

Copepods come in first: rapid colonization of new temporary ponds

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Published in:

Fundamental and Applied Limnology - Archiv für Hydrobiologie (2007) 168/4: 289-297.

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Abstract

The sequence in which new colonists reach an empty habitat can be crucial for future development and species structure of communities. It is therefore important to assess species composition and abundance in the initial stages of habitat existence. In the present study we focussed on colonization of newly constructed temporary ponds in Doñana, Southwest Spain, created by removing 30 or 60 cm of top soil. To confirm that no egg-bank was present, we conducted hatching experiments using sediment cores from six new ponds and two reference sites. Hatching was not recorded in the sediment of the new ponds with the exception of two rotifer individuals. In contrast, in the reference sites a maximum of 103 individuals per sample hatched, including cyclopoids, cladocerans, ostracods and rotifers. In the field, water samples were collected from seven ponds after 19 days of their first hydroperiod. Cyclopoid copepods, mostly *Metacyclops minutus*, had arrived and dominated all sampled ponds. Other taxa were the monogonont rotifer *Brachionus plicatilis* in four ponds, and the cladoceran *Moina brachiata* in one pond. The abundance of zooplankton was negatively correlated with conductivity, suggesting that ponds with higher conductivity undergo delayed colonization. We suggest that fast dispersal and dominance of certain cyclopoid copepods during early colonization is related to their ability to store sperm and fast individual development. Given that cyclopoid copepods can survive drought periods in the sediment of temporary ponds, first and early colonization by cyclopoids is likely to have a profound effect on the propagule bank and future plankton communities when the ponds refill.

Introduction

Rotifers and cyclopoid copepods are among the first zooplankton to colonize experimental tanks (COHEN & SHURIN 2003, CÁCERES & SOLUK 2002, JENKINS & BUIKEMA 1998). JENKINS & BUIKEMA (1998) found that, in newly colonized experimental ponds, copepods were second in number and biomass to rotifers. Although cladocerans have generally not been found to enter experimental ponds or mesocosms until the third month of their existence, it has recently been suggested that cladocerans are faster dispersers than copepods (DEJEN et al. 2004).

Priority effects of initial zooplankton colonists may strongly determine species composition (ROBINSON & DICKERSON 1987, JENKINS & BUIKEMA 1998). Experimental introduction of zooplankton species into established aquatic communities indicates that local biological interactions might preclude invasion of existing communities (SHURIN 2000). The arrival sequence of species is thus a crucial factor shaping the structure of zooplankton communities, and it is important to study colonist species and their abundance during the first weeks of habitat existence in order to understand the determinants of community structure in the long term.

The colonization by cladocerans of newly created ponds in Belgium has recently been studied, beginning in the third month of pond existence (LOUETTE & DE MEESTER 2004, LOUETTE & DE MEESTER 2005). In contrast, despite the obvious speed with which some cyclopoid copepods enter experimental systems (JENKINS & BUIKEMA 1998, CÁCERES & SOLUK 2002), little is known about their colonization of new habitats under natural conditions in the field. The main modes of dispersal of copepods remains unclear, although the possibilities of bird, insect and wind mediated dispersal have been suggested (FIGUEROLA & GREEN 2002, HAVEL & SHURIN 2004, GREEN & FIGUEROLA 2005), as well as dispersal via hydrological connections (MICHELS et al. 2001, FRISCH & THRELKELD 2005).

Isolated, newly constructed ponds that fill with rainwater offer an ideal opportunity to study initial colonization and community succession. While a propagule bank starts to build up during the first year of existence of new ponds (LOUETTE & DE MEESTER 2005, VANDEKERKHOVE et al. 2005), the sediment of older waterbodies contains dormant stages of major zooplankton taxa and is an important source for annual recruitment (HAVEL et al. 2000, GYLLSTROM & HANSSON 2004). The dormancy stage differs between taxa: resting eggs are generally produced by rotifers, cladocerans and calanoid copepods while in other copepod taxa dormancy is expressed in more advanced developmental stages, e.g. in copepodids or adults of cyclopoids, and in adults of harpacticoids (GYLLSTROM & HANSSON 2004, SANTER 1998).

