

Morphology and ecology of *Daphnia middendorffiana*, Fisher 1851 (Crustacea, Daphniidae) from four new populations in the Alps

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ABSTRACT

Daphnia middendorffiana has an arctic, circumpolar distribution, with some isolated southerly populations restricted to mountainous areas, including the Alps. In this paper, new records of *Daphnia middendorffiana* on the Alps are reported. The species was regularly recorded in several samples collected from 2006 to 2009 in four high altitude lakes in the Gran Paradiso National Park (GNP) during the ice free period. This is the third finding for the Alps and the described populations are the largest. Chemical and morphometric features of the lakes are provided, the morphology of the *Daphnia middendorffiana* from GNP is described as well as some aspects concerning its ecology. The studied lakes are small, oligotrophic (total phosphorus ranged from 0 to 7 $\mu\text{g L}^{-1}$) and well preserved from acidification risk (pH ranged from 6.45 to 8.14). *D. middendorffiana* is the largest zooplanktonic crustacean inhabiting the Alpine lakes in GNP reaching 3.43 mm in length; the morphological analysis noted some differences within the studied populations; however there is a clear resemblance to the only Alpine population previously described (from Central Alps, Bognanco Valley, Lake Campo IV) and to the arctic populations. *D. middendorffiana* in GNP lives at low density levels, reaching higher densities in late August and early September. No males have been found during the sample campaign confirming its ability to produce asexual viable diapauses eggs. This study confirms the attitude of this species for cold and oligotrophic waters and increases the current knowledge on the geographical distribution, morphology and ecology of this species in Alpine environments. The finding of *D. middendorffiana* in the GNP poses interesting issues concerning the phylogeography of Alpine *Daphnia middendorffiana*, as well as raising need for conservation efforts aimed at keeping the populations safe from several global and local threats, such as climate warming and the ecological impact of alien species.

Key word: zooplankton, isolated population, introduced fish, Gran Paradiso National Park, Alpine lake

1. INTRODUCTION

Daphnia middendorffiana has an arctic, circumpolar distribution (Brooks 1957; Manuilova 1964; Hobaek & Weider 1999) and is one of the most widespread *Daphnia* species in arctic freshwater ecosystems, extending its range to the northernmost land in Greenland (83°30'N) (Haney & Buchanan 1987). There are also isolated southerly populations in the Rockies (Brooks 1957), a record (as *D. gibbosa*) from Poland (Hellich 1877) and some other populations are reported in Argentina (Vega & Clause 2000). *D. middendorffiana* has been reported also in the Alps and its presence is known since several decades (Brooks 1957; Ferrari 1967). In spite of the extensive zooplankton taxonomic surveys conducted through the Alps (Tonolli & Tonolli 1951; Giussani *et al.* 1986; Angeli *et al.* 2002; Angeli & Tavernini 2006) just two records were known for this species until now: Lake Campo IV (2293 m a.s.l.) in the Bognanco Valley, Italy (Ferrari 1967, 1970) and Lake Antermoia (2496 m a.s.l.) in the Avisio catchment, Italy (Angeli & Tavernini 2006).

Four new records of *D. middendorffiana* from high altitude fishless lakes in the Gran Paradiso National Park (GNP) are reported for the first time in this paper. The examined species was found during a monitoring

campaign that included several other lakes, regularly sampled since 2006-2007. These lakes were sampled since 2006 or 2007. The results concerning *D. middendorffiana* presented in this paper are supported by data collected in four years of monitoring activities (2006-2009). Particular emphasis is given to the morphology of the studied populations and its variability as a diagnostic taxonomic trait (Margaritora 1985; Haney & Buchanan 1987; Manuilova 1964) and as an adaptive ecological feature (Balseiro & Vega 1994).

2. METHODS

2.1. Study area

Gran Paradiso National Park (GNP) is located between 45°25' and 45°45' N and between 7° and 7°30' W in the Western Italian Alps. The protected area shows a large altitudinal extension (between 800 and 4061 m) and a typical Alpine climate.

D. middendorffiana was found in 4 lakes belonging to the catchments of rivers Orco and Dora di Savarenche, all included within the territory of GNP: the lakes Nivolet Superiore, Trebecchi Inferiore, Trebecchi Superiore and Lillet are all above 2500 m a.s.l. and they are not affected by hydromorphological alterations (Tiberti *et al.* 2010). In this paper, toponyms of the lakes will be replaced by the following abbreviations:

Tab. 1. Geographic, morphometric and chemical features of GPNP studied lakes and catchment characteristics (Tiberti *et al.* 2010). Z_m : maximum depth; A: area; L: perimeter; V: volume; B: catchment area. Geology - AG: catchment entirely composed by Acidic Gneiss; geology - CS: catchment dominated by thick covering of Calcareous Schists. Average values of the main chemical features during the zooplankton sampling campaign: C: conductivity at 20 °C; Alk: total alkalinity; TP: total phosphorus; TN: total nitrogen; TOC: total organic carbon.

