124 m. It lies on the foothills of the Alps (190 m a.s.l.), at the end of a prealpine valley, Val Camonica. The inflow and outflow of water in the lake is due to River Oglio that passes through the lake. The theoretical renewal time of the lake is about 4.2 years. It is 'warm monomictic' lake having a circulation of the water column annually > 4 $^{\circ}$C during late-winters or early spring. However, being very deep, this lake is oligomictic and circulates completely only irregularly during very windy and very cold winters. It is naturally oligotrophic lake but, over the last 40 years, the increase in nutrient loadings has resulted in a dramatic worsening of the trophic conditions of the lake (Garibaldi et al. 2003).

The fish assemblage of Lake Iseo was described in "Osservatorio Laghi Lombardi" (2005) as including 17 native species and 8 non-native species. During the last decade, the fish assemblage has shown an almost constant structure, dominated by pelagic species of shad (*Alosa fallax lacustris*) and whitefish (*C. lavaretus*). Other species, which are mainly littoral taxa, have moderate densities. These are: bleak (*Alburnus arborella*), burbot (*Lota lota*), eel (*Anguilla anguilla*), european chub (*Leuciscus cephalus*), pike (*Esox lucius*), perch (*Perca fluviatilis*), arctic char (*Salvelinus alpinus*), rudd (*Scardinius erythrophthalmus*), tench (*Tinca tinca*), italian roach ''triotto'' (*Rutilus erythrophthalmus*) and brown trout (*Salmo trutta lacustris*). The available fish data support the hypothesis that they did not change either in terms of the pressure of predation on *Daphnia* or in their effects on the populations of their prey, in fecundity, proportion of adults, and adult body size.

Field program and laboratory analyses

Water samples for abiotic and biological analyses were collected every 4 weeks from 2001 to 2009 in the deepest zone of the lake between Tavernola and Siviano (45°43’11’’N and 10°03’46’’E). Transparency was estimated using Secchi disk, and water temperature and dissolved oxygen were measured using underwater multiparametric probes (WTW Multi 3410). The samples for chemical analysis were collected, using a Van Dorn bottle (1700 mL), from the surface to bottom water and at depths of 10, 20, 30, 50, 75, 100, 150, 200 m. The total phosphorus (TP), pH and conductivity were measured in the laboratory, following standard methods (Leoni et al. 2007). The depth profiles of pH, conductivity, and oxygen were used for determining the time and amount of mixing in Lake Iseo. The depth of the spring mixing was taken to be the depth at which an upper epilimnetic layer had almost-uniform water temperature, and below which there was a lower heterogeneous layer of rapidly changing temperature values (Goldman and Jassby 1990, Salmaso 2005). The reported values of mixing depths do not represent exact estimates because of their dependence on the sampling frequency and on incomplete synchrony in some years in the depth and time changes of all the selected variables.

On all occasions, biological samples were collected in the euphotic layer from the 20 m depth to the surface. To analyze chlorophyll-*a* concentration and phytoplankton community, an integrated sampling was done with a special sampler: Schröder sampler from Züllig - CH9424 Rheineck, Switzerland- volume collected = 750 ml (Schröder, 1969). Chl- *a* concentration was determined by spectrophotometry after extraction of the chlorophyll pigments with 90% acetone. Phytoplankton was analysed on subsamples preserved in acetic Lugol’s solution. The organisms were counted on Zeiss Axiovert 135 inverted microscope using Utermöhl’s technique and phytoplankters were identified mostly to species level. The smallest algae were counted at 400×, and the less abundant at 250×, until reaching a number of at least 200 individuals for the most abundant species. To obtain about 85%, precision, between 30 and 90 randomly selected fields per sample were counted.

Phytoplankton total biovolume was estimated from the density data of the different species and the original measurements of the average cell volume of the species (Smayda 1978).

Zooplankton samples were collected by vertical tows in the water column from the 20 m depth to the surface with a Wisconsin type net of 25 cm diameter and 200 μm mesh. Each sample was obtained by pooling three replicate hauls (3 hauls corresponding to 2.94 m^3 of total filtered water).

Zooplankton samples were filtered on a 100 μm mesh nylon net and fixed by immersing in 95% ethanol before preserving in a 5% neutralized ($CaCO_3$) formaldehyde solution. The use of alcohol prevents carapace ballooning and the loss of eggs/embryos from the brood pouch (Manca et al. 2000). In the laboratory, Cladocera were identified and counted mostly to the species level. For details of procedures used in the field and laboratory see in Garibaldi et al. (2003) and Leoni et al. (2007). All monthly samples of Cladocera were analysed and density of each species was calculated. Clutch size and adult body length of *Daphnia* were analysed on samples collected from March to June when their densities are the highest. Abundance of daphnids and number of females carrying eggs were counted in at least 25% of the total zooplankton sample volume. The number of eggs or embryos per brood pouch was counted for 51±28 females per sample, depending on females carrying egg density. Body length of daphnids was measured on 2700 organisms, c.a. 100 animals per sample, using a PC, connected to the microscope, and having image analysis software (Leoni and Garibaldi 2009). Size at maturity for individuals of the *Daphnia longispina* group was determined according to Caramujo et al. (1997). The smallest adult size class (primipara) was considered as the size class with at least 5% of the total number of egg bearing females was observed. The minimum amount of 5% ensured that the smallest adult size class would not be set or established by a single precociously reproducing female.