For the present study we used a set of new temporary ponds in Doñana, Southwest Spain. For pond construction, 30 or 60 cm of the top soil were removed. Given the rich diversity of zooplankton present in temporary ponds of Doñana (FAHD et al. 2000, SERRANO & FAHD 2005, FRISCH et al. 2006), there is high potential for colonization of the new ponds. We aimed to confirm the absence of a propagule bank prior to flooding by conducting hatching experiments from pond sediments. Secondly, we aimed to assess species composition and dominance structure of the zooplankton species present after initial colonization in the earliest stages of pond existence (within 19 days). This generally represents the depth in which a propagule bank, if present, is located (GYLLSTROM & HANSSON 2004). Given the evidence from mesocosm studies (JENKINS & BUIKEMA 1998, CÁCERES & SOLUK 2002), we hypothesized that cyclopoid copepods and rotifers would be the main early colonists in the new ponds. As far as we are aware, this is the first study of its kind in Mediterranean habitats.

Methods

Study area

Caracoles estate (37°07'N, 6°31'W) is a former marshland area of 2,700 ha, which was incorporated into Doñana National Park in February 2004. Until then it had been cultivated mainly with cereals for over 20 years. The area was levelled and drained by ditches in the 1970s. Until the winter of 2004-2005, waterbodies were limited to drainage ditches and small, highly ephemeral rainpools. The regional zooplankton species pool was studied in spring 2004, including the natural marshes and neighbouring wetlands of Doñana (FRISCH et al. 2006). Between summer 2004 and spring 2005, a total of 96 ponds of three different diameters (60, 125 and 250 m) and two depths (30 and 60 cm) were excavated (Fig. 1, and SANTAMARÍA et al. 2005). For pond construction, 30 or 60 cm of soil was removed across the whole pond area by heavy machinery, with a narrow, sloped border (less than 1 m wide). Laser technology was used to level the bottom of the ponds.

Doñana has a Mediterranean climate with rainfall concentrated between October and May. The 2004-2005 winter following pond construction was exceptionally dry, and most of the 96 new ponds did not contain water at any time. Precipitation occurred between 27 February and 1 March 2005 during which a total of 43.2 mm of rainwater was recorded at the meteorological station 30 km north of the study area (Aznalcazar, 37° 09' 10" N, 06° 16' 19" W, source: Junta de Andalucía, Consejería de Agricultura y Pesca). Rainwater accumulated in several ponds for the first time since their construction, covering only part of the designated pond basin (Table 1 and Fig. 2). The shallow temporary lakes Lucio de Marilópez (300 ha) and Lucio del Lobo (120 ha) within the Doñana National Park were flooded artificially with groundwater during winter and spring for waterbird management. Both lakes are located within 2 km of the new ponds, and are a major potential source of colonists (Fig. 1).

Sediment samples

To confirm the absence of a propagule bank prior to inundation, we sampled sediment of six of the 30 cm deep ponds on 26 October 2005 (Fig. 1). These ponds were chosen to be representative of all 96 new ponds and therefore contained ponds from both northern and southern groups. These ponds had not held surface water prior to collection of sediment cores. Three replicate sediment cores were collected in each pond, along a transect from centre to border, using a metal corer (depth 16 cm, diameter 8 cm). For comparison with the propagule banks of natural sites and to test suitability of hatching conditions, six replicate sediment cores were collected in each of two reference sites (Fig. 1) when they had been dry for four months: Lucio del Lobo (25 October 2005) and Lucio de Marilópez (27 October 2005).

Each core was subdivided into four layers (0-4, 4-8, 8-12, 12-16 cm) and placed separately into plastic zip-block bags to avoid cross-contamination. In the laboratory, samples were flooded in plastic containers (volume 1 L) with 500 ml of dechlorinated tap water and homogenised with a spoon. The completely submerged samples were incubated for seven days in a climate chamber at 15°C and 12:12 h light, to resemble field conditions at the time of sampling. The overlying water was filtered after 3 and 7 days of incubation and the filtrate preserved in 30% ethanol. Due to spatial constraints in the climate chamber, only the samples of the top 4 cm of all sampled replicates (n=30) were incubated on the day of sampling, while the other samples (n=90) were stored at 15°C in the dark, and incubated successively within one month of sampling. Six controls with dechlorinated tap water were included with each trial, to monitor possible cross-contamination or aerial contamination during the experiment. With the exception of ostracods and nauplii of calanoid copepods, hatching animals were identified to genus or species level. Following the hatching experiment, all sediment samples of the new ponds were washed over a 100 µm metal sieve and the filtrate examined visually for copepodids or ephippia.

