

Phenology of *Daphnia* in a Northern Italy pond during the weather anomalous 2014

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ABSTRACT

This note reports a comparison between *Daphnia* phenology in the weather anomalous 2014 and a previous three years period (2011-2013), in a shallow water body of Northern Italy (Bodrio del pastore III) where we recorded *D. pulex*. In 2011-2013, *Daphnia* population showed 1-2 density peaks from mid spring to early summer, it declined in July-August and did not recover, from ephippia, until the following spring. The seasonal dynamics was probably related to the species thermal tolerance. Males and ephippial females appeared at the beginning of growth season according to a typical feature of *Daphnia* populations from temporary habitats. The presence of the *Chaoborus* larvae resulted in juvenile adaptive predator-avoidance cyclomorphosis. In 2014, in the study area, mean winter air temperature was much warmer than average recorded during the past three years while it was much colder than average in July and August. This reflected the relatively rainy and cloudy summer months: the winter and summer precipitations total was above the previous three years average. In 2014, *Daphnia* was found all over the year and showed a maximum peak of density in November. The general increase of *Daphnia* was related to a shift in *D. pulex* population phenology, seasonal growth started earlier and lasted longer, and to the occurrence of *D. longispina*. Both species were identified by genetic markers and phylogenetic analyses of ND5 sequences placed isolates from the Bodrio del pastore III into the European *D. pulex* group. Both populations reproduced by cyclical parthenogenesis and showed cyclomorphosis. However, *D. pulex* produced more males and ephippial females than *D. longispina*. Their seasonal dynamics were quite different: *D. longispina* dominated in late summer while *D. pulex* showed the highest density in November. The presence of *D. pulex* in the Bodrio is important in the framework of conservation ecology especially because we have showed that it is native European strain instead of the invasive North American clone that replaced native *D. pulex* throughout Africa and was already recorded in Italy. We provide some indications and discuss how *Daphnia* phenology of shallow lakes of temperate areas may be susceptible to inter-annual variability in weather conditions.

Key words: Interannual meteo-climatic variability; *Daphnia pulex*; *D. longispina*; recruitment from ephippia; shallow water.

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INTRODUCTION

Growing evidence for phenological changes of plankton in response to climate change come from empirical, experimental and theoretical research over recent years (Edward and Richardson, 2004; Winder and Schindler, 2004; Hays *et al.*, 2005; Visser and Both, 2005; Richardson, 2008; Vadadi-Fülöp and Hufnagel, 2014). Zooplankton is a key component of aquatic food webs and the climate change may cause different adverse effects on species diversity by means of elimination of sensitive species, replacement of sensitive species/strains by less sensitive ones, shifts in food-web interactions; acclimation of species/strains to stress, selection of tolerant genotypes, outcome of predator-prey interactions by altering the ability of prey to detect predators or to implement defensive responses both in marine and freshwater habitats (Riessen *et al.*, 2012; Vadadi-Fülöp *et al.*, 2012; Vadadi-Fülöp and Hufnagel, 2014). Shallow water bodies are highly sensitive to climate warming due to substantial changes in thermal and hydrological regimes; changes in

phenology may be important to ecosystem function because the level of response may vary across functional groups and multiple trophic levels (Scheffer *et al.*, 2001; Meerhoff *et al.*, 2007; Feuchtmayr *et al.*, 2009; Landkildehus *et al.*, 2014).

In a previous paper we reported the occurrence of *Daphnia pulex* in a permanent water body, Bodrio del pastore III, in Northern Italy (Rossi *et al.*, 2014). During a three year study (2011-2013), the dynamics of this population was characterised by an increase in density from spring to early summer, a decline in July-August and a recover, presumably from ephippia, only in the following spring (Fig. 1). The seasonal dynamics was probably related to the species thermal tolerance. Male and ephippial females appeared at the beginning of growth season, before the density peak, according to a typical feature of *Daphnia* populations from temporary habitats. The presence of the predaceous larvae of *Chaoborus flavicans* probably account for seasonal variation of juveniles morphology that showed neck-teeth and/or elongated tail spines as typical predator-