Statistical analyses

Environmental parameters and female body length were standardized into z-score (see Larsen and Marx 2000), while phytoplankton biovolume, *Daphnia* density and clutch size were log-transformed. Levene's test has been used to assess the equality of variances in different samples (Levene 1960).

Spearman's coefficient of rank correlation was performed on all data set: seven parameters measured every 4 weeks during 2001-2009 (c.a. 700 values); maximum vertical mixing depth measured in spring during 2001-2009 (nine values). The correlation was calculated to measure the intensity of association observed between parameters to prevent spurious correlations in subsequent analyses. In fact the inclusion of highly correlated variables into the same model may inflate the standard error estimates. Therefore, the inclusion enlarges the P value for a predictor that significantly affected the response variable. In this study parameters showing correlation higher than 60% were excluded from the statistical models.

Selected data were analyzed using Hierarchical Linear Model (HLM-2 level models). This analysis estimates variance-covariance parameters and regression relationships from data that are hierarchically structured, as different cohorts of females in the same sample. Furthemore, females sampled at the same time, cannot be considered statistically independent because they have realistically experienced same environmental conditions (McMahon and Diez 2007). In HLM analyses we first built a FULL model including all the independent variables that, at each hierarchical level, may affect the dependent variable under scrutiny. Finally, we removed all non-significant predictors in two steps to obtain a FINAL model. The rationale for this procedure was that we aimed at reducing the number of steps in the simplification procedure thus not inflating the probability of Type I errors due to multiple statistical test (Whittingham et al. 2006). Random terms were not removed as our object was to maintain the hierarchical structure of the data and as only nested models could be compared by a likelihood ratio test (Golden 2000). The random coefficients of a HLM in which the predictors are included represent 'conditional variance components' i.e., the amount of variance that is not explained by the predictors (Singer 1998). The "effect of single mother" was included as a random grouping factor to take into account that each female can differently react to environmental parameters (predictors).

The assumption of independence of residuals was evaluated inspecting autocorrelation function (ACF) plots.

Statistical analyses were performed with SPSS 17.0 (SPSS Inc. Chicago, Illinois).

Results

In Lake Iseo, during the 9-year study period from 2001 to 2009, the lake fully circulated only in March 2005 and March 2006. In the other years, mixing depths had ranged between 30 m and 200 m, with no-regular pattern (Fig. 1a). Usually, the thermal stratification that began in spring was well-established between June and August, with the thermocline depth varying from 10 to 20 m. During the maximum summer stratification, the thickness of the euphotic zone was reduced to around 10 m, but during the autumn-winter months euphotic zone depth was as deep as 30 m. The weighted mean of water temperature over the water column from surface to 20 m depth varied similarly over 2001-2009: it ranged from 5.9+1.7 °C in winter to 19.6+3 °C in summer. Concentrations of dissolved oxygen in the top 20 m show only slight fluctuations during 9 years, with mean annual value of 9.7±1.6 mg L^{-1}. TP concentrations in the top 20 m layer gradually decreased from late spring, summer to autumn-winter, followed by a marked increase of TP in late winter, when the annual highest values were usually recorded. During the incomplete mixing years, in epilimnion (0-20 m) the spring maximum TP-values ranged between 21 and 32 μg P L^{-1}. In contrast, during 2005 and 2006 when mixing was complete, maximum TP values increased to 73 μg P L^{-1} and to 58 μg P L^{-1}, respectively (Fig. 1b). Spearman's correlation analysis showed a highly significant positive relationship between epilimnetic TP concentration and water mixing depth in spring ($r=0.902$, $P<0.0001$). In the top 20 m, the conductivity maxima were usually recorded during winters while minimum values occurred in summers. Conductivity was positively correlated with inorganic nutrients (e.g. TP: r=0.753, P<0.0001) and, negatively, with temperature (r=-0.74, P<0.0001) and pH (r=-0.222, P=0.015).