		NIVSUP	TREINF	TRESUP	LILLET
Latitude N		45°28'41"	45°30'08"	45°30'07"	45°28'00"
Longitude E		07°08'55"	07°08'48"	07°08'40"	07°12'26"
Altitude	(m)	2538	2723	2729	2765
Z_m	(m)	17.1	8.1	7.5	13.2
A	(m ²)	34482	14812	14172	36249
L	(m)	986	493	565	846
V	(10 ³ m ³)	162.1	61.8	48.9	233.1
B	(ha)	29.11	43.76	23.66	91.86
Geology		AG	CS	CS	AG
pH		7.07	7.93	7.98	7.38
C	(μ S cm ⁻¹)	21.69	87.58	100.55	43.90
Alk	(meq L ⁻¹)	0.20	0.94	1.07	0.40
TP	(μ g L ⁻¹)	2.7	2.3	1.4	2.0
TN	(mg L ⁻¹)	0.18	0.12	0.13	0.27
TOC	(mg L ⁻¹)	0.58	0.48	0.45	0.25

Nivolet superiore - NIVSUP, Trebecchi Inferiore - TREINF, Trebecchi Superiore - TRESUP, Lillet - LILLET. Main geographical, morphological and watershed data are based on Tiberti *et al.* (2010) (Tab. 1). Chemical features (Tab. 1) are partially based on Tiberti *et al.* (2010) and available only for the last two years of study.

The lakes are placed in two geologically separated areas: the first is entirely dominated by acidic gneiss (surrounding NIVSUP and LILLET), while the second is dominated by thick covering of calcareous schists variously metamorphosed (surrounding TRESUP and TREINF) (Leporati *et al.* 1999). The geology affects the vegetation development in the watershed (Scotta *et al.* 1999) as well as the hydrochemistry of the lakes (Tiberti *et al.* 2010). The studied lakes are well preserved by acidification risk (pH ranges from 6.45 to 8.29 and alkalinity from 0.16 to 1.08 meq L⁻¹) and their conductivity is low (from 20.3 to 101.5 μ S cm⁻¹). Concerning the nutrient contents, according to the Redfield ratio, phosphorus is the phytoplankton growth limiting element and is assumed to have a leading role in shaping the biotic community (Tiberti *et al.* 2010); its concentration is an index of oligotrophy and ultraoligotrophy. In the studied lakes the ice cover lasts for most of the year, from November until late June or even late July. Measurements of temperature profiles show that only deeper lakes (LILLET and NIVSUP) undergo stable stratification during the ice free period, while shallow lakes (TREINF and TRESUP) mostly show slightly depth-decreasing temperature gradients, suggesting that thermal stratification would be likely unable to offer resistance to wind mixing effects (Tiberti *et al.* 2010). Low values of oxygen saturation can occur at the bottom just after surface ice melting (17% oxygen saturation was observed in NIVSUP on June 30, 2008), probably because of Winter oxygen consumption under

the ice cover, while all the lakes were well oxygenated late in the season (Tiberti *et al.* 2010).

2.2. Sampling and analytical methods

Monitoring activity in NIVSUP, TREINF and TRESUP started during 2006 and one year later in LILLET. Zooplankton samples were collected at least twice every year during the ice free period until 2009. Unfortunately only one zooplankton sample is available for TREINF and TRESUP in 2006. The first sample was collected at the beginning of summer (June or July), the last late in the season (August or September) to observe the zooplankton community both in its early stages and in the mature stages. Moreover, during summer 2009, NIVSUP and TRESUP were sampled at regular intervals of time (every 15 days) to obtain results on the ecological dynamics of *D. middendorffiana* and the whole zooplankton community during the season.

Zooplankton samples were collected in the deepest point of the lakes. The zooplankton net (an open net with 48 μ m mesh size) was towed vertically from the bottom to the surface. Sample fixation in 4% formaldehyde was carried out in the field as early as possible to avoid damage to animal tissues by bacterial action and autolysis (Goswami 2004). Zooplankton taxa in the samples were identified following Dussart (1969), Harding & Smith (1974), Barioni & Gelmini (1983), Stella (1984) and Margaritora (1985). The number of individuals of each taxon in each sample were counted following Edmonson (1971) in order to obtain density data.

2.3. Morphological analysis

A sample of 163 *D. middendorffiana* specimens, collected over four years at different times from all the studied lakes, underwent more detailed morphological

analysis. A series of relevant morphological traits (Haney & Buchanan 1987) was observed, such as the pigmentation of the carapace, the shape of the head and of the abdominal processes, the presence of a gape between ventral margin of the head and carapace, the number of anal teeth, the number of denticles in the middle pecten and the number of eggs in the brood chamber. Moreover the body length of 302 *D. middendorffiana* specimens have been measured (from the top of the head to the base of the tail spine) while the tail spine length was measured in a smaller subsample of 126 *D. middendorffiana* specimens.

