Diversity of floodplain copepods (Crustacea) modified by flooding: species richness, diapause strategies and population genetics

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With 5 figures and 3 tables

Abstract: We examined the impact of lateral and longitudinal hydrological connectivity on copepod diversity (local species richness, diapause strategies and population genetics) in ponds of a North American floodplain. Results of a General Linear Model (GLM) which included three environmental pond variables showed that species richness in these ponds was significantly influenced only by pond type (permanent ponds, temporary ponds with connections to other waterbodies during floods, isolated temporary ponds). Local species richness in connected temporary ponds had significantly higher scores of local richness compared to isolated temporary ponds (means 7.4 and 2.7, respectively). Such enhancement of species diversity in connected ponds appears to be facilitated by increased dispersal during floods and also pertains to the diversity of diapause strategies in the ponds studied. Species with and without diapause were present in connected temporary ponds, but absent from isolated temporary ponds, which contained only species capable of diapausing during the dry season. To explore the significance of hydrological connectivity of longitudinal dispersal between upstream and downstream sites, we used RAPD markers in populations of a calanoid copepod. The increase of average heterozygosity \(H\) (0.301 to 0.477) in an upstream-downstream direction and generally low genetic distances (Nei’s \(D < 0.05\)) suggested maintenance of gene flow by flood-mediated dispersal between connected ponds. Lower heterozygosity (0.289) of the population in a downstream pond protected by levees suggested the interruption of gene flow when dispersal by flooding is blocked.

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Key words: hydrological connectivity, pond type, dispersal, genetic distance, gene flow.

Introduction

Riverine floodplains are complex systems which encompass a diversity of lentic, lotic, permanent and temporary waterbodies (Ward et al. 2002). Hydrological connectivity in riverscapes on longitudinal and lateral gradients resulting from fluvial dynamics increases habitat diversity in waterbodies of floodplains and results in spatial heterogeneity which directly influences biodiversity patterns (Amoros & Bornette 2002). Riverscapes are characterized by high $\alpha$-, $\beta$-, and $\gamma$-diversity and high diversity of life history strategies (Amoros & Bornette 2002). Hydrological dynamics of floodplains are caused by frequency, duration and intensity of flooding by the main river. For example, large rivers such as the Amazon have a monomodal flood pulse with predictable amplitude and timing while smaller rivers generally have a polymodal flood pulse and less predictable floods (Junk 1997). Alternation of aquatic and terrestrial periods which result from the flood pulse represent a unique feature of floodplains and distinguish these habitats from other transitional areas between land and water with relatively constant water levels (Junk 1997).

In floodplains and other wetlands, seasonal lateral connectivity during inundations potentially facilitates dispersal of copepods between otherwise disconnected waterbodies (Elgmork 1964, Frisch 2002). Ponds newly formed by levee breaks in the floodplain of the Missouri River were rapidly colonized by a diversity of crustacean zooplankton species (Havel et al. 2000). A common strategy of both terrestrial and aquatic invertebrates living in transient habitats is to produce a diapause stage that is resistant to or tolerant of adverse conditions such as drying or flooding (Wiggins et al. 1980, Adis & Junk 2002). Diapause stages are frequently found in floodplain cyclopoid copepods (Frisch 2002) and calanoid copepods (Libman & Threlkeld 2000). While within calanoid copepods diapause eggs are produced, cyclopoid copepods express diapause in one of the juvenile (copepodid) stages or more rarely as adults (reviewed in Santer 1998). Species from both groups are able to survive terrestrial conditions for a certain period of time varying from months to years in cyclopoids (Hairston & Cáceres 1996, Frisch 2002) and decades to centuries in calanoids (reviewed in Williams-Howze 1997). In the present study we explore the impact of pond hydroperiod and connectivity on copepod richness and on the contribution of diapausing and non-diapausing species. We hypothesize that lateral hydrological connectivity increases diversity both with regard to species richness and diapause strategies in periodically connected temporary habitats compared to isolated temporary ponds.
Longitudinal hydrological connectivity in rivers and associated floodplains can be expected to affect the genetic population structure of resident organisms. Greater heterozygosity in downstream populations results from active, downstream directed dispersal in the mosquitofish *Gambusia holbrooki* (Hernandez-Martich & Smith 1997). Flooding facilitates substantial transport of passively dispersing zooplankton. Such “washout” effects have been reported from floodplains around the world (Saunders & Lewis 1988, Vásques & Ray 1992, Michels et al. 2001). Here, we examine heterozygosity and genetic distances between populations of the calanoid copepod *Skistodiaptomus pallidus* (Herrick) 1879 in relation to pond location on a longitudinal gradient. We test the hypothesis that hydrological connectivity leads to washout of individuals, and translates into increasing heterozygosity from upstream to downstream locations.