Chl-*a* and phytoplankton biovolume were positively correlated (r=0.420, P<0.0001). Chl-*a* was generally lower in 2003 than in other years with mean annual values of 4.7±3.3 μg L^{-1} in 2003 vs 6.7 3 μg L^{-1} in the other years (Fig. 1d). Mean annual total biovolumes of phytoplankton usually exceeded 2000 mm^3 m^{-3}, except in 2003. Peak values of the mean integrated biovolume varied narrowly between $9.5*10^3$ mm^3 m^{-3} in April 2001, $10.7*10^3$ mm^3 m^{-3} in August 2005, and $10.0*10^3$ mm^3 m^{-3} in March 2008 (Fig. 1c). During nine-year study, 59 taxa of phytoplankton belonging to seven taxonomic groups were identified. Among these, Bacillariophyceae, Conjugatophyceae and Cyanobacteria were best represented in terms of biovolume. The temporal development of the algal biovolume followed a fairly regular trend during the years. Large colonial diatoms (*Aulacoseira* spp., *Fragilaria crotonensis*, *Melosira* spp. and *Asterionella formosa*), had a regular seasonal succession in late winter and spring. Conjugatophyceae (mostly *Mougeotia* sp.), developed mainly in spring and summer and Cyanobacteria (mostly *P. rubescens*) developed in autumn. The species so far mentioned, during the study period, alternated cyclically in the phytoplankton dominance of Lake Iseo. Chlorophyceae were exclusively represented by Chlorococcales. In spring 2005 or 2006, among forty-three genera of phytoplankton *Melosira* sp., *Fragilaria* sp., *Asterionella* sp. (diatoms) and *Mougeotia* sp. (Conjugatophyceae) peaked; *Gemellicystis* sp. (Chlorophyceae) reached biovolume values that are unusual in Spring (Fig. 2).

Five taxa of Cladocera were identified in Lake Iseo during the study period: *Daphnia longispina* group, *Eubosmina* sp., *Diaphanosoma brachyurum* and two predatory species, *Leptodora kindtii* and *Bytothrephes longimanus*. *Daphnia* was the dominant Cladocera taxon, except in June-September 2005 and in August 2007 when *Eubosmina* sp. and *Diaphanosoma brachyurum*, respectively, dominated the community (Fig. 3). The seasonal trend in fluctuations of *Daphnia* densities was comparable in different study years. It was characterized by a well-defined spring pulse (April-June) when annual density maxima occurred. In September 2008 and 2009, and in October 2007, secondary maxima were observed. Very high density of daphnids, 33605 ind m^{-3}, was reached in May 2005 and the study period maximum of 43473 ind m^{-3} was observed in May 2006. Other years (Fig. 3), population density never so high: it ranged from 25000-20000 ind m^{-3} (in 2001 and 2007) to 4500-6500 ind m^{-3} (2004 and 2008).

The study of reproduction of *Daphnia* spp. was limited to the spring-early summer season, when the population reached its annual maximum. In this period, parthenogenetic reproduction occurred widely and females with eggs comprised up to 50% of the total adult female population; in 2001, 2002 and 2008 the females with eggs exceeded the 75% of the total adult population. In 2005 and 2006 parthenogenetic females were 40% and 60%, respectively, of adult population. The proportion of adult females decreased in late spring (Fig. 4a). The sexual reproduction was sporadically observed: only in April 2006 the females with ephippia constituted 28% of the total adult females. In other months, such females did not exceed 3% (Fig. 4a).

The mean body length of *Daphnia* usually ranged from about 1.3 mm to 1.6 mm, except in May 2004 when about 67% of the individuals were < 1.2 mm and in June 2003, May 2007 and late spring 2009 when the mean body length considerably exceeded 1.7 mm (Fig. 4b).

The mean clutch size (egg number in brood chamber) was highest in 2005, reaching 6.9 eggs per egg-carrying female. In 2005, some females carried up to thirteen eggs per brood chamber. In 2006, the mean clutch size was 4.4, slightly more than in 2007 (mcs=4.0), while in other years, rarely exceeded three (Fig. 4c).

highly correlated variables into the same model may inflate the standard error estimates.

In order to identify significant predictors of clutch size an HLM analysis was performed (see paragraph in ‘‘Statistical Analyses’’). The FULL model for clutch size included as predictors among physical, chemical and biological features: female body length, temperature, total phosphorus, Secchi disk transparency, phytoplankton biovolume and *Daphnia* density (Table 1). Few parameters, highly correlated with the previous, were excluded from the model to not inflate the standard error estimates: mixing vertical depth, pH and conductivity. This model also included the interactions between female length and all other parameters (e.g. body length*phytoplankton biovolume), as several experimental studies have shown that reproduction in *Daphnia* spp. may be efficiently controlled by multiple factors producing different effect in their mean clutch size for a given adult body length (Table 1). Temperature was entered in the statistical models as a covariate. After removing all non-significant predictors using a backward stepwise procedure, in FINAL model (Table 2), three significant predictors could be identified for clutch size: TP, body length and phytoplankton biovolume.

Discussion

This research of demographic and reproductive parameters in Lake Iseo allowed us to investigate the role of nutrients and depth of water mixing in controlling the population density of *Daphnia longispina* group, and also the Cladocera community structure, comparing the data of the complete-mixing years of the water column with those of incomplete mixing. We assume that the interannual variations in the extent of spring vertical mixing affect epilimnetic nutrient replenishment (TP), phytoplankton quantity and quality. The novel finding from the correlative study of environmental parameters and population dynamic of *Daphnia* in Lake Iseo, a deep oligomictic lake, is that the depth of vertical mixing during late-winter is a consistent predictor of the success of this taxon. In addition, our long-term field study provides an evidence that reproduction in *D. longispina* group may be predicted precisely from food availability, i.e. food concentration. During the complete mixing years, the average mean body length of mother does not appear fully responsible for the number of eggs per brood chamber, even if significant predictor of clutch size. In the spring periods, the demography and reproduction of *Daphnia* group do not seem to be related to rise in water temperature, but rather in food quality.