2.4. Data analysis

The factors influencing some biometric variables such as the body length (Size) and the length of tail spine (Spine), were determined with linear mixed-effects models (LME) fitted by Restricted Maximum Likelihood (REML) to avoid pseudoreplication due to repeated sampling of the same lakes at different times. The LMEs were fitted following Zuur *et al.* (2009) using the nlme package (Pinheiro *et al.* 2010) of the statistical environment R version 2.12.1 (R Development Core Team 2010). A "beyond optimal model", where the fixed component contains all explanatory variables and as many interactions as possible, has been used to find the optimal structure of the random component evaluated considering the difference between the Akaike Information Criteria (AIC difference). Once the optimal random structure was found, the optimal fixed effect structure was determined with a stepwise method by comparing the same models fitted with Maximum Likelihood (ML) with a Likelihood Ratio Test. Only the results of the optimal model refitted by RELM are provided in this article. The number of eggs or embryos carried by the measured individual (Eggs), the size of the measured individuals (Size), the year of sampling (Year), the number of days since June 15th of the year of sampling (Day) and biologically meaningful interactions among these variables were included to the "beyond optimal model" as fixed effects. In the model in which "Size" is fitted as dependent variable, "Eggs" was included among the fixed effects as a binary variable (presence/absence of eggs or ephippia). The lake identity (Lake) was included in all models as a random effect.

3. RESULTS

3.1. Morphological features

A detailed morphological analysis was conducted on 163 *D. middendorffiana* specimens. Distinct brown pigmentation extending to the dorsal exoskeleton and often to the proximal segments of antennae is the most striking feature of *D. middendorffiana* and has been regularly observed in all the studied populations. The head of the observed individuals was separated from the valves by a gap and sloped quickly after the optic vesicle, producing an acute angle with body axis; a slight

spinulation of ventral and dorsal margin of valves was always present and was more marked in the tail spine region. In the large, mature individuals the 1st post abdominal process was lint and longer than the 2nd. The number of anal teeth ranged between 11 and 23, but there were some differences in the range of variation depending on the studied lakes (12-22 in NIVSUP; 11-23 in TREINF; 17-22 in TRESUP; 11-18 in LILLET). The number of denticles in the middle pecten ranged between 5 and 11; as in the case of anal teeth, some differences were detectable among the studied lakes: while in NIVSUP and LILLET there were up to 11 denticles, in TREINF and TRESUP the number of denticles did not exceeded seven. The number of parthenogenetic eggs retained inside the brood chamber could reach 15 (in a large, 3.35 mm long, *D. middendorffiana*, sampled in NIVSUP on 24 June, 2007) but it was usually lower.

The length (Size) of 306 *D. middendorffiana* specimens (tail spine excluded) ranged from 0.60 mm (13/7/07, LILLET) to 3.43 mm (24/6/2007, NIVSUP). LME models with "Size" as dependent variable were improved by adding the random effect Lake (AIC difference = 112.11), showing size variability depending on the population of origin. The LME models indicated the size was negatively related with the number of Days from the 15th of June and was positively affected by the presence of Eggs, while Year and all the interactions terms among variables were dropped from the initial model (Tab. 2), suggesting that the size of *D. middendorffiana* is significantly larger at the beginning of the ice free period and in egg carrying females.

Tab. 2. Fixed effect results from Linear Mixed Effect models with body length (Size) of *Daphnia middendorffiana* as the dependent variable, Lake as a random grouping factor, a random intercept, and Day, Year and Eggs as independent variables. Model is based on 306 observations from 4 lakes.

	Coeff.	df	F	p
Terms included in the model				
Day	-0.76	1.296	37.68	≤0.0001
Eggs		1.296	8.77	≤0.01
Rejected terms				
Year	0.01	1.294	0.14	0.70
Eggs*Day	0.00	1.295	0.75	0.38

The tail spine length (range: 0.05-0.58 mm) from 126 *D. middendorffiana* specimens was analyzed in the same way. Adding the random effect Lake improved the models (AIC difference = 17.51). Spine length was inversely proportional to Size (Fig. 1), showing a negative allometric development of tail spine; the main results are summarized in table 3.