Water samples and faunal analysis

Water samples were collected on 17-18 March 2005 in seven ponds that had held a small amount of water for 19 to 20 days. In some ponds several puddles were present. Ponds dried up about one week after sampling. Due to the shallow water depth in most ponds (< 5 cm), we used a 500 ml plastic cup to sample without disturbing the sediment. Samples were filtered (63 µm nylon net), and the filtrate preserved in 6% formaldehyde. Where possible, a volume of 10 L was sampled in each pond (see Table 1). When several puddles were present, subsamples were collected in several of these and combined to the total volume. Temperature, DO, conductivity, pH, pond depth and water surface area were recorded during sampling (Table 1). During the much wetter 2003-2004 winter (FRISCH et al. 2006, FRISCH et al. unpublished data) branchiopods and copepods were identified in a number of samples from temporary waterbodies elsewhere within the Caracoles estate prior to pond construction. The species list of these samples is included here (Table 4) for comparison between previously established species in the estate, and pioneer species colonizing the new ponds.

Adults were identified to species level, using EINSLE (1993), ALONSO (1996) and KOSTE (1978). All individuals were counted under a light microscope. Juvenile copepods were counted in subcategories: calanoid nauplii, cyclopoid nauplii, copepodid stages C1- C4, and fifth copepodid stage (C5). Samples with less than 400 individuals per litre were counted without subsampling. At higher densities, subsamples representing at least 1/6th of the total sample was counted. This subsample always contained at least 200 individuals of the most frequent category (cyclopoid nauplii in all cases). The complete sample was screened microscopically to encounter rare taxa.

Results

Hatching experiment

Of all 72 sediment samples (18 cores by four depths) of six new ponds, two samples contained one hatchling each. Both samples were from replicate cores of pond 0N3 (sediment depth 0-4 cm). Both hatchlings were rotifers, one individual of *Brachionus plicatilis* OF Müller 1786, and one of *B. calyciflorus* Pallas, 1766. No hatching was recorded in the other 70 samples from the new ponds. In contrast, zooplankton hatched from all 12 cores from reference sites. A maximum of 62 and 103 Ind/sample hatched from the top layer (0-4 cm) of cores from Lucio de Marilópez (n=6 cores) and Lucio del Lobo (n=6 cores), respectively. Hatchlings included monogonont rotifers (*Brachionus angularis* Gosse, 1851, *B. plicatilis*, *B. quadridentatus* Hermann 1783, *B. urceolaris* OF Müller 1773, *B. variabilis* Hempel 1896), cladocerans (*Alona rectangula* (Sars, 1862), *Alonella excisa* (Fischer, 1854), *Ceriodaphnia quadrangula* (Müller, 1874)), cyclopoid copepods (*Metacyclops minutus* (Claus) 1863), calanoid nauplii and ostracods (details in Table 2). The mean number of hatching individuals was approximately 6900 Ind/m² in Lucio de Marilópez and 5800 Ind/m² in Lucio del Lobo. Abundance of individuals (Table 2) hatching from the reference sediment samples did not differ between reference sites for any of the taxa separately nor for the total number of hatching individuals (Mann-Whitney-U test, p >0.05). Abundance of hatchlings from the deeper layers of the reference sites was generally much lower (Fig. 3).

Visual examination of filtered sediment confirmed the absence of propagules from all replicate cores of the new ponds with the exception of 0S3, which contained ehippia cf. *Moina* in the top 4 cm (7 and 4 ehippia in two of the three replicates). In contrast, the cores of the reference sites contained larger numbers of unhatched propagules (e.g. *Daphnia* and *Moina* ehippia, copepod eggs, ostracods), but their number was not recorded.

Colonization of new ponds

Within two weeks, the new ponds were colonized by copepods (*Metacyclops minutus* and *Megacyclops viridis* (Jurine) 1820), rotifers (*Brachionus variabilis* (Hempel, 1896) and an unidentified monogonont species) and cladocerans (*Moina brachiata* (Jurine, 1820)). Ostracods were not recorded. While cyclopoid copepods were present in the water of all ponds studied, rotifers were only found in four, and cladocerans only in one of the seven new ponds (Table 3).