Evidence about effects of the spring vertical water mixing depth

The study on Lake Iseo reveals the importance of the degree of the overturn in controlling population density and clutch size of *Daphnia* group and, indirectly of the Cladocera community structure and their implications for the pelagic foodweb, since zooplankton is often observed as the crucial link between phytoplankton primary producers one hand, and the higher consumer levels, including planktivorous fish, on the other (e.g. Gulati et al. 1982, Williamson and Stoeckel 1989). The cladoceran densities were two/three-fold higher during years with complete overturn, e.g. as in spring-summer 2006, than those with incomplete mixing. The relatively high population density in spring 2007 may be due to the hatching of many resting eggs produced in 2006.

The observed increases in cladocerans density in May 2005 and May 2006 were driven mainly by *Daphnia* spp: their densities in these years were 33605 and 43474 ind m^{-3}, respectively. Such an increase in *Daphnia* densities can be explained, among potential alternative explanatory factors (e.g. resting egg hatching), by possibly an increase in hatching of resting eggs or by the increase in clutch size. The latter can be well predicted by the TP concentration in 0-20 m layers (HLM: $t=2.56$, $P=0.01$). In turn, the increase in P concentration in surface layers, recycled from the P-rich deeper water strata, is strongly related ($r=0.902$, $P<0.0001$) with the depth of the spring vertical water mixing (Salmaso 2010, 2011). Thus, the resulting nutrient regime, depending of the mixing depth, can affect the structure of the pelagic food web: starting with the phytoplankton development, and its feedback for development and growth of the zooplankton community. Our field study clearly demonstrates that Cladocerans are very sensitive to the changes in lake's trophic status and food quality. In fact, the variations in zooplankton species composition and community structure, especially the increase in relative abundance of Cladocera, reflect changes in trophic conditions of lakes (Brodersen et al. 1998, Manca et al. 2000). A major factor that determines reproduction of *Daphnia* in nature is the composition of its food, which undergoes pronounced seasonal changes due to the succession of algal species and seasonally varying ratios of algae to detritus biomass (Schatz and McCauley 2007, Koch et al. 2009).

Evidence about effects of food

The importance of food quality - i.e. presence of certain phytoplanktonic taxa, diatoms and green algae- and food quantity on success of zooplankton is well known (see Gulati and DeMott 1997). On the other hand, the response of *Daphnia* to food quality is well-supported (Gulati and DeMott 1997, Weers and Gulati 1997a, DeMott et al. 2001, Sterner 2008). Daphnids, with their relatively the highest P requirements among the planktonic cladoceran taxa, are apparently more sensitive to P limitation than other cladoceran taxa (Sterner and Hessen 1994; Schulz and Sterner 1999). Several studies demonstrate strong positive relationships between clutch size of *Daphnia* and high algae P content of lake bioseston (review by Gulati and DeMott 1997, Acharya et al. 2004). In addition, in experiments with P enrichment of food using animals from the field show that some of the growth reduction in the field is directly attributable to P deficiency of food (DeMott et al. 1998, Elser et al. 2001, Boersma and Kreutzer 2002). Phosphorus appears also to influence zooplankton indirectly, as several workers consider the *Daphnia* reproduction limitation by polyunsaturated fatty acids (PUFA) of the food a secondary effect of P limitation (e.g. Weers and Gulati 1997b, Bednarska and Slusarczyk 2013). Both P and PUFA, separately or in conjunction, are likely to explain in general the present results in Lake Iseo. During the full mixing years, we observed both elevated concentration of phosphorus and relative high biovolume of diatoms in phytoplankton, which are usually high in PUFA content and are thus good-quality food for freshwater zooplankton (Gulati and DeMott 1997). In fact, for 2005 and 2006, when the lake got fully mixed in late winter/early spring, average values of TP in the 0-20 m layer were 51 and 47 μg P L^{-1}, respectively. These concentrations are much higher than those observed during the years of incomplete mixing, when the mean range from 12 to 23 μg P L^{-1}. The enrichment of trophogenic layers with P produced different effects on a limited number of phytoplankton groups. The greater importance assumed by the various algal groups in each year may be due to changes in the meteoclimatic conditions during the whole vegetative season, but also to the different pool of nutrients recycled from the deep to the surface layers as a result of late winter and spring circulation processes. In 2005 and/or 2006, after the major enrichment episodes, three species of diatoms and one of Chlorophyta peaked during April-May (Fig. 2) followed by maxima for zooplankton, especially of *Daphnia* population. In both these years, increases in egg production and in population density of *Daphnia*, followed with seasonal increases in P and good-quality food availability (e.g. *Melosira* - Bacillariophyta), support earlier reports (Winder and Schinder 2004; Kagami et al. 2007). On the other hand, the relatively lower biovolume of *Aulacoseira* spp. in 2005-2006 than in 2001-2002-2004, may have favoured the development of *Daphnia* popolations. In fact, the filamentous genus *Aulacoseira* is know to have clogging abilities that affect fish-nets and filter-beds but also the filter combs of *Daphnia* (Weyhenmeyer et al. 2008).