3.2. Ecological features

A total of 40 zooplankton samples were analyzed since 2006 (14 from NIVSUP, 7 from TREINF, 13 from

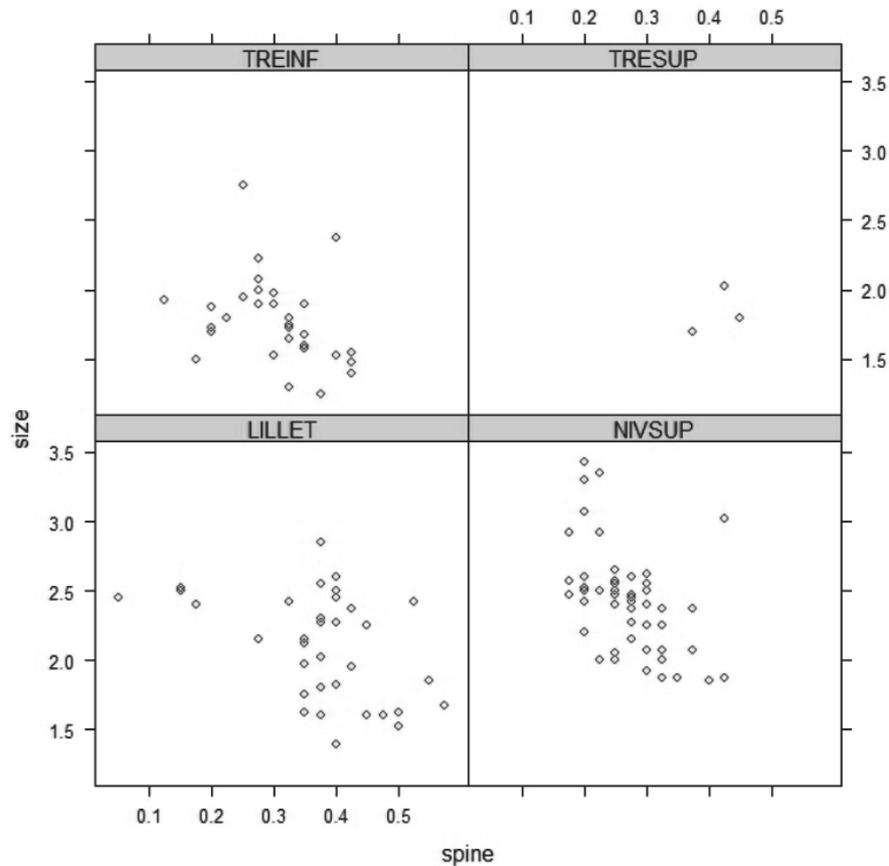


Fig. 1. Negative allometric development of tail spine in *Daphnia middendorffiana* populations from GPNP.

TRESUP and 6 from LILLET). *D. middendorffiana* was regularly found in samples from all four study sites (Fig. 2; Tab. 4). Compared with other zooplanktonic species, *D. middendorffiana* lives in fairly low densities (Tab. 4).

Tab. 3. Fixed effect results from Linear mixed Effect models with tail spine length (Spine) of *Daphnia middendorffiana* as the dependent variable, Lake as a random grouping factor, a random intercept, and Size, Day, Year and Eggs as independent variables. Model is based on 126 observations from 4 lakes.

	Coeff.	df	F	p
Terms included in the model				
Size	-0.10	1.118	24.61	≤0.0001
Day	0.01	1.118	6.34	≤0.05
Eggs	0.00	1.118	4.25	≤0.05
Rejected terms				
Eggs*Day	0.00	1.114	0.03	0.86
Year	0.00	1.115	0.12	0.73
Size*Day	0.00	1.116	0.30	0.59
Size*Eggs	-0.02	1.117	2.44	0.12

The maximum density was found in NIVSUP, reaching 314 individuals m^{-3} (August 20, 2008), but it never exceeded one hundred individuals in all other samples. In comparison, a very similar species such as *Daphnia gr. longispina* can exceed 10,000 individuals

m^{-3} within the same samples. *D. middendorffiana* has been found also in samples collected early in the season, often when the lakes were still mostly covered by ice. However most of the specimens were sampled later in the season. Some information concerning the ecological dynamics of *D. middendorffiana* have been obtained in lakes NIVSUP and TRESUP, where its density was measured every 15 days during 2009, confirming that the highest densities are registered between mid-August and early September (Fig. 2).

The studied populations of *D. middendorffiana* share the same environment with other zooplanktonic crustaceans and rotifers (Tab. 5): LILLET is the only lake where *D. middendorffiana* does not live in simpatry with *Daphnia gr. longispina* (in TREINF and TRESUP some individuals have been identified as *Daphnia rosea* belonging to the *Daphnia longispina* species complex), but otherwise it always coexists with *Arctodiaptomus alpinus* and *Cyclops gr. abyssorum*. In a few cases also *Eucyclops serrulatus*, *Chydorus sphaericus*, *Alona quadrangularis* and *Acropaerus harpae* were found in the studied lakes. The zooplankton community in GPNP lakes is complemented by some species of rotifers such as *Keratella quadrata* (the most common rotifer in the studied lakes), *Polyarthra gr. dolichoptera*, *Synchaeta gr. stylata-pectinata*, *Notholca squamula*, *Notholca labis*, *Keratella cochlearis* and *Lecane gr. lunaris*.