avoidance morphological traits (Rossi *et al.*, 2014). Recently, the invasive hybrid lineage of the American *D. pulex* and American *D. pulicaria*, invaded Africa and was already recorded in Northern Italy and Sardinia (Mergeay *et al.*, 2006; Fadda *et al.*, 2011; Marková *et al.*, 2013). In this study, we used a diagnostic mitochondrial DNA gene to identify if *D. pulex* population from Bodrio del pastore III represents this invasive hybrid clone or if it belongs to native European *D. pulex* strain. Moreover, in 2014 our survey continued with the aim of evaluating the role of *C. flavicans* larvae in the *Daphnia* population dynamics and cyclomorphosis (Rossi *et al.*, 2014).

We are now reporting this note on the peculiar *D. pulex* phenology and on the occurrence of *D. longispina* in the weather anomalous 2014. The 2014 was the warmest year across global land and ocean surfaces since records began in 1880 (<http://www.ncdc.noaa.gov/sotc/global/>). The winter and summer precipitations total was above the 20th century average, marking the wettest summer even recorded, and the wettest since 1989.

METHODS

The Bodrio del pastore III (28 m asl, 45° 00' 05" N 10° 19' 26" E, Hayford ellipsoid) is a water body located in cultivated lands in Cremona province and originated by erosion and eversion from the old Po river-bed surface. It is considered a permanent water body, it has a surface area of about 3130 m², and a maximum depth of 6.30 m. Its volume is affected by River Po level, aquifer, precipitation, runoff and river inundations (Rossi *et al.*, 2014).

Bi-weekly surveys were carried out from March to December 2014 and zooplankton was collected by 105 µm-mesh net. From 2 to 16 L of water were filtered for each sample according to the water volume and depth. Each sample was preserved in 95% ethanol. During each survey, water temperature was measured a few centime-

tres below the surface and maximum depth was recorded. The water volume was estimated by considering the Bodrio conical shape according to the formula: $V = (S * h)/3$ where S is the surface area and h is the maximum depth. Meteorological data about daily air temperature and rainfall were collected from the weather station *LaCrosse WS2350* of Gussola.

To check the species identity of *D. pulex* from Bodrio del pastore III, a diagnostic mitochondrial DNA gene (sub-unit 5 of the NADH; *ND5*) was used to sequence 10 *Daphnia* isolates sampled in March, May and June. Total genomic DNA was extracted from *Daphnia* stored in 95% ethanol using the QIAGEN DNeasy Tissue Kit (Valencia, CA). Approximately 711 bp long fragment of *ND5* gene was amplified and sequenced in accordance with the PCR and sequencing conditions described by Dufresne *et al.* (2011). Additional data for the *D. pulex* complex were included from previously published sequences (Mergeay *et al.*, 2006; Fadda *et al.*, 2011; Marková *et al.*, 2007, 2013). To check the species identity of different *Daphnia* species (namely *D. longispina*) diagnostic mitochondrial DNA gene (12S rRNA) was used for isolates sampled in March, May and June. Nucleotide sequence data from the present study have been deposited in the GenBank database (accession number KR233296). The maximum-likelihood tree was constructed using MEGA software ver. 6 (Tamura *et al.*, 2013), and based on HKY+I nucleotide substitution model selected according to the Bayesian information criterion (BIC), with the base frequencies A, 0.19; C, 0.18; G, 0.21; T, 0.42; and the proportion of invariable sites equal to 0.490. Bootstrap support was estimated from 1000 replicates.

Abundance of *Daphnia* was enumerated using 100% of each sample and the number of juveniles, adult males, adult females, ovigerous females and ephippial females was recorded. In each sample, to check the morphological species identity, the analysis of at least 100 individuals was made and *D. pulex* was classified by the presence of the pecten on postabdominal claw (Margaritora, 1985). Individuals were classified, photographed and measured using a dissecting microscope and a stereomicroscope and the Nis-Elements D 3.1 software (NIKON NIS Elements Imaging Software).