The group of cyclopoid nauplii was by far the most numerous, followed in density in most ponds by the early copepodid stages C1 to C4 (Table 3). *Metacyclops minutus* was the most frequent cyclopoid in the study area (present in five out of seven ponds) whereas *Megacyclops viridis* only occurred in one pond. It can be assumed that juvenile stages belonged to the respective cyclopoid species recorded in a pond, as adults of these two species did not occur together. In all ponds, the relative abundance of adult cyclopoids was below 3%. The maximum density of juvenile cyclopoids including nauplii was 1136 Ind/L in pond 6S3 (Table 3). Although rotifers were present in five ponds, their densities were below 2 Ind/L. The cladoceran *Moina brachiata* was only present in one pond at low density (0.2 Ind/L, Table 3). Both the total number of zooplankton individuals and that of cyclopoid copepods was significantly and negatively correlated to conductivity (Spearman correlations, $R = -0.83$, $p = 0.01$), but not to any other environmental variable recorded.

During spring 2004, which preceded pond excavation and other restoration measures, a total of 13 crustacean species were recorded in drainage ditches and small puddles of the Caracoles estate. These included five copepods, six cladocerans, an anostracan and a notostracan (Table 4). Of these crustaceans, only two species colonized the new ponds during the present study (*Metacyclops minutus* and *Moina brachiata*).

Discussion

The cyclopoid copepod *Metacyclops minutus* was the dominant first colonist in the new ponds. Although this species produces drought-tolerant resting copepodid stages (MAIER 1992) and could thus have hatched locally from pond sediments, our data support the absence of local propagule banks prior to flooding, at least for cyclopoids. While no *Metacyclops minutus* hatched from sediment of the new ponds, this species and other zooplankton taxa hatched from sediment cores of reference sites, confirming their survival in dry sediments during July-October, with maximum air temperatures of 44°C in July and 41°C in August 2004, and also confirming the suitability of the experimental hatching conditions. Although two individuals of two rotifer taxa hatched from one of the new ponds, it is possible that a rotifer eggbank did not exist immediately after pond construction but that eggs entered this pond by wind dispersal (HAVEL & SHURIN 2004) during the dry phase of the ponds. As the study ponds were built in an area that had been drained for over 20 years, and the top 30 - 60 cm of soil had been removed to create the ponds, we suggest that *M. minutus* and other zooplankton species present colonized the ponds from external source populations. The high number of ponds in neighbouring areas where *Metacyclops minutus* is present (FRISCH et al. 2006) may have facilitated its dispersal into the new ponds. The dispersal mode for cyclopoid copepods remains unknown, although transport by insects and birds have been suggested (HAVEL & SHURIN 2004, GREEN & FIGUEROLA 2005). The study ponds were too small, dry and isolated to attract ducks and other waterbirds at the time of this study, but wagtails and other passerines as well as Odonata and other insects frequently visited the ponds. In addition to overland vectors, possible dispersal via hydrological connections must be taken into account, because we cannot rule out the possibility that a propagule bank was present in the sediments adjacent to the inundated ponds, so that the cyclopoids could have entered the ponds via drainage from the surrounding catchment. This would be an alternative explanation for the observed high colonization rates of *Metacyclops*, although we have no evidence that

rainfall was heavy enough to cause drainage into the ponds, and the pools present in the pond basins appeared to be originating from direct rainfall. However, dispersal via drainage would not explain the strong dominance of cyclopoids over other zooplankton which are equally likely to be present in established propagule banks, as evidenced by the diversity of taxa hatching from sediments of reference sites and by the variety of species recorded in temporary pools in Caracoles estate before pond construction (Table 4).

To obtain an estimated number of colonists per pond we modelled population growth for *M. minutus* (Table 5) using development times from MAIER (1992). Due to the short generation times of *M. minutus*, most individuals recorded in the ponds could be offspring of a few founder females. According to our model, a population with 19050 nauplii can be produced in the second generation within 19050, starting with one fertilized female. According to this estimate and assuming fertilized females to be the dispersal stage, the number of colonists required to obtain the number of nauplii observed was between 0.002 and 12.7 in six of seven ponds. In contrast, 200 colonists must be assumed for pond 5S4, suggesting either a massive dispersal event in this pond, or a very patchy distribution of nauplii during sampling. The estimated value of 0.002 colonists for pond OS2 suggests this pond was colonized much less than 20 days before sampling. Although these results must be interpreted with caution due to the assumptions involved, our results suggest a higher colonization rate for *M. minutus* compared to the findings of VANDEKERKHOVE et al. (2005), who estimated a minimum rate of 7.2 ephippia pond⁻¹year⁻¹ for cladocerans.