Tab. 4: Zooplankton community structure, *Daphnia middendorffiana* density \pm sd and its mean length \pm sd; DAPMID: *Daphnia middendorffiana*; N: number of measured individuals; NAU: nauplii; ARC: *Arctodiaptomus alpinus*; CYC: *Cyclops* gr. *abyssorum*; EUC: *Eucyclops serrulatus*; DAP: *Daphnia* gr. *longispina*; CHY: *Chydorus sphaericus*; ACR: *Acropaerus harpae*; ALO: *Alona quadrangularis*; ROT: rotifera; KER: *Keratella quadrata*; POL: *Polyarthra* gr. *dolichoptera*; KCO: *Keratella cochlearis*; NSQ: *Notholca squamula*; NLA: *Notholca labis*; LLU: *Lecane* gr. *lunaris*; SYN: *Synchaeta* gr. *stylata-pectinata*; (+): <10 individuals m^{-3} ; (*): from 10 to 100 individuals m^{-3} ; (**): from 100 to 1000 individuals m^{-3} ; (***) : from 1000 to 10000 individuals m^{-3} ; (****): >10000 individuals m^{-3} .

Lake	Data	DAPMID N m^{-3}	Length mm	N	NAU	ARC	CYC	EUC	DAP	CHY	ACR	ALO	ROT
NIVSUP	12/07/06	4 \pm 8.8	1.85	1	**	**	**	+	*				KER****; POL+; KCO*; NSQ*
NIVSUP	27/08/06	34 \pm 11.3	2.02 \pm 0.25	15		*	**		**				KER*; KCO *
NIVSUP	24/06/07	2 \pm 2.9	3.15 \pm 0.17	6	**	**	**	+	*				KER**; POL*; KCO +
NIVSUP	20/08/07	314 \pm 76.2	2.06 \pm 0.30	106		**	***	*	***			+	
NIVSUP	30/06/08				**		*						KER****; POL**; NSQ**
NIVSUP	01/09/08					**	**	*	*		+	+	KER**; POL***; NSQ*
NIVSUP	08/07/09	8 \pm 4.4	2.65 \pm 0.17	4	**		**		*				KER***; KCO +; NSQ+
NIVSUP	21/07/09				**	+	**		+				KER***
NIVSUP	03/08/09	2 \pm 5.4	2.55 \pm 0.50	5	+	**	**		*				KER**
NIVSUP	19/08/09				*	***	**	+	**				KER**
NIVSUP	02/09/09	52 \pm 27.0	2.04 \pm 0.52	8	**	**	***		***				KER**; POL+
NIVSUP	17/09/09	20 \pm 14.1	2.35 \pm 0.18	5	*	**	**	*	***				KER*
NIVSUP	02/10/09	7 \pm 10	2.34 \pm 0.41	2	**	*	**		***				KER+; POL*; NSQ+
NIVSUP	14/10/09				*	+	*		**				POL**
TREINF	16/07/06	31 \pm 26.8	2.08 \pm 0.48	9	**	**	***		**	*			KER**; KCO *
TREINF	30/06/07				**	**	**		*				KER***
TREINF	22/08/07	15 \pm 9.9	1.65 \pm 0.33	9	*	*	***		*			*	KER*
TREINF	10/07/08				**	**	***		*				KER***; POL+; NSQ+
TREINF	23/08/08	21 \pm 2.3	1.41 \pm 0.24	13	**	*	**	+	***	+			KER*; POL**; NSQ+
TREINF	07/07/09				**	*	**		*	+			KER**; KCO *
TREINF	18/08/09	91 \pm 23.8	1.90 \pm 0.37	27	*	***	**	*	***	*		+	KER**
TRESUP	15/07/06	20 \pm 13.7	1.76 \pm 0.59	6	***	***	***	+	**				KER**; KCO **
TRESUP	30/06/07	2 \pm 3.1	2.00	1	**	**	***		*	+			KER***
TRESUP	22/08/07					*	**		***				*
TRESUP	09/07/08	14 \pm 3.6	1.37 \pm 0.67	4	***	**	***		*		+	+	KER****; POL*; NSQ*
TRESUP	23/08/08				**	*	**	+	**			*	KER**; POL**; NSQ*
TRESUP	07/07/09				***	*	***		+				KER***; KCO +; NSQ+
TRESUP	20/07/09				***	**	***	+	**				KER***; KCO +; NSQ+
TRESUP	04/08/09	4 \pm 9.9	2.12 \pm 0.06	3	**	***	**		**				KER**
TRESUP	18/08/09	20 \pm 20.8	1.86 \pm 0.19	5	*	**	***		***	+			KER**; NLA+
TRESUP	01/09/09	7 \pm 14.8	2	1	*	*	**		****				KER**
TRESUP	18/09/09				*	**	*		***	+			
TRESUP	01/10/09					*	**		****			*	KER*
TRESUP	13/10/09				+	+	+		*	+			KER*
LILLET	03/07/07	3 \pm 4.2	1.67 \pm 0.92	4	***	**	*						KER*; SYN*
LILLET	18/08/07	24 \pm 1.9	1.49 \pm 0.51	26	**	***	**						KER*
LILLET	13/07/08	1 \pm 0.7	1.24 \pm 0.81	4	***	+	+						KER*; POL*; SYN**
LILLET	26/08/08	48 \pm 5.9	1.44 \pm 0.57	28	**	***	**		+				KER***; POL***
LILLET	28/07/09				***		*						KER**; KCO +; NSQ+; NLA+; SYN*
LILLET	29/08/09	16 \pm 9.2	0.97 \pm 0.21	13	**	**	*						KER*; KCO +

Females carrying parthenogenetic eggs were found throughout the summer, even early in the season. Individuals with diapausing eggs (or ephippia) were found only once in NIVSUP on 2 September, 2009. Throughout the sampling campaign, no male has been identified.