RESULTS

In most 2014, mean monthly air temperatures were much warmer than average recorded during the past three years while they were much colder than average in July and August (Fig. 2). This likely reflected the relatively (wet) rainy and cloudy summer months acting to moderate temperatures. The winter and summer precipitations total was above the previous three years average. Abundant rainfall and the Po River flood accounted for the Bodrio volume that varied between 2380 m³ (October 24th)

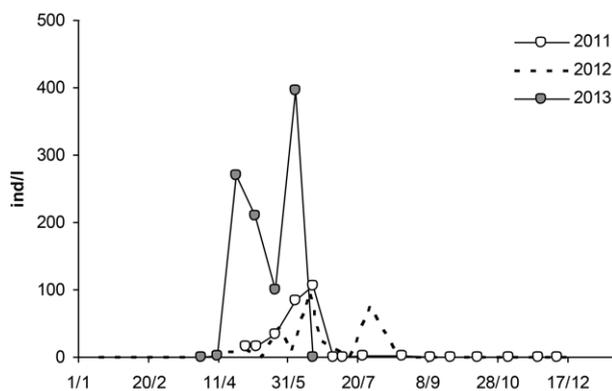


Fig. 1. Monthly variation of *D. pulex* total density in the period 2010-2013.

and 4948 m³ (November 21st). As in the previous three years the *Daphnia* population was probably re-established from resting eggs (Fig. 3), but in 2014 the annual peak densities varied substantially. Early seasonal peak was anticipated, with respect to the previous study period, to late winter and a second late spring peak was recorded just before a sharp population decrease in June-August: further bout of density and the maximum annual peak were recorded in late August and early November, respectively.

Totally 10 individuals from Bodrio del pastore III sampled in different periods of the year were used in phylogenetic analyses. Whereas the *ND5* is a diagnostic mitochondrial gene for species from *D. pulex* complex and the *ND5* oligonucleotide primers are specific for *D. pulex* group, only five isolates were amplified in PCR reaction and sequenced. Five isolates shared the same *ND5* haplotype that clustered with European *D. pulex* clade (Fig. 4). The species identity for the additional five isolates was checked by sequencing the *12S* rRNA mitochondrial gene. The phylogenetic analyses placed all *12S* rRNA sequences within the *D. longispina* group (data will be published elsewhere).

In fact, in 2014, *D. pulex* coexisted with *D. longispina* and the population dynamics of the two species were quite different. At the beginning of the season both species were

present in similar proportions while different percentage of *D. pulex* and *D. longispina* were responsible of the density increase of *Daphnia* population later on ($\chi^2 = 273$, $df = 9$, $P < 0.01$) (Fig. 3). However, it is important to note that in May and June the accurate morphological discrimination of *D. pulex* and *D. longispina* isolates was prevented by the presence of epibionts on the postabdominal claw of most individuals. For this reason the percentage of different species in spring samples may be not completely reliable. *D. pulex* and *D. longispina* coexistence was confirmed by genetic analyses of *Daphnia* isolates sampled in March, May and June 2014. *D. pulex* disappeared in July and August, reappeared in September and in November when became dominant being the major responsible of the highest annual density peak. *D. longispina* was recorded throughout all the sampling period, with the exception of April 24th (only two individuals were recorded on July 27th and August 7th) and showed the highest densities at the end of August.

Males were recorded throughout all the sampling period, with the exception of April 24th, July, August 7th, September 28th and October 24th. Density of adult and juveniles males was between 0.2 and 139 ind L⁻¹, on June 20th and November 9th, respectively. Free ephippia and/or ephippial females were also recorded throughout all the