Also several studies on Dutch shallow lakes (Gulati et al. 1992) indirectly support our conclusion that in summer, when poor-quality food, mainly filamentous Cyanobacteria, (*Planktothrix rubescens* in case of Lake Iseo), dominant the bioseston, the daphnids are rare and bosminids (*Eubosmina* in case of Lake Iseo) are the dominant crustacean zooplankton.

Effects of "mother" body length (MBS)

It is generally assumed that clutch size variability within *Daphnia* populations in field should be explained by changes in MBS (Gliwicz and Boavida 1996, Pietrzak 2011). In our study, we found a positive correlation between MBS and egg production in Lake Iseo (HLM: $t= 10.33$, $P<0.01$), even though MBS was not higher in 2005 and 2006 when number of offspring in a clutch was maximum. Several studies demonstrated that clutch size is controlled by a combination of different "forces" and high variability in the number of eggs per clutch in our field data suggested that other factors must also be at work (Gliwicz and Boavida 1996, Hülsmann 2011, Pietrzak 2011). In Lake Iseo, it seems likely that maximum clutch sizes for different body length classes are mainly controlled by food availability.

Conclusions

We investigated in Lake Iseo, an oligomictic-meromictic lake (Italy), the effects of changes in mixing depths during late winter and early spring periods from 2001 to 2009 on clutch size and population development of *Daphnia longispina* group, the most typical taxon of Cladocera and primary consumers. Our study underscores the effects of complete mixing period on P enrichment of in upper water. This P increase in the surface water layers leads to an increase in phytoplankton primary production rates, and in reproduction and population growth rates of zooplankton, especially *Daphnia* spp. This is apparently the first long-term field study that highlight the importance of vertical water mixing depth on Cladocera dynamics and *Daphnia* phenology in meromictic/oligomictic deep lake. Furthermore, our results are consistent with previous field and laboratory studies relating to food and reproductive strategy of Cladocera species. The changes in both the concentration and quality of food affect the clutch size of the *Daphnia* spp. as well as their density of population in spring.

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Table and Figure legends

Table 1 Full hierarchical linear model of *Daphnia* clutch size in relation to physical, chemical and biological features of Lake Iseo -sampled monthly from 2001-2009. The first three rows of the table describe components of the random part of the model (Cov. par.= covariance parameters and z=z-value), while remaining rows describe the fixed part of the model (effect and t-value).

Table 2 Final hierarchical linear model of *Daphnia* clutch size in relation to physical and biological features of Lake Iseo -sampled monthly from 2001-2009. The first two rows of the table describe components of the random part of the model (Cov. par.= covariance parameters and z=z-value), while remaining rows describe the fixed part of the model (effect and t-value).

Fig. 1 Annual variation of spring vertical mixing depth of Lake Iseo (2001-2009) and dissolved oxygen concentration (DO), in surface and bottom layers, during water mixing period (a). Temporal variations in the 0-20 m layer of total phosphorus (b), phytoplankton biovolume (c) and chlorophyll-a (d).

Fig. 2 Maximum values of the biovolumes ($mm^3\ m^{-3}$) of the principal taxonomic algal genera in the trophogenic layer (0–20 m). Values computed in April-May for the period 2001–2009.

Fig. 3 Seasonal variations of the density of *Daphnia longispina* group and the remaining filter-feeding Cladocera, collected in the 0-20 m layer of Lake Iseo (2001-2009).

Fig. 4 Temporal variation of reproductive females (a), body length (b) and clutch size of *Daphnia longispina* group (c) in Lake Iseo (2001-2009). Minimun clutch size is not showed, as always equal to 1.

Table 1

Cov. par. or effect	Coefficient	SE	df	z or t	P
Residual	0.215	0.008		26.930	<0.001
Intercept	0.120	0.040		3.024	0.002
Body length	<0.001	<0.001		1.664	0.096
Intercept	-1.309	0.961	23.909	-1.363	0.186
Total Phosphorus	0.015	0.005	20.495	2.789	0.011
Body length	0.003	0.003	22.153	0.859	0.399
Transparency	0.052	0.054	22.498	0.957	0.349
Temperature	<0.012	0.026	22.988	0.047	0.963
Phyto biovolume	0.514	0.231	22.981	2.221	0.036
Daphnia density	-0.064	0.116	22.263	-0.550	0.588
Body length*Total Phosphorus	<0.001	<0.001	14.163	0.192	0.851
Body length*Transparency	<0.001	<0.001	15.450	0.539	0.5985
Body length*Temperature	<-0.001	<0.001	21.878	-0.253	0.802
Body length*Phyto biovolume	<-0.001	<0.001	19.188	-0.377	0.710
Body length**Daphnia* density	<-0.001	<0.001	21.414	-0.803	0.431