**Methods**

**Study area and pond characteristics**

The Little Tallahatchie River (LTR) is a fourth order river located in northern Mississippi, USA. The area has a humid climate with an annual mean temperature of 16.6 °C and high precipitation with an average annual sum of 1270 mm (Blackmarr 1995). The river was impounded by the construction of Sardis Dam (34° 24′ 32″ N, 89° 47′ 45″ W) in 1939 as part of a flood control program or the lower Yazoo River basin. The upstream part of the floodplain is located east of Sardis Reservoir (Fig. 1). Flooding is of short duration and occurs several times per year at high river levels which connect floodplain waterbodies for a limited number of days (flash floods with high flow velocity). During these flood periods, changes in the reservoir level lead to comparable seasonal inundation of reaches in the immediate proximity of the reservoir. Parts of the floodplain closest to Sardis Reservoir, west of Lee Creek (Fig. 1) may be flooded by water backing up from the reservoir at high lake water levels, creating long-lived connections between waterbodies in this area. Reaches immediately downstream of Sardis Dam (Fig. 1) do not flood unless water passes over the dam’s emergency spillway, which has occurred three times since 1939 (17 March–19 June 1973; 20 May–20 June 1983; 1 May–11 July 1991). Fish are observed frequently in the permanent waterbodies and ponds that are hydrologically connected to them.

For the study on species richness and diapause strategies we collected samples from three pond types. These were categorized by hydroperiod and connectivity (permanent waterbodies, temporary ponds with connection to other waterbodies during floods, and temporary ponds isolated from flooding). Permanent waterbodies included oxbow lakes, permanent creeks and a permanent swamp (for numbers of ponds in each category and other pond characteristics see Table 1). We hereafter use the term pond for all waterbodies. Of the 20 ponds studied, 15 ponds were situated in the upstream part of the floodplain and five were located adjacent to Sardis Reservoir. Long-term data on hydroperiod of ponds was generally unavailable. We therefore used own obser-
Fig. 1. Map of the Little Tallahatchie River and Sardis Reservoir. The floodplain (black contour) is located upstream to the east of Sardis Reservoir (black area). At high water levels, Sardis Reservoir backs up into the western part of the floodplain (grey area). Triangles in the enlarged map details mark the locations of *Skistodiaptomus pallidus* populations sampled for the analysis of population genetics.


divations on dry periods and flooding of ponds for the categorization of pond types. In addition, we consulted topographical maps to determine ponds that were isolated from flooding due to their elevation. We measured pond size (depth and area) on one occasion after flooding. The size of floodplain waterbodies fluctuates strongly during the year and therefore these variables are regarded as rough estimators only. Temperature, DO, pH and chlorophyll-a concentration were recorded on each sampling date (see Table 1 for a summary of pond variables).

**Flood duration and frequency**

Daily gage data were available for a 30-year period for the Little Tallahatchie River, gage at Etta (source: United States Geological Survey, gage # 07268000). The river gage is in immediate proximity of the upstream floodplain. Monthly flood duration was defined as the number of days per month on which gage levels exceeded flood stage (river levels at which the floodplain is inundated). Flood frequency was calculated as the average number of floods per year from the same data set. Floods were defined as separate events when river gages were below flood stage for a minimum of 10 days. Differences of flood duration between months were analyzed by 1-way ANOVA (StatSoft Statistica 6.0), using years as replicates.