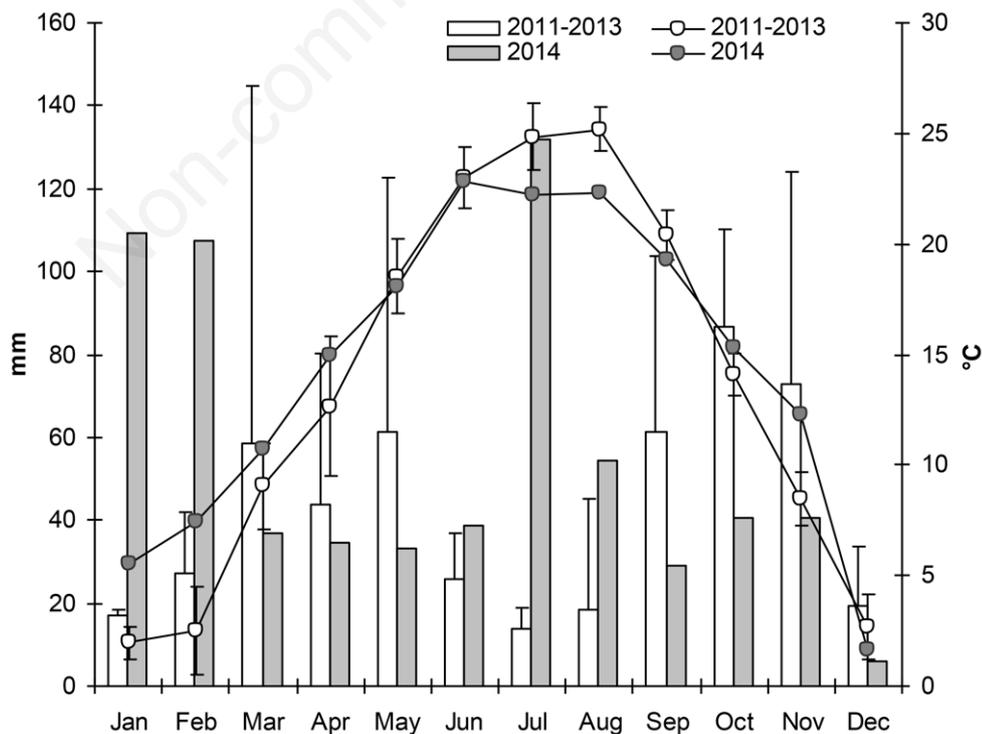


Fig. 2. Monthly variation of rainfall and air temperatures in 2014 and mean values (with standard deviation) recorded during the period 2011-2013 in the sampling area.

sampling period with the exception of July 4th and September. Their densities were between 0.3 and 19 ind L⁻¹, on August 28th and November 21st, respectively. Male and ephippial females frequencies were mainly supported by *D. pulex* with 95 % of total males and 87% of total ephippial females. The highest densities of *D. longispina* males and ephippial females were recorded on August 28th (2.29 ind L⁻¹) and November 9th (3.67 ind L⁻¹), respectively.

Chaoborus flavicans larvae were found in 5 samples (March 30th, April 10th, May 9th, August 28th and September 28th) and their density was near or below 1 ind L⁻¹. Seasonal variation was recorded in the frequency of *Daphnia*, most juveniles, showing neckteeth on the dorsal part of the carapace (Fig. 3). Most juveniles of both *D. pulex* and *D. longispina* showed neckteeth through March and May. Percentage of individuals with the typical predator-avoidance morphological trait decreased in June and most *Daphnia* juveniles did not show neckteeth in late autumnal samples.

DISCUSSION

The global anomaly of weather in 2014 was confirmed by our local data on mean air temperature and on precipitations. In general, mean air temperatures were the highest in the last three years and the winter and summer

precipitations was above the 2011-13 years average, making July and August the coldest of our four year survey period. The Bodrio del pastore III permanently hold water and, in 2014, its volume was larger and less fluctuating than in the past three years. Temperature and precipitations rise altered mixing regimes and seasonal variation of the Bodrio volume and represent major forcing on the abiotic and biotic factors accompanying interannual meteo-climatic variability. In general, the seasonal cycle of *Daphnia* depends on temperature and summer oxygen depletion (Alekseev, 2007). In accordance with general reports and with data from the Bodrio in the period 2011-2013, high *Daphnia* density population was recorded when temperature was between 15 and 22°C and, notwithstanding the reduction of mean temperature, a significant *Daphnia* population decline was recorded in late spring and early summer (Margaritora, 1985; Havel, 1985; Verbitskii et al., 2009; Rossi et al., 2014). In 2014, early seasonal peak of *Daphnia* was anticipated to late winter, a second late spring peak was recorded just before a sharp population decrease in June-August, and two further peaks were recorded in late August and early November. Peak abundance in November 2014 was higher than the maximum peak previously recorded in the cool June 2013 (Rossi et al., 2014). The shift in *D. pulex* population phenology, which seasonal growth started earlier and lasted