In cyclopoids, multiple clutches may be fertilized by sperm received in a single mating, during which a spermatophore is attached to the female, making re-mating unnecessary (MAIER 1995). Thus a new habitat can potentially be colonized by a single fertilized female. We suggest that a combination of the ability to store sperm and fast development allow some cyclopoid copepods to be successful early colonists. Other cyclopoid genera with similarly short generation times, such as *Eucyclops* or *Tropocyclops* are known to be among the first zooplankton to colonize new waterbodies (EINSLE 1993, CÁCERES & SOLUK 2002, JENKINS & BUIKEMA 1998, REID & JANETZKI 1996). However, whether ponds were colonized by repeated arrival of cyclopoids, or whether populations are founded by one or few individuals should be clarified in future population genetic studies.

Despite the reported capability of *Metacyclops minutus* to inhabit a wide salinity spectrum between 0 and 25 ppt (ALONSO 1990), our results suggest a delay of colonization in ponds with higher salinities. We found a negative correlation between the abundance of cyclopoid copepods and conductivity, while other environmental factors measured had no apparent relation to the abundance of zooplankton. Negative relationships between abundance and salinity have been found previously in other microcrustaceans inhabiting shallow Mediterranean wetlands (GREEN *et al.* 2005). This is likely to be related to a limited halotolerance of most freshwater zooplankton species, which often occur within certain ranges of salinity (ALONSO 1990). Therefore, the arrival of propagules in a brackish pond might not translate into colonization. Fecundity and hatching rates of zooplankton can be negatively influenced by high conductivities (CASTRO-LONGORIA 2003) thus reducing population growth rates of colonists.

Many cyclopoid copepods have the capacity to rapidly colonize the water column after rehydration, both by hatching from the copepodid bank and by dispersing from outside sources (FRISCH 2002, FRISCH & THRELKELD 2005). *Metacyclops minutus* has long been known to withstand considerable drought (CLAUS 1894). The density of dormant C4 stages can reach 1200 Ind /m² (MAIER 1992). They can be expected to be the first and fastest species to recolonize the water column of the ponds when they reflood over the years, influencing future habitat conditions and zooplankton community structure via priority effects (HAVEL & SHURIN 2004). We aim to test this hypothesis in the following years.

Although our results suggest a high dispersal capacity for cyclopoids, this may be scale dependent. Our results and those of previous studies (JENKINS & BUIKEMA 1998, CÁCERES & SOLUK 2002) might be explained by high dispersal capacity for cyclopoids over shorter distances of <10 km. However, it remains possible that cladocerans have a greater capacity to be dispersed over long distances of >100 km or >1000 km by waterfowl, as suggested by DEJEN et al. (2004), e.g. because their resting eggs can survive for long periods in a bird's gut. Bearing in mind the lack of previous information about copepod dispersal compared with other groups such as cladocerans and anostracans (see BILTON et al. 2001, BOHONAK & JENKINS 2003 for reviews), our results highlight the need for further research into the dispersal mechanisms and population and molecular ecology of copepods.

Acknowledgments

This work was financed by the Spanish Ministerio de Medio Ambiente Doñana 2005 restoration project and via a postdoctoral fellowship from the Ministerio de Educación y Ciencia to DF. We are grateful to ARANTZA ARECHEDERRA for her assistance with the hatching experiment, to RICARDO DÍAZ-DELGADO for helping with the preparation of Fig. 1, and to LUIS SANTAMARÍA for useful discussions.

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Table 1. Pond details and volume of water samples collected in seven new temporary ponds (Caracoles estate, Doñana National Park). “Size” is an estimation of the pond basin area that was covered with water on the sample date and “Depth” represents maximum water depth measured in the respective ponds.