**Species richness**

For measuring species richness we collected five to ten litres of water from each pond, comprised of several smaller samples (details in Frisch 2002). All ponds were
Table 1. Pond characteristics of the Little Tallahatchie floodplain summarized by pond type (P = permanent ponds, F = temporary ponds with connection to other ponds during floods, T = temporary ponds isolated from flooding). Means and SD were obtained from two sample dates. Numbers of ponds studied for each pond type are given in parenthesis.

<table>
<thead>
<tr>
<th>Pond type (N)</th>
<th>P (8)</th>
<th>F (9)</th>
<th>T (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>7.8</td>
<td>2.3</td>
<td>6.6</td>
</tr>
<tr>
<td>Temp. (°C)</td>
<td>22.3</td>
<td>5.0</td>
<td>20.4</td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td>0.3</td>
<td>7.0</td>
</tr>
<tr>
<td>Chl-a (µg/L)</td>
<td>47.1</td>
<td>43.0</td>
<td>64.3</td>
</tr>
<tr>
<td>Area (m²)</td>
<td>4031.3</td>
<td>1679.1</td>
<td>852.9</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>1.4</td>
<td>0.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

sampled twice between February and May, except for two flooded temporary ponds which had already dried on the second sample date. Samples were collected in a maximum depth of 50 cm, filtered over a 64 µm nylon gauze and preserved in a 5% formalin solution. The number of copepod species was determined from at least 25% of the volume of each sample. Species were identified according to Wilson & Yeatman (1959), Reid (1988, 1992), Reid et al. (1989), Reid & Reed (1994), Dodson (1994), and Reid & Moreno (1999). We used a GLM (General Linear Model) with indicator variable coding of the categorical predictor (overparameterized model) for analysis of the effects of pond type (categorical predictor with three levels), and three environmental factors (temperature, pH and chl-a concentration as continuous predictor variables) on local species richness. DO was excluded from the GLM because it was correlated with hydroperiod. Local species richness was defined as the total number of species present in each pond over the study period. Environmental factors were averaged over the sample dates separately for each pond. On one sample date, data for DO, pH and chl-a concentration were unavailable for three ponds. These data were replaced by the means of all available data for the respective variables prior to the calculation of averages. To specify the effect of pond type on species richness, Bonferroni post-hoc tests were employed between the three pond types. All statistical analyses were performed with StatSoft Statistica 6.0.

Composition of diapause strategies

For analysing the composition of diapause strategies, we determined the number of species over the entire study period for each pond. Diapause classification (with or without diapause) was based on our own observations or on data in the literature. A species list with details on dormancy periods is provided in Table 2.

The relationship between diapause strategy and pond type (isolated temporary, flooded temporary, permanent) was analyzed by a Multivariate Analysis of Variance (MANOVA, StatSoft Statistica 6.0) with the two strategies “with diapause” and “with-
Table 2. Calanoid and cyclopoid copepod species recorded in the Little Tallahatchie River (LTR) floodplain. Information is given on presence of species (+) in ponds of different pond types in the LTR floodplain (P = permanent, F = temporary with connection during floods, T = temporary, isolated), and on reported season of planktonic activity and putative diapause season in the Southern USA (spr = spring, s = summer, w = winter, n-seas = nonseasonal, no = no diapause, ? = no data). Nonseasonal diapause was characterized either by production of resting eggs during more than one season or by the capability of being terminated whenever aquatic habitats are available.