4. DISCUSSION

4.1. Morphology

The observed morphology was consistent with the one reported by Ferrari (1967) from Lake Campo IV (central Italian Alps) or by Haney & Buchanan (1987) from the Arctic regions (Tab. 5). The pigmentation, the dorsal margin of head, the abdominal processes, the gape between head and carapace and the body length show the same features both in the Alpine and Arctic populations. However, the GPNP populations share with the Arctic populations the same number of anal teeth

and denticles in the middle pecten, markedly higher than in Campo IV populations. Variability in both the number of anal teeth and denticles in the middle pecten was found also within the GPNP populations: for example, the maximum number of anal teeth was 18 in LILLET, very similar to the number observed in Campo IV. The same situation was recorded for the number of denticles of the middle pecten: in TREINF and TRESUP its number did not exceeded seven (in Campo IV it was six), while in NIVSUP and LILLET there were up to 11 denticles. Any morphological variations within GPNP populations and among GPNP, CampoIV and arctic populations could be caused either by environmental pressures or genetic differences, which should be cleared out by further genetic analysis. Both morphological and genetic variability can be used as valuable information for studying *D. middendorffiana* distribution and geographical separation.

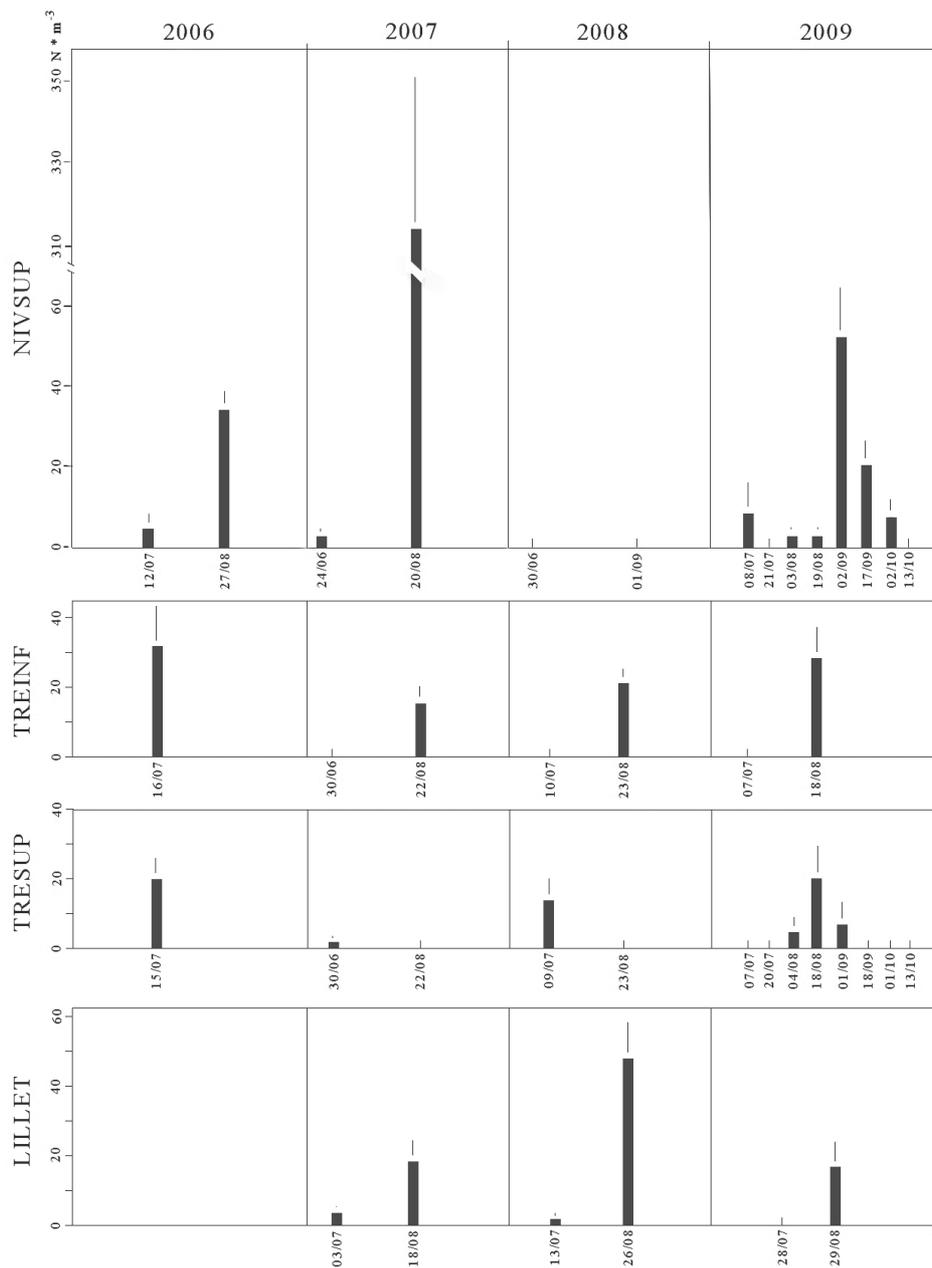


Fig. 2. Date of sampling and abundances + 1se of *Daphnia middendorffiana* in GPNP populations.

Tab. 5. A comparison of morphology of *Daphnia middendorffiana* from GPNP, Campo IV (Ferrari 1967) and Arctic populations (Haney & Buchanan 1987).

	This study	Campo IV lake	Arctic
Pigmentation	Yes	Yes	Yes
Dorsal head margin	Nearly straight, acute angle with body axis	Nearly straight, acute angle with body axis	Nearly straight, 45° angle with body axis
Gap	Present	Present	Usually present
Spinulation	Extends on to anterior half of the margin	Extends on to anterior half of the margin	Often extends on to anterior half of the margin
Abdominal processes	Length of 1 st longer than the length of 2 nd	Length of 1 st longer than the length of 2 nd	Length of 1 st about two time the length of 2 nd
Anal teeth	23	17	24
Middle pectin	5-11	6	5-10
Maximum Size	3.43 mm	More than 3 mm	Up to 3.5 mm
Tail spine	Short, 2-34 % body length	Short, 10-24 % body length	Up to 33% length of valves; rarely short
Tail spine negative allometric development	Yes	Yes	Yes
Maximum number of retained eggs	15	16	-

Ferrari (1970) observed up to 16 parthenogenetic eggs retained inside the brood chamber in specimens from Campo IV population. The results from the present study confirm this observation, as the maximum number of parthenogenetic eggs retained inside the brood chamber was 15, but usually lower.

