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Resilience of mollusc communities of the River Saone (eastern France) and its two main tributaries after the 2003 heatwave

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SUMMARY

1. Global climate change can increase the mean and variability of temperature and may increase the occurrence of extremes such as heatwaves. Both gradual and abrupt warming may perturb freshwater communities, but understanding of ecological resilience to such events is limited.
2. We report the response of mollusc communities in the River Saone and its two main tributaries to the European heatwave of 2003 and determine the extent of community recovery over 8 years following this rare extreme event, during a period of gradual warming.
3. The 2003 heatwave had a major impact on the density and species richness of mollusc communities across the study area. After the heatwave, abiotic conditions favoured recolonisation by molluscs, yet full recovery of community states (defined as the combination of structure x density x species richness) was not observed at any site.
4. Given the profound changes observed in the mollusc community structure in the Saone River and in its two main tributaries and the observed slow pace of recovery relative to that observed elsewhere for pulse disturbances (typically $<$ 3 years), we suggest that global change, including warming, may preclude community recovery from pulse disturbances, such as extreme events, and instead yield communities comprising new combinations of species.

Key-words: climate change, community shift, disturbance, extreme climatic event, recovery.

Introduction

Global climate change can lead to gradual shifts in ecological communities. For instance, environmental warming can alter species distributions, local community composition and phenology over decades (Parmesan and Yohe, 2003; Root et al., 2003). However, where key thresholds are crossed, gradual environmental change may also cause abrupt shifts in community structure (van Nes and Scheffer, 2004). Slight erratic change in the environment, combined with gradual change, may be sufficient to invoke such shifts. However, extreme climatic events may have more pronounced physical and ecological impacts, as evidenced by research on heatwaves (Mouthon and Daufresne, 2006) and floods (Daufresne et al., 2007). Extreme events can be defined as statistically rare or unusual weather or climatic occurrences, such as extremes of precipitation or temperature, which can have severe natural impacts on the environment (IPCC, 2012). Future shifts in the occurrence of extreme events could challenge the resilience of freshwater ecosystems, either by themselves or in combination with underlying gradual environmental change.

Resilience can be defined as the amount of disturbance a community can undergo and still remain within the same state (Holling, 1973) or as the ability of a community to recover and return to its original state (Klein et al., 2003). Although freshwater ecosystems contain

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species adapted to environmental stressors, climate change could produce novel future regimes or combinations of stressors that exceed the capacity of communities to recover. Thus, from a management perspective, it is important to lessen anthropogenic pressures that could impair resilience to anthropogenic or natural disturbances, including extreme climatic events such as heatwaves.

One of the most notable recent extreme climatic events in Europe was the heatwave of summer 2003 (Schar et al., 2004; Stott et al., 2004; Trigo et al., 2005; Twardosz and Kossowska-Cezak, 2013). Heatwaves of such magnitude are exceptionally rare, occurring only once every several thousand or million years (Schar et al., 2004). Although research capturing the ecological consequences of the 2003 event for freshwater ecosystems is scarce (but see Jankowski et al., 2006; Mouthon and Daufresne, 2006; Daufresne et al., 2007; Wilhelm and Adrian, 2007; Jöhnk et al., 2008; Wegner et al., 2008), we know that the very hot summer of 2003 caused intense thermal stratification in European eutrophic, temperate lakes, depleted hypolimnetic oxygen and stimulated blooms of harmful cyanobacteria. These harsh conditions caused the mass mortality of fish and birds in the Netherlands (Jankowski et al., 2006; Jöhnk et al., 2008) and suppressed the abundance of Dreissena polymorpha larvae in Müggelsee, a shallow eutrophic lake in Germany (Wilhelm and Adrian, 2007). In large lowland rivers in France, the heatwave caused a rapid, substantial change in the structure of mollusc communities, as well as drastic declines in species richness and density (Mouthon and Daufresne, 2006). Coupled with major droughts and floods, it led to the development of assemblages of tolerant and invasive taxa (Daufresne et al., 2007). The heat wave also increased parasitism and mortality of fish in an experimental study in the lake Grosser Plöoner See in Germany (Wegner et al., 2008).

The occurrence of hot extremes is expected to increase in the future (IPCC, 2012, 2013), and thus, understanding how aquatic communities recover from such disturbance is a priority for research. According to Stott et al. (2004), the heatwave of 2003 was a manifestation of global warming. Freshwater communities were exposed to a short-term pulse disturbance (heatwave) overlying a ramp disturbance (environmental warming) intensifying steadily over time (see Lake, 2000). How the impact of these two disturbances combined to influence the resilience of the freshwater communities remains uncertain. For instance, the recovery time of most aquatic invertebrates to a pulse disturbance is <3 years (Resh et al., 1988; Niemi et al., 1990; Yount and Niemi, 1990; Lake, 2000; Watanabe et al., 2005). However, Mouthon and Daufresne (2006) and Daufresne et al. (2007) did not observe clear signs of recovery of macroinvertebrate communities in the 3–4 years after the heatwave, and since then, more research has been undertaken to track the extent of recovery. In this paper, we analyse the change in structure of the mollusc communities in the Saone River and its two main tributaries, the Doubs and Ognon, over 8 years following the heatwave. The aim of the study was to evaluate the resilience of the different river systems to the heatwave within the context of global warming.

Methods:

Study area

The River Saone (catchment area 