<table>
<thead>
<tr>
<th>Species</th>
<th>LTR</th>
<th>Present in plankton</th>
<th>Diapause reported</th>
<th>Putative diapause season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>F</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Calanoida</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diaptomus sanguineus (SA FORBES 1876)</td>
<td>+</td>
<td>w, spr⁶</td>
<td></td>
<td>yes⁶</td>
</tr>
<tr>
<td>Osphranticum labronectum (SA FORBES 1882)</td>
<td>+</td>
<td>?</td>
<td></td>
<td>yes⁸</td>
</tr>
<tr>
<td>Skistodiaptomus pallidus (HERRICK) 1879</td>
<td>+ +</td>
<td>n-seas⁷</td>
<td></td>
<td>yes⁷</td>
</tr>
<tr>
<td>Cyclopoida</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acanthocyclops robustus-vernalis group</td>
<td>+ +</td>
<td>n-seas¹,³,⁹</td>
<td></td>
<td>yes²,⁴,⁵,¹⁴</td>
</tr>
<tr>
<td>Acanthocyclops venustoides (COKER) 1934</td>
<td>+</td>
<td>w, spr¹</td>
<td></td>
<td>yes¹⁵</td>
</tr>
<tr>
<td>Diacyclops bisetosus (REHBERG 1880)</td>
<td>+</td>
<td>w, spr⁴,⁵</td>
<td></td>
<td>yes⁴,⁵,¹⁵</td>
</tr>
<tr>
<td>Diacyclops haueri (KIEFER) 1931</td>
<td>+ +</td>
<td>w, spr¹,⁹,¹⁴</td>
<td></td>
<td>yes⁹,¹⁴,¹⁵</td>
</tr>
<tr>
<td>Diacyclops navus (HERRICK) 1882</td>
<td>+ +</td>
<td>n-seas¹⁰,¹²</td>
<td></td>
<td>yes¹⁵</td>
</tr>
<tr>
<td>Diacyclops thomasi (SA FORBES 1882)</td>
<td>+ +</td>
<td>w, spr¹</td>
<td></td>
<td>yes¹⁴</td>
</tr>
<tr>
<td>Ectocyclops phaleratus (KOCHE) 1838</td>
<td>+</td>
<td>w, spr¹⁰</td>
<td></td>
<td>yes²,⁸</td>
</tr>
<tr>
<td>Eucyclops agilis (KOCHE) 1838</td>
<td>+ +</td>
<td>n-seas¹,ⁱ⁰</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Eucyclops elegans (HERRICK) 1884</td>
<td>+ +</td>
<td>?</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Macrocyclops albidus (JURINE 1820)</td>
<td>+ +</td>
<td>n-seas¹,⁵,¹⁰</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Macrocyclops ater (HERRICK) 1882</td>
<td>+ +</td>
<td>?</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Macrocyclops fuscus (JURINE 1820)</td>
<td>+ +</td>
<td>n-seas¹,⁹</td>
<td></td>
<td>yes⁹,¹³</td>
</tr>
<tr>
<td>Megacyclops latipes (LOWNDES) 1927</td>
<td>+</td>
<td>w, spr¹</td>
<td></td>
<td>yes²,⁴</td>
</tr>
<tr>
<td>Mesocyclops americanus DUSSArt 1985</td>
<td>+</td>
<td>s⁹</td>
<td></td>
<td>yes⁹,¹⁴</td>
</tr>
<tr>
<td>Mesocyclops edax (SA FORBES 1890)</td>
<td>+ +</td>
<td>n-seas¹</td>
<td></td>
<td>yes²,¹¹</td>
</tr>
<tr>
<td>Microcyclops varicans (GO SARS 1863)</td>
<td>+ +</td>
<td>?</td>
<td></td>
<td>yes⁸,¹⁴,¹⁵</td>
</tr>
<tr>
<td>Orthocyclops modestus (HERRICK) 1883</td>
<td>+ +</td>
<td>s⁹</td>
<td></td>
<td>yes⁹,¹³,¹⁴</td>
</tr>
<tr>
<td>Paracyclops fimbriates poppei (REHBERG) 1880</td>
<td>+</td>
<td>w, spr¹</td>
<td></td>
<td>yes²,¹⁵</td>
</tr>
<tr>
<td>Tropocyclops prasinus (FISCHER 1860)</td>
<td>+</td>
<td>s¹,¹⁰</td>
<td></td>
<td>yes¹⁰</td>
</tr>
<tr>
<td>Tropocyclops prasinus mexicanus KIEFER 1938</td>
<td>+ +</td>
<td>?</td>
<td></td>
<td>yes²</td>
</tr>
</tbody>
</table>


out diapause” as dependent variables and ponds as replicates. MANOVA was followed by Bonferroni post-hoc tests to examine the differences of each strategy separately between pond types.
Population genetics