The body length of *D. middendorffiana* is significantly larger at the beginning of the ice free period and in egg carrying females. Margaritora (1985) suggested that the life cycle of *D. middendorffiana* is restricted to the ice free period, from June to October. However the presence early in the season of large, often eggs carrying *Daphnia* shows that they have developed under the ice, suggesting that the life cycle of the species is longer of at least a few weeks. Likewise, Poulsen (1940) and Hilliard & Tash (1966) observed adult females wintering in deep lakes and producing a subitaneous generation in the spring, just before dying. Probably, as in the arctic populations, even Alpine *D. middendorffiana* rely on both diapausing and subitaneous eggs when the depth of lakes prevents the freezing of the whole water body.

The studied populations share a negative allometric development of tail spine with the population from Campo IV as well as with Arctic and Andean populations. This inverse proportion between body size and spine length has been linked to predation risk: *D. middendorffiana* shields itself from predation by developing a long spine in the juveniles and a large body size in the adults (Balseiro & Vega 1994). Both the tail spine and the large body size are considered to be defenses against invertebrate predators (Dodson 1974; Luecke & O'Brien 1983; Dodson 1984; Balseiro & Vega 1994), commonly found in fishless lakes (Knapp *et al.* 2001).

4.2. Ecology and distribution

The finding of *D. middendorffiana* in the lakes of the GPNP increases the current knowledge of the geographical distribution of this species with particular reference to its southern range. As in the case of the populations in the Rockies, also in Europe the southern populations live on mountains, where *D. middendorffiana* can meet its ecological requirements. The findings described in this study are the third for the Alps and the studied populations are the most numerous. This means that this species, though rare, is widespread in the Italian Alps with a remarkable longitudinal range. Moreover, while in its main geographical range, *D. middendorffiana* can occur in lakes, ponds and also in semi-permanent and shallow bodies of water (Haney & Buchanan 1987; Vega & Clause 2000), the sampling campaign in GPNP was conducted only in the lakes, while the surrounding smaller aquatic environments were neglected. The same is true for almost all samples collected during every extensive zooplankton taxonomic survey through the Alps (Tonolli & Tonolli 1951; Giusani *et al.* 1986; Angeli *et al.* 2002; Angeli & Tavernini 2006). Therefore other populations of *D. middendorff-*

fiana could occur in the considerable number of ponds and puddles that typically surround the studied lakes. To better understand the ecology of Alpine populations of *D. middendorffiana*, future sampling sites should include all these environments.