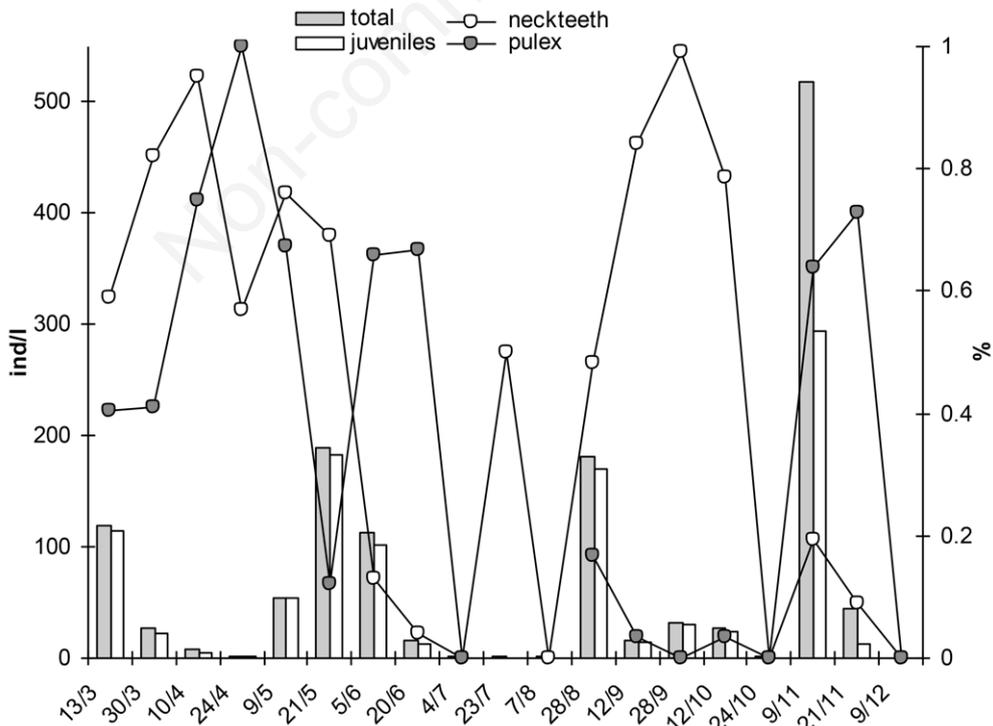


Fig. 3. Monthly variation of total density, juvenile density, neckteethed individuals percentage and *D. pulex* percentage in *Daphnia* population of the Bodrio del pastore III in 2014.

longer, may be linked to a direct or indirect effect of winter temperature rise (Manca and DeMott, 2009; Manca, 2011; Manca *et al.*, 2015). Warming may promote initial population growth by increasing fecundity *per se* and may be regarded as a factor able to worsen the effects of eutrophication (Schindler, 2001; Winder and Sommer, 2012). Peak density level may be positively correlated with increase of nutrient concentration and of food sources (Schalau *et al.*, 2008; Manca *et al.*, 2015). Hatching cues and associated emergence dynamics from resting stage have been given minor attention but winter warming may alter the interaction between photoperiod and temperature, and have direct, different effects on the emerging population of *Daphnia* (Vadadi-Fülöp and Hufnagel, 2014). In our case, earlier seasonal timing of *Daphnia* should be linked to cues for hatching of resting eggs (induced by late winter warming) (Chen and Folt, 2002; Gerten and Andrian, 2002). Shallow lakes of temperate areas has strong dependence on the *resting egg-bank*, as the probability of active overwintering daphnids decreases with lake depth (De Senerpont Domis *et al.*, 2007; Dupuis and Hann, 2009). In *D. catawba* and *Epischura lacustris* from Norford Lake (Vermont, USA) spring thermal stress induced the early hatch of resting eggs (Chen and Folt, 2002). In 2014, autumnal unusual density peak of *D. pulex* was related to a delayed recruitment from ephippia that might be activated by anomalous thermal summer regime or by summer inundation due to the Bodrio largest

volume (Stross, 1987; Rossi *et al.*, 1998; Larsson, 1991; Clark *et al.*, 2012).