RAPDs (Randomly Amplified Polymorphic DNA; Welsh & McClelland 1990, Williams et al. 1990) were used to assess the genetic structure of populations of *Skis-todiaptomus pallidus* from seven ponds along the LTR River (Fig. 1). A main criticism of RAPDs is that profiles can be difficult to replicate (Jones et al. 1997). To overcome these difficulties, only bands from samples that gave clear banding patterns were used. Random subsampling returned identical banding patterns. Non-readable samples were discarded and repeated. All twenty primers from Operon Technologies (Alameda, CA) kit B were tested, and only primer OPB 10 (four marker alleles: opb.a, opb.b, opb.c, opb.f) gave reproducible results under the polymerization conditions outlined below.

PCR Protocol: Genomic DNA from individual copepods (*n* = 16–32) collected from 7 populations was analyzed using primer OPB10 (5′CTGCTGGGAC). DNA was extracted from individual copepods (females, eggs removed, males or C5 copepodids) by placing them in 1.5 ml microfuge tubes containing 20 µl of lysis buffer (0.2 µmol L⁻¹ Tris pH 8, 1 µmol L⁻¹ KCl, 0.5 % Tween 20, 200 µg ml⁻¹ proteinase K) for one hour at 65 °C followed by heating at 95 °C for 20 minutes to heat inactivate the proteinase K before PCR (C. Lee, pers. comm.). One µl of the proteinase-treated lysate was added to 24 µl of PCR reaction mixture containing 2.5 µl PCR buffer (Perkin-Elmer) 1 µl of dNTP, 2 µl of primer, 0.125 µl (100 units ml⁻¹) TAQ polymerase (Stoffel-fragment; Perkin-Elmer) and 3.75 µl of 0.094 µmol L⁻¹ MgCl₂. The amplified PCR products were separated by electrophoresis in 1.5 % agarose gels run at 6 volts per centimetre, stained with ethidium bromide and photographed.

We used the methods developed by Lynch & Milligan (1994) to estimate allele frequencies, within-population gene diversity (heterozygosity), and Nei’s Distance D for all population pairwise comparisons. To test the relationship between heterozygo-

![Fig. 2. Monthly means of flood duration in the LTR floodplain (lines), ± 1 SE (boxes) and 95 % Confidence Intervals (whiskers). Means, SE and 95 % C. I. were calculated from 30 years of monthly flood duration (days above flood stage, for details see Methods).](image)
sity and hydrological distances among hydrologically connected populations, we used Pearson Product-Moment correlation analysis. A Mantel-test was used to test for correspondence between Nei’s distances and hydrological distances between populations using the computer program IBD (BOHONAK 2002). Hydrological distances between waterbodies were measured as straight lines from the respective waterbody to the river channel and along the river channel.

Results

Monthly flood duration and frequency

The results of 1-way ANOVA showed a significant effect of month on flood duration \( F_{(11, 284)} = 6.88, p < 0.001 \). Monthly flood duration was generally longer in the winter and spring months (Fig. 2). Between December and June mean monthly flood duration was between 1.2 to 2.4 days. Flooding from July to November was on average less than one day.

Local species richness

The total numbers of species in each of the pond types was 16 in permanent ponds, 19 in flooded temporary ponds and five in temporary isolated ponds (Table 2). Eight species were shared between permanent and flooded temporary ponds, but were absent from isolated temporary ponds. All species found in isolated temporary ponds were also present in other pond types (Table 2).

Table 3. Summary of GLM results on local species richness (dependent variable), pond type (categorical predictor variable with three levels: P = permanent, F = temporary with connection during floods, T = temporary, isolated), and the continuous predictor variables temperature, pH and chl-a concentration. (A) Test of model with \( R^2 \) as the variance explained by the model. (B) Parameter estimates of the predictor variables.