Table 2

Cov. par. or effect	Coeff.	SE	df	z or t	P
Residual	0.219	0.008		27.177	<0.001
Intercept	0.112	0.034		3.271	0.001
Body length	0.001	<0.001	1481.288	10.322	<0.001
Total Phosphorus	0.013	0.004	23.303	2.887	0.008
Phyto biovolume	0.430	0.152	23.387	2.834	0.009

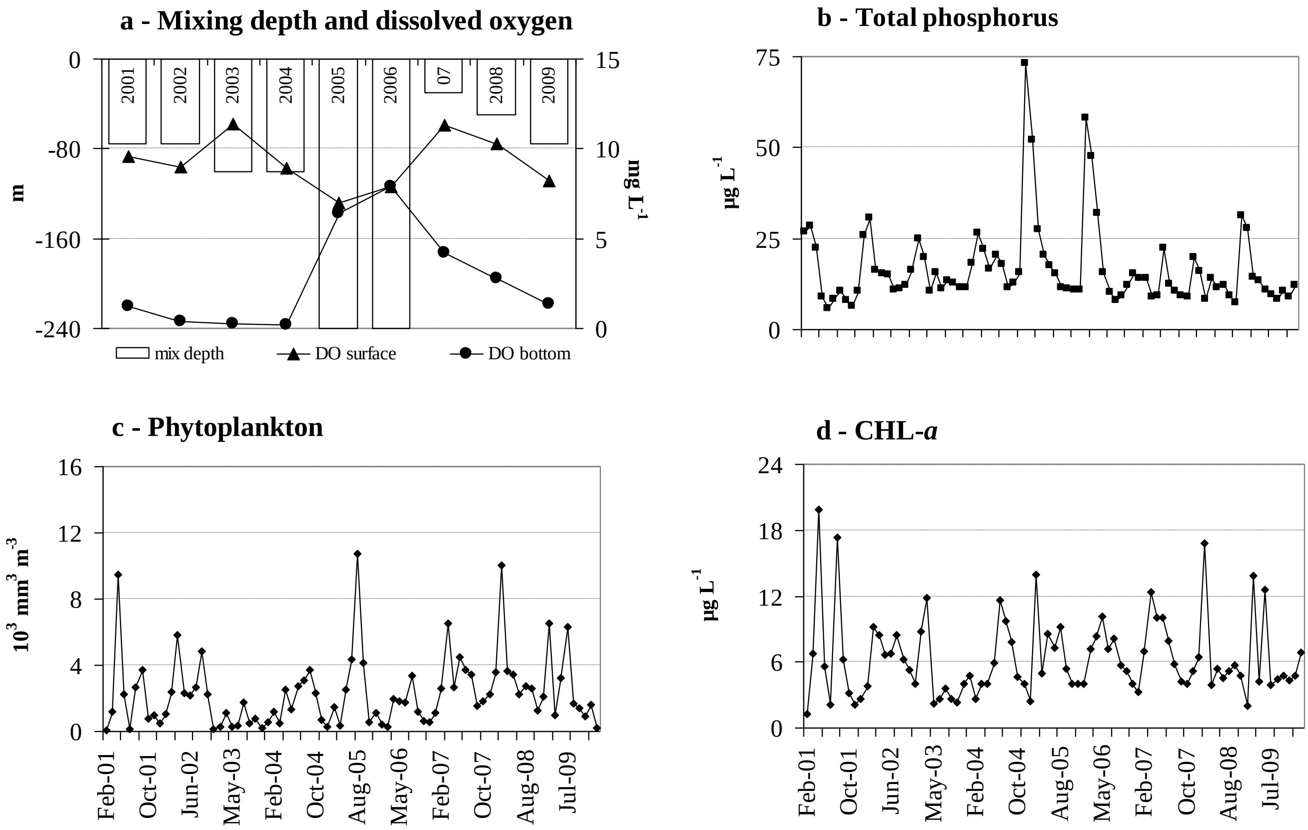
a - Mixing depth and dissolved oxygen
m
mg L-1
2001
2002
2003
2004
2005
2006
07
2008
2009
mix depth
DO surface
DO bottom
b - Total phosphorus
µg L-1
c - Phytoplankton
10^3 mm^3 m^-3
d - CHL-a
µg L-1
Feb-01
Oct-01
Jun-02
May-03
Feb-04
Oct-04
Aug-05
May-06
Feb-07
Oct-07
Aug-08
Jul-09