Pond	Date	Temp. (°C)	DO (mg/L)	Cond. (mS/cm ²)	pH	Size (m ²)	Depth (m)	Sample Volume (L)
6S3	17/03/05	25.2	14.0	1.80	8.9	24	0.02	10
8S3	17/03/05	27.0	14.8	10.50	8.9	300	0.05	10
4S4	17/03/05	27.4	12.1	8.16	8.7	20	0.03	8
5S4	18/03/05	14.1	15.3	1.79	8.9	200	0.04	10
9S1	18/03/05	21.4	15.7	5.76	9.5	174	0.03	10
0S2	18/03/05	17.6	18.2	49.30	8.7	8	0.04	10
10S2	18/03/05	19.6	13.6	1.38	8.5	1	0.03	3

Table 2. Means and ranges of zooplankton individuals hatching from sediment samples of the top sediment layer (0-4 cm) of the reference sites Lucio de Marilópez and Lucio del Lobo.

The sample surface of each core was 50.3 cm². The mean number of hatchlings was calculated per m².

	Rotifers	Cladocerans	Calanoid nauplii	Cyclopoids	Ostracods	total
Marilópez						
sample N	6	6	6	6	6	6
sample mean	3.3	0.5	14.2	0.3	16.5	34.8
range	0.0 - 12.0	0.0 - 1.0	0.0 - 36.0	0.0 - 2.0	2.0 - 49.0	2.0 - 62.0
SD	4.6	0.5	15.6	0.8	16.5	22.4
mean per m ²	663.2	99.5	2818.4	66.3	3282.6	6929.9
Lobo						
sample N	6	6	6	6	6	6
sample mean	2.8	1.2	18.2	0.7	6.3	29.2
range	0.0 - 7.0	0.0 - 3.0	1.0 - 74.0	0.0 - 3.0	1.0 - 17.0	2.0 - 103.0
SD	2.5	1.2	28.2	1.2	6.2	37.5
mean per m ²	563.7	232.1	3614.1	132.6	1260.0	5802.5

Table 3. Density (Ind/L) of copepods (*Metacyclops minutus*, *Megacyclops viridis*), cladocerans (*Moina brachiata*) and monogonont rotifers (*Brachionus variabilis*, and an unidentified species) in seven new temporary ponds (Caracoles estate). Ostracods were not recorded in the ponds. For copepods, developmental stages are given as follows: calanoid (cal) and cyclopoid (cyc) nauplii, cyclopoid copepodids C1 to C4, cyclopoid copepodid 5 (C5) and adults. Calanoid copepods older than the nauplii stage and juvenile cladocerans were not present in the ponds studied.

Pond	Cal nauplii	Cyc nauplii	Cyc C1-C4	<i>M. minutus</i> C5	adult	<i>M. viridis</i> C5	adult	<i>Moina brachiata</i> adult	Rotifers
6S3	-	1010.0	125.6	0.1	3.6	-	-	-	1.3
8S3	-	6.6	0.7	-	-	-	0.1	-	0.4
4S4	-	47.0	-	-	0.3	-	-	-	-
5S4	0.2	947.5	41.0	-	1.4	-	-	0.2	1.0
9S1	-	80.6	15.2	-	2.2	-	-	-	0.3
0S2	-	0.2	-	-	-	-	-	-	-
10S2	1.7	461.7	11.7	-	2.4	-	-	-	-

Table 4. Crustacean species recorded in temporary waterbodies of the Caracoles estate during the 2003-2004 winter (FRISCH et al. 2006, FRISCH et al. unpubl.). Ostracods were recorded, but not identified.

Branchiopoda

Cladocera

Alona rectangula (Sars, 1862)

Daphnia magna (Straus, 1820)

Daphnia pulex (Leydig, 1860)

Dunhevedia crassa (King, 1853)

Moina brachiata (Jurine, 1820)

Moina salina (Daday, 1888)

Anostraca

Chirocephalus diaphanus Prevost 1803

Notostraca

Triops cancriformis Bosc 1801

Copepoda

Calanoida

Arctodiaptomus wierzejskii (Richard) 1888

Copidodiaptomus numidicus (Gurney) 1909

Cyclopoida

Diacyclops bisetosus (Rehberg) 1880

Metacyclops minutus (Claus) 1863

Harpacticoida

Cletocamptus retrogressus Shmankevich 1875

Table 5. Calculation of *M. minutus* population growth, assuming 1 fertilized female founded the population on day 1 and individual fecundity of 30 eggs per day (MAIER 1995).