(A) Test of Whole Model SS vs. Residual SS

<table>
<thead>
<tr>
<th>Model</th>
<th>( F_{(df1, df2)} ) ratio</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R^2 = 0.53 )</td>
<td>( F_{(5, 14)} = 3.12 )</td>
<td>0.042</td>
</tr>
</tbody>
</table>

(B) Parameter Estimate

<table>
<thead>
<tr>
<th></th>
<th>SE</th>
<th>( t )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>21.509</td>
<td>16.85</td>
<td>1.28</td>
</tr>
<tr>
<td>Temperature</td>
<td>-0.096</td>
<td>0.17</td>
<td>-0.55</td>
</tr>
<tr>
<td>pH</td>
<td>-2.447</td>
<td>2.12</td>
<td>-1.16</td>
</tr>
<tr>
<td>Chl-a</td>
<td>0.014</td>
<td>0.01</td>
<td>1.37</td>
</tr>
<tr>
<td>Pond type (P)</td>
<td>5.773</td>
<td>1.75</td>
<td>3.29</td>
</tr>
<tr>
<td>Pond type (F)</td>
<td>4.184</td>
<td>1.58</td>
<td>2.65</td>
</tr>
<tr>
<td>Pond type (T)</td>
<td>0.000</td>
<td>(aliased)</td>
<td></td>
</tr>
</tbody>
</table>
Diversity of floodplain copepods

Fig. 3. Local species richness of calanoid and cyclopoid copepods in the three pond types (P = permanent, F = flooded temporary, T = isolated temporary) of the LTR floodplain. The boxplot shows means (lines), ± 1 SE (boxes), ± 1 STD (whiskers).

The GLM, which included pond type and three environmental variables to predict species richness, was significant (Table 3 A). Of the predictor variables only pond type significantly explained species richness (Table 3 B). Local species richness of temporary isolated ponds (mean 2.7, Fig. 3) was lower than of flooded temporary ponds (mean 7.4, p = 0.02, Bonferroni post-hoc test) and of permanent ponds (mean 8.0, p = 0.01, Bonferroni post-hoc test). Flooded temporary ponds and permanent ponds did not differ in species richness (p = 1.00, Bonferroni post-hoc test).

Composition of diapause strategies

The MANOVA revealed a significant effect of pond type on the composition of species with and without diapause (Wilks test, $F_{(4, 32)} = 6.00, p = 0.001$). The number of species without diapause was higher in permanent ponds compared to flooded temporary ponds (Fig. 4, means 2.3 and 1.0 species respectively, $p = 0.03$, Bonferroni post-hoc test) and in isolated temporary ponds (0 species, $p = 0.01$, Bonferroni post-hoc test). The number of species with diapause was equal in permanent and flooded temporary ponds (means 4.9 and 6.0, respectively, $p = 0.53$, Bonferroni post-hoc test), but higher in flooded temporary ponds than in isolated temporary ponds (mean 2.7, $p = 0.02$, Bonferroni post-hoc test).

Population genetics of *Skistodiaptomus pallidus*

RAPD markers were used to study population genetics of *Skistodiaptomus pallidus* in four pond populations in the LTR floodplain upstream of Sardis Reservoir (US-1, US-2, US-7, US-8) and three pond populations downstream of Sardis (DS-B, DS-BT1, DS-F). We observed increasing heterozygosity of the marker alleles in an upstream-downstream direction (Fig. 5 A) with the ex-
Fig. 4. Composition of diapause strategies in the three pond types (P = permanent, F = flooded temporary, T = isolated temporary) of the LTR floodplain. The boxplot shows means (lines) of species number in the two categories (black = without diapause, white = with diapause), ± 1SE (boxes), ± 1 STD (whiskers).