D. middendorffiana can successfully compete with other zooplankton species in the studied lakes, which provide a cold and oligotrophic suitable environment. Even if *D. middendorffiana* is considered eurythermal, it has a thermal range for respiration, approximately 5 centigrade degrees lower than that for temperate zone *Daphnia* sp. (Yurista 1999) and this feature advantages its presence in Alpine lakes. Moreover the life history of *D. middendorffiana* enables this species to cope with a very short ice-free season by speeding up the production of diapause eggs. Unlike most species of *Daphnia*, *D. middendorffiana* is known to be facultatively asexual and to produce viable diapauses eggs without males (Zaffagnini & Sabelli 1972; Hebert 1981; Yurista 1999), as well as producing ephippia eggs prior to shifting to parthenogenetic reproduction (Stross *et al.* 1980; Yurista & O'Brien 2001). This ability is also confirmed by the results of this study, where no males were found during the whole sampling campaign. However individuals carrying diapauses eggs were found only once and late in the season, while parthenogenetic individuals were found both early and late in the season. As observed by Edmondson (1955) and Zaffagnini & Sabelli (1972) the ability to produce asexual diapausing eggs does not imply the impossibility to produce males, but on the Alps, this ability has never been observed in the wild.

All these adaptation for cold temperatures and short seasons enabled *D. middendorffiana* to survive in GPNP lakes. However, in the future, *D. middendorffiana* might have to compete with species expanding their range under the predicted temperature increase due to climate change in the Alpine area (Magnuson *et al.* 1997; Holzapfel & Vinebrooke 2005). The finding of new populations of *D. middendorffiana* can be of help in the design of conservation plans for this species in the Alpine environment in a context of global changes.

A common factor for all the aquatic ecosystems containing *D. middendorffiana* population, is the absence of predatory fish (Vega & Clause 2000): a considerable number of studies describes the impact of fish introduction in originally fishless lakes, which results in the loss of native species and the degeneration of natural food-web structure (Knapp *et al.* 2001; Eby *et al.* 2006). One of the most obvious prediction is that direct selective predation by visually hunting fish would result in an abundance reduction of more visible, larger and pigmented preys which can lead to their local extinction (Brooks & Dodson 1965; O'Brien 1987; Brancelj 1999; Knapp *et al.* 2001; Eby *et al.* 2006). *D. middendorffiana* is by far the largest zooplankton species occurring in the GPNP lakes and is strongly pigmented, potentially

becoming an easy prey. Its presence in the studied lakes can be therefore assumed to depend on the natural absence of fish. Lake Nivolet Inferiore, for instance, is only a few meters distance from NIVSUP and the two lakes are connected by a short river, nevertheless, *D. middendorffiana* has never been found in this lake (personal observation), where a population of *Salvelinus fontinalis* (an alien salmonid from north America) was introduced. To preserve the population of *D. middendorffiana* in GPNP, it is absolutely necessary to prevent any further introduction of fish. The presence of the national park provides some protection against this threat. Moreover, in accordance with the institutional conservation objectives of GPNP, the establishment of new populations of *D. middendorffiana* should be encouraged through a program of eradication of alien fish. Indeed *D. middendorffiana* could naturally recover following fish removal from egg banks (Manca & Armiraglio 2002) or thanks to the dispersal of adults and resting eggs (by water flow, wind or animal vectors).

5. CONCLUSIONS

This investigation confirms the preference of *D. middendorffiana* for cold and oligotrophic waters and increases the current knowledge of the geographical distribution, morphology and ecology of this species in Alpine environments, rising interesting issues related to its phylogeography. In fact the most interesting feature concerning *D. middendorffiana* Alpine populations is probably related to their geographical isolation: a remarkable distance exists between arctic and Alpine populations, which suggests fascinating, although speculative, hypothesis on the phylogeny of the Alpine *D. middendorffiana*. A recent series of studies (Weider & Hobaek 1994; Weider & Hobaek 1997; Colbourne *et al.* 1998; Hobaek & Weider 1999; Weider *et al.* 1999a, b; Weider & Hobaek 2003; Paland *et al.* 2005; Adamowicz *et al.* 2009) has examined the phylogeography of the arctic *Daphnia pulex* species complex (to which *D. middendorffiana* belongs) discovering that, in spite of a slow rate of morphological evolution in the Daphniidae, morphologically similar taxa showed high levels of genetic divergence. These studies often involved the arctic populations of *D. middendorffiana* from North America, Asia and Europe, including the mountain population from the Rockies, but the Alpine population was never analyzed in this global context.

The finding of *D. middendorffiana* in the GPNP also raises the need for conservation efforts aimed at keeping the populations safe from several global and local threats, such as climate warming and the ecological impact of alien species. A deeper understanding of the distribution of *D. middendorffiana* in the GPNP would enhance the ability to address the threats to its conservation. Moreover the presence of *D. middendorffiana* in fishless lakes should encourage the implementation of programs for the eradication of alien fish, for the benefit of the whole biotic community.

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