Development times were assumed at 1 day from egg to nauplii, 5 days for naupliar development and 2 days for copepodid development, based on data reported for 20°C by MAIER (1992). Mortalities from eggs to adults was set at 60%, of which 40% were estimated to occur in the naupliar phase and 20 % in the copepodid phase, exceeding experimental data available for other copepods, which estimate total juvenile mortality at 40 to 50% (FRISCH 2001, SARVALA 1979, ELBOURN 1966). Adult mortality was neglected because presumably the temporary ponds were predator-free and the life span of adult cyclopoids can exceed 20 days (HOPP et al.1997).

Day	Females	Eggs	Nauplii	Copepodids
1	1	30	0	0
2	1	30	30	0
3	1	30	60	0
4	1	30	90	0
5	1	30	120	0
6	1	30	150	0
7	1	30	150	18
8	1	30	150	36
9	7	210	150	36
10	13	390	330	36
11	19	570	690	36
12	25	750	1230	36
13	31	930	1950	36
14	37	1110	2850	36
15	43	1290	3750	144
16	49	1470	4650	360
17	91	2730	5550	576
18	169	5070	7530	792
19	283	8490	11670	1008
20	433	12990	19050	1224
21	619	18570	30750	1440

Table 6. Estimated number of *Metacyclops minutus* nauplii and required colonists per pond. For an approximation of nauplii /pond we multiplied Ind/L by estimated pond volume (water surface area*maximum depth/2). Number of colonists were based on the number of nauplii (19050 Ind) in a model population on day 20 after its foundation on day 1 (Table 5).

Pond	Nauplii/pond	Colonists	Pond vol. (L)
6S3	242400	12.7	240
8S3	49500	2.6	7500
4S4	14100	0.7	600
5S4	3790000	199.0	4000
9S1	210366	11.0	2610
0S2	32	0.002	160
10S2	6926	0.4	15

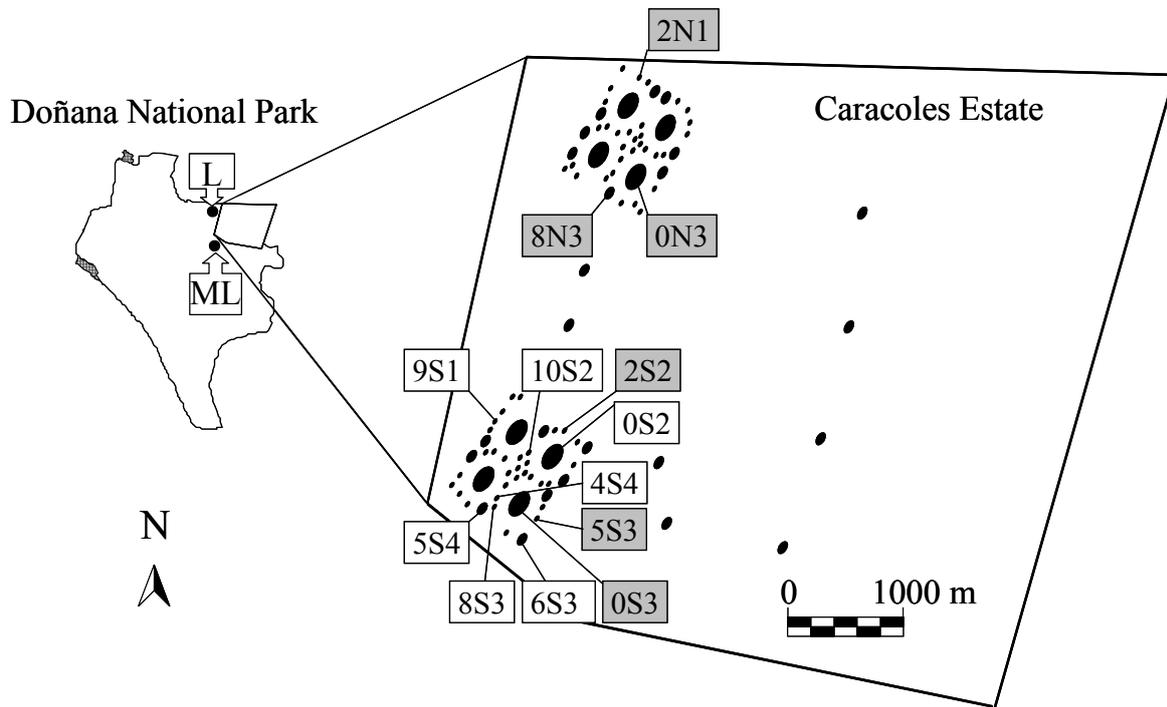


Fig. 1: Study area, showing the outline of Doñana National Park and the location of the two reference sites sampled for sediment (L = Lucio del Lobo, ML= Lucio de Marilópez). The map detail shows the area of the newly constructed ponds, of which different diameters are indicated by dot sizes. Labels indicate the ponds sampled for the present study (white labels: water, grey labels: sediment).