ception of the downstream pond DS-B which is completely isolated from floods by a man-made dam. A correlation analysis between heterozygosity and hydrological distances among the hydrologically connected populations (all ponds except for DS-B) was significant ($r = 0.869$, $p = 0.025$). Nei’s distance (Nei’s $D$) calculated from allele frequencies generally showed very low to negative distances between populations (Fig. 5 B). The results of a Mantel-test performed on all pairs of Nei’s distances and geographical distances were not significant. Plotting of pairwise Nei’s $D$ against hydrological distance (Fig. 5 B) showed that Nei’s $D$ was higher within upstream than within downstream populations. Between these groups, Nei’s $D$ between the two populations furthest upstream (US-1, US-2) and downstream populations tended to be higher than those of pairs between lower upstream populations (US-7, US-8) and downstream populations (Fig. 5 B).

**Discussion**

The results of our study support our hypothesis that lateral connectivity increases diversity of both species and diapause strategies. Flooding enhances species diversity in temporary ponds by creating connections, which could function as corridors to otherwise isolated habitat patches. Thus permanent and flooded temporary ponds of the LTR floodplain support a higher number of species than isolated temporary ponds. Ward et al.’s (1999) extension of Connell’s (1978) intermediate disturbance hypothesis proposed that high species
Fig. 5. (A) Average within-population heterozygosity $H_j$ of four RAPD marker alleles for seven pond populations of *Skistodiaptomus pallidus* in reaches of the Little Tallahatchie River. Error bars denote estimated SE of $H_j$ for dominant markers (Lynch & Milligan 1994). The line shows the linear relationship between average $H_j$ of populations with hydrological distance on an upstream-downstream gradient (excluding DS-B, correlation analysis, $r = 0.869$, $p = 0.025$). (B) Nei’s $D$ (corrected for dominant markers) of pairs of all seven pond populations of *Skistodiaptomus pallidus* in reaches of the Little Tallahatchie River in relation to hydrological distances between ponds. Symbols denote distances between different groups of populations. Filled circles = upstream–upstream, open circles = downstream–downstream, filled triangles = upper upstream (US-1, US-2) – downstream, open triangles = lower downstream (US-7, US-8) – downstream.
diversity also pertains to intermediate levels of connectivity (measured by the ease with which species move from one habitat to another) and results from maximized habitat heterogeneity and reduced habitat fragmentation. Frequency of disturbance and connectivity may act antagonistically (Amoros & Bornette 2002): the floods which create connectivity can be severe disturbances decreasing species diversity, while hydrological connectivity may at the same time increase biological connectivity resulting in greater species richness in connected waterbodies. Invasibility of pond communities declines with increasing resident species diversity and might be precluded by local processes in communities with high species richness (Shurin 2000). Cottenie & De Meester (2003) found that local environmental variables primarily explained species richness of cladocerans in a set of highly interconnected ponds, whereas connectivity and dispersal only acted secondarily. In the LTR floodplain, local processes in the connected temporary ponds appear to be dominated by strongly increased dispersal probability via hydrological connections during floods. Our results suggest that connectivity plays an important role specifically in temporary ponds, where species richness is low when these ponds remain isolated, perhaps because these ponds can continuously be inhabited only by specialist species with drought resistant stages (Wiggins et al. 1980).

The life-cycle of copepods plays a crucial role in the colonisation of temporary ponds. All species found in isolated temporary ponds in the present study produce a diapause stage during the dry season and are able to hatch after dry periods (Wyngaard et al. 1991, Frisch & Threlkeld, in press). Although diapause is thought to be a strategy to bridge adverse conditions in general, resulting e.g. from food shortage, presence of predators (reviewed in Santer 1998) or washout caused by flooding (Libman & Threlkeld 1999), it is a prerequisite for survival in temporary ponds when coupled with drought tolerance. The presence of species without diapause exclusively in flooded temporary ponds and permanent ponds of the LTR floodplain supports the hypothesis of dispersal via hydrological connections.