Figure 1

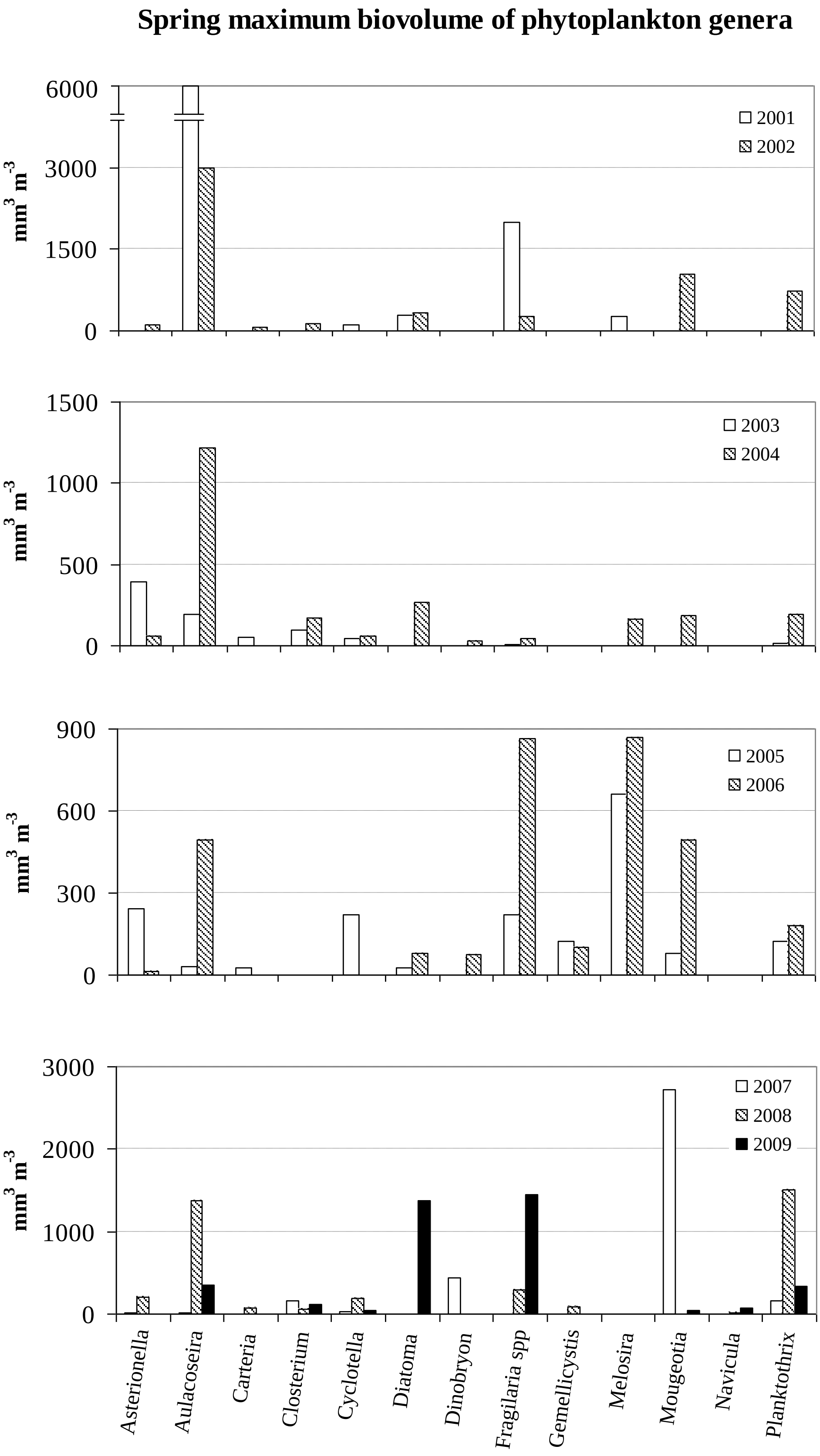
Spring maximum biovolume of phytoplankton genera
mm3 m-3
6000
3000
1500
0
2001
2002
1500
1000
500
0
2003
2004
900
600
300
0
2005
2006
3000
2000
1000
0
2007
2008
2009
Asterionella
Aulacoseira
Carteria
Closterium
Cyclotella
Diatoma
Dinobryon
Fragilaria spp
Gemellicystis
Melosira
Mougeotia
Navicula
Planktothrix

Figure 2

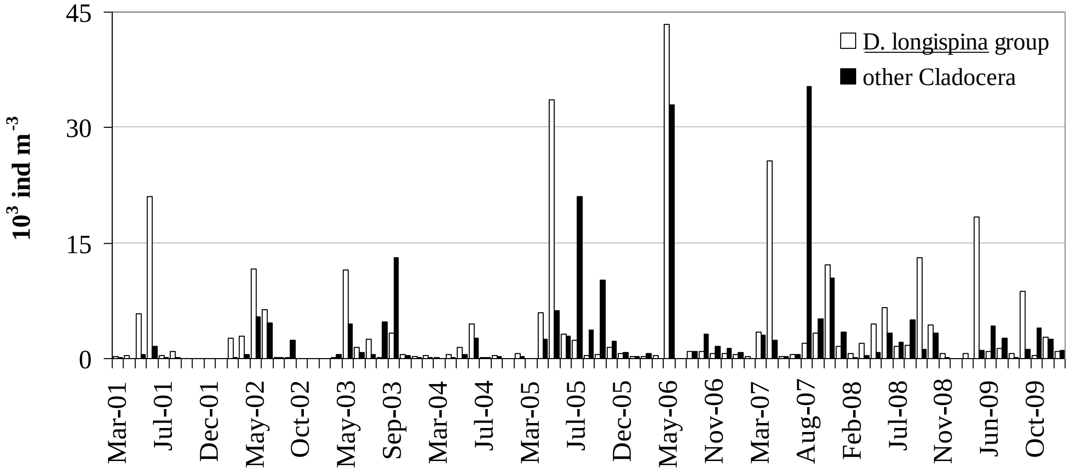
45
30
15
0
10^3 ind m^-3
D. longispina group
other Cladocera
Mar-01
Jul-01
Dec-01
May-02
Oct-02
May-03
Sep-03
Mar-04
Jul-04
Mar-05
Jul-05
Dec-05
May-06
Nov-06
Mar-07
Aug-07
Feb-08
Jul-08
Nov-08
Jun-09
Oct-09