A further and important anomaly with respect to the previous three years was the occurrence of *D. longispina* that, especially in September, accounts for the *Daphnia* population density peak and leads to a general increase of *Daphnia* in the Bodrio. The morphological re-examination of the stored zooplankton samples confirmed that *D. longispina* was not present in the Bodrio del pastore III in the period 2011-2013. Actually we found *D. longispina* in other two pools in the study area and we can assume that it came from there by recent dispersal, possibly linked to increased winter precipitation and subsequent floods (Rossi *et al.*, in preparation). But we cannot rule out that *D. longispina* occurrence in 2014 was due to re-emergences from stored ephippia. In the second case we would highlight the common mechanism between the new record of *D. longispina* in the Bodrio and the anticipated and unusual autumnal recruitment of *D. pulex* from ephippia.

D. pulex and *D. longispina* have very similar ecological characteristics and interspecific differences in life history traits are dependent on temperature and food conditions (Hanski and Ranta, 1983). Their coexistence in rock pools was reported and shifts in competitive advantage with environmental changes were described (Bengtsson, 1986; 1987). In the Bodrio, *D. pulex* and *D. longispina* seasonal dynamics was quite different: *D. longispina* dominate in late summer while *D. pulex*

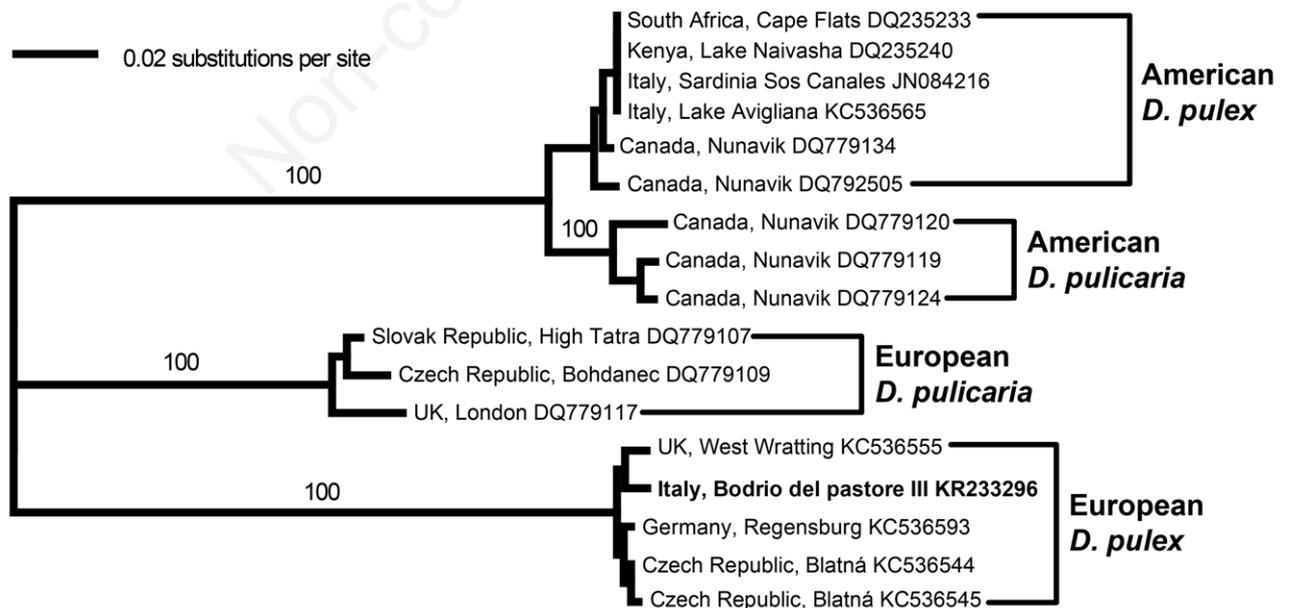


Fig. 4. A maximum likelihood (ML) phylogeny of *ND5* sequences showing the relationship of haplotype carried by *Daphnia* from the Bodrio del pastore III with other members of the *Daphnia pulex* complex. Numbers along branches correspond to ML bootstrap support values if higher than 70.