A factor likely to influence the prevalence of certain diapause strategies in wetlands is the season of available aquatic habitat. Habitat availability decreases sharply during summer when floods are rare and aquatic habitat is restricted to permanent ponds. Analogous to a previous study (Frisch 2002), a large number of species in the LTR floodplain are able to survive dry periods in summer. Predation could be another important factor in the permanent and flooded temporary ponds where fish were frequently observed during the study period. Activity of predators is likely to increase in the summer months and may generally favour the presence of species with summer diapause as known from other examples (summarised in Santer 1998).
Information on species-specific diapause timing from other locations, such as listed in Table 2 has to be treated with caution when applied to the LTR populations, because the timing may vary between populations of the same species (Williams-Howze 1997). However, these data can be useful for making predictions of diapause patterns in relation to habitat properties. For example, species reported by others to diapause in winter (Table 2) occurred in permanent and connected temporary ponds but not in isolated temporary ponds in the study area. A large number of species from the LTR floodplain diapause non-seasonally in other locations of the Southern USA (Table 2). It might be predicted that irregular hydrodynamics as in the LTR floodplain favour a flexible diapause strategy characterized either by production of resting eggs during more than one season or by the capability of being terminated whenever aquatic habitats are available. However, we need detailed studies on the phenology of the respective populations to substantiate such predictions.

Dispersal between connected habitats on a longitudinal gradient was studied by using RAPD markers in populations of the calanoid copepod *Skistodiaptomus pallidus* from ponds along the LTR River. Heterozygosity is expected to increase with dispersal and gene flow (Bohonak 1999) as observed in downstream populations of the mosquitofish *Gambusia holbrooki* (Hernandez-Martich & Smith 1997). Our results support the notion of downstream directed dispersal by an increase in heterozygosity from upstream to downstream ponds. For fish populations, dam construction eliminating river flooding can result in losses of genetic diversity and local extinctions (Nehlsen et al. 1991, Kynard 1997). For the exchange of copepod individuals Sardis dam appears to be unimportant as a barrier. However, complete isolation from flooding by man-made structures such as the levees surrounding the downstream pond DS-B appear to be effective in reducing gene flow. Individuals of this pond population had lower heterozygosity compared to other downstream ponds.

Genetic distances are expected to be low when dispersal between populations is high (Bohonak 1999). However, our results suggest no relation between geographic and genetic distances for the populations studied. Non-significant results from a Mantel-test performed on geographic distances and Nei’s D between populations of *S. pallidus* could be caused either by the extremely low Nei’s D we found between populations or by imprecise estimation of hydrologic distances. In particular, our estimation of hydrological distances appears to be incorrect with regard to the frequency in which ponds are connected. Considering only upstream-downstream pairs of populations, Nei’s D tended to be lower for those pairs that were formed with the two upstream ponds with a frequent and long-lived connection to Sardis Reservoir. This suggests that Sardis Reservoir may function as a connecting element between downstream and upstream populations, in particular those that are more fre-
quently linked to the reservoir through lake water backing up into the up-
stream floodplain. Our observations give rise to questions about the impact of
lateral versus longitudinal dispersal of zooplankton in floodplains and need to
be addressed in further studies. Upstream populations laterally connected dur-
ing floods might serve as source populations for increased downstream genetic
diversity. Despite the low number of available markers, our results are consis-
tent with the concept of dispersal between connected ponds. Small Nei’s dis-
tances between populations in general suggest ample gene flow within the
study area. The present results support the idea that flood events and resultant
connectivity between ponds facilitate dispersal between floodplain populations
and may act as a homogenizing factor in sufficiently connected populations.

In conclusion, the pattern observed in the aspects of species diversity, di-
apause strategies and population genetics, support the notion of enhanced di-
versity caused by dispersal between hydrologically connected waterbodies in
natural systems. Temporary ponds seem to be affected in particular with re-
gard to species diversity and diapause strategies, both of which are less diverse
when temporary ponds are isolated from flooding. Copepod assemblages of
isolated temporary ponds rely on species specialised for survival during dry
periods. Connected ponds, on the contrary, support a diverse copepod commu-
nity and might be the source for genetic diversity on both lateral and longitu-
dinal spatial gradients.

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