Figure 3

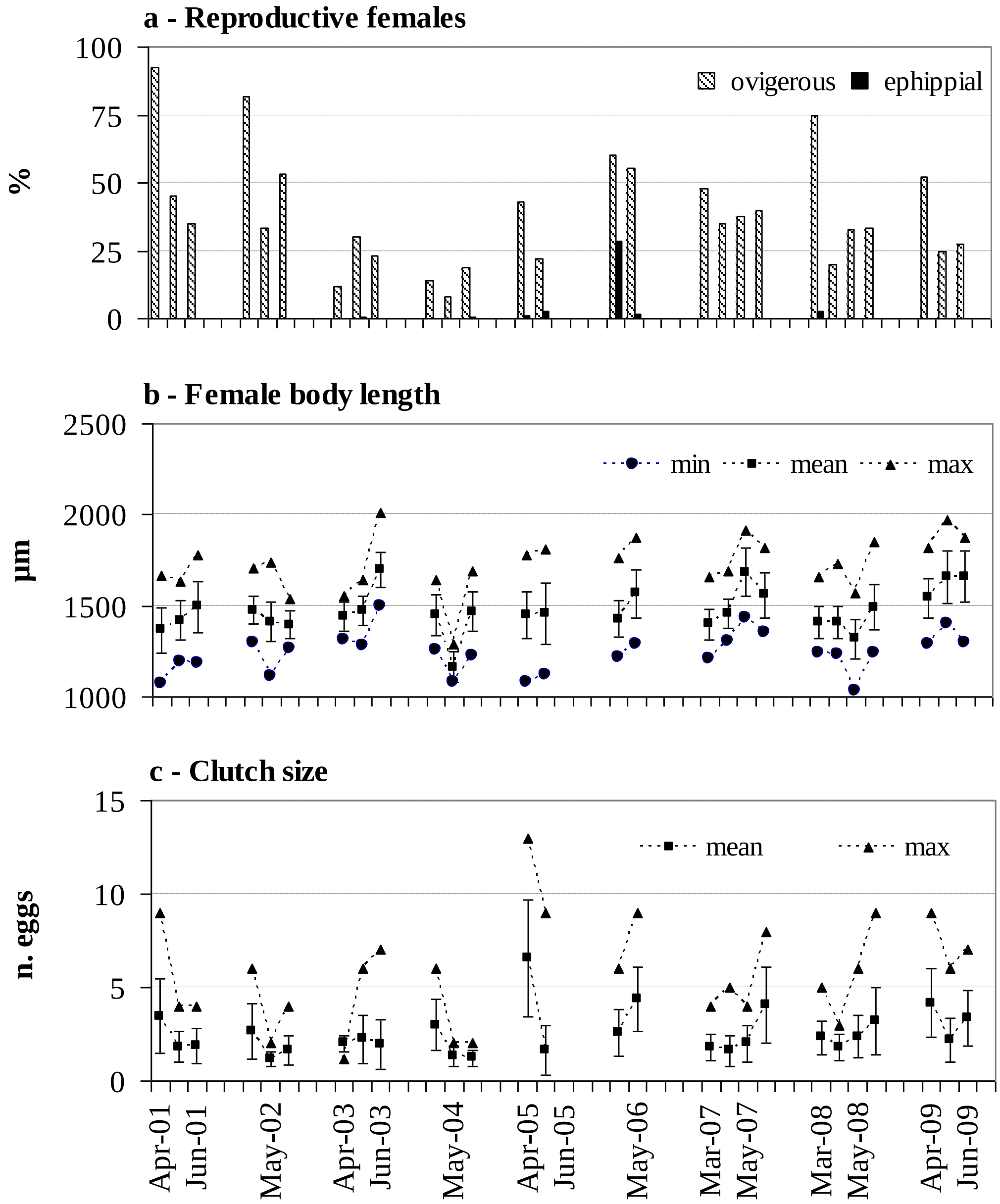
a - Reproductive females
%
ovigerous
ephippial
100
75
50
25
0
b - Female body length
µm
min
mean
max
2500
2000
1500
1000
c - Clutch size
n. eggs
mean
max
15
10
5
0
Apr-01
Jun-01
May-02
Apr-03
Jun-03
May-04
Apr-05
Jun-05
May-06
Mar-07
May-07
Mar-08
May-08
Apr-09
Jun-09

Figure 4