Fig. 2: Photograph of the pond 8S3, with the outline of the designated pond basin (dotted white line) on 17 March 2005. As with the other sampled ponds, only part of the designated pond basin was covered with water. In 8S3, a third of the surface was flooded at a maximum water depth of 5 cm (see Table 1).

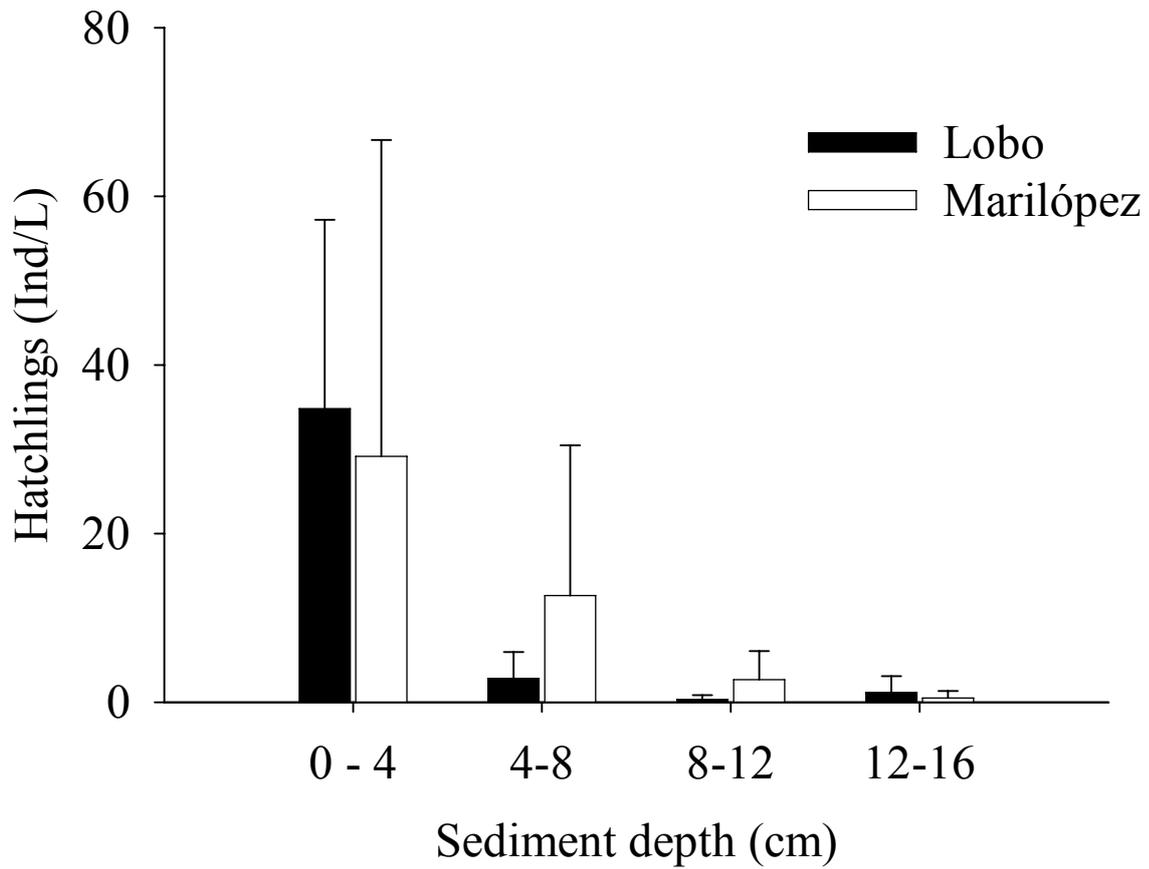


Fig. 3: Vertical distribution of zooplankton hatching within a week from sediment cores of the reference sites Marilópez (ML) and Lobo. The graph shows mean (columns) and SD (bars) for 6 replicate samples of four depths in each reference site.