showed the highest density in November. However morphological and genetic analysis confirmed the coexistence of both species in spring when the presence of epibionts on the postabdominal claw prevented a complete morphological discrimination of the two species and a reliable evaluation of their percentages. Epibionts were observed throughout the sampling period in 2014. The prevalence of infection increased from March to June, then epibionts changed anatomical units and were found mainly on the surface of carapace and on the antennae in autumn. We had not insight that infection degree was higher in *D. pulex* than in *D. longispina* or *viceversa*. The presence of epibionts on freshwater zooplankton is a very common phenomenon (Carman and Dobbs, 1997) and free swimming ciliates of the genus *Carchesium* were recorded in the Bodrio also in the previous study period. A high epibiont burden should indicate high food availability because the epibiont dispersal stage is actively and intensively grazed by *Daphnia* (Pérez-Martínez *et al.*, 2001). Several studies have shown that virulence varies with temperatures and epibionts can influence zooplankton individual and population dynamics in many ways, although the available data show considerable variability (Barea-Arco *et al.*, 2001; Mitchell *et al.*, 2005).

In the Bodrio, both *D. pulex* and *D. longispina* reproduce by cyclical parthenogenesis but difference in sexual phase timing and importance between the two species was recorded. In 2014, as well as in 2011-2013 period, very early and continuous production of males and ephippia, also independent of population density, was observed in *D. pulex* (Rossi *et al.*, 2014). A relatively limited investment in sexual reproduction and mismatch between the presence of males and ephippial females was recorded in *D. longispina*. Temperature, photoperiod, food availability (epibiont burden and/or edible phytoplankton biomass) were related to sexual reproduction: different threshold for males and ephippia production and both interspecific and intraspecific variation in sex allocation were reported (Stross, 1987; Hobaek and Larsson, 1990; Larsson 1991; Spaak 1995; Deng, 1996; Innes and Dunbrack, 1993; LaMontagne and McCauley, 2001; Slusarczyk 2001; Koch *et al.*, 2009). We cannot exclude that the mismatch between males and ephippial females production in *D. longispina* might be an effect of the meteo-climatic anomaly (Yurista and O'Brien, 2001; Sarmaja-Korjonen, 2003; De Senerpont Domis *et al.*, 2007; Lampert *et al.*, 2012; Conde-Porcuna *et al.*, 2013). According to our preliminary evaluation and due to the low density, *C. flavicans* should not affect *Daphnia* juvenile mortality or selective survivorship of the two different *Daphnia* species. However, *Chaoborus* larvae might account for seasonal variation of juvenile morphology in both *Daphnia* species. It is well known that, in presence of invertebrate predators *D. pulex* and *D. longispina*

groups, developed neckteeth as inducible defences (Havel, 1985; Colbourne *et al.*, 1997; Kotov *et al.*, 2006; Juračka *et al.*, 2011). Accordingly, we observed that the proportion of juveniles bearing neckteeth was a positive function of predator density and decreased in autumnal samples.

Finally, the finding of native European *D. pulex* strain in the Bodrio is important in the framework of conservation ecology. In this study we have showed that, to date, the Bodrio has not been colonized by the invasive North American asexual clone that has spread and replaced the native *D. pulex* throughout Africa, Spain and that has been recently recorded in Sardinia and Piedmont (Northern Italy) (Mergeay *et al.*, 2006; Vergilino *et al.*, 2011; Fadda *et al.*, 2011; Crease *et al.*, 2012; Marková *et al.*, 2013).

CONCLUSIONS

Overall, our results suggest that coexistence of *D. pulex* and *D. longispina*, their biotic interactions, for instance with epibionts and predators, and/or shifts of their competitive advantage may depend on water temperature and hydroperiod. We do not claim that our present data prove a causality between weather and *Daphnia* dynamics anomaly in 2014 but we discuss about possible relationship between inter-annual meteo-climatic and *Daphnia* phenology variability. Our results encourage in continuing and extending our monitoring for evaluating direct and indirect impact of climate change on biological communities of shallow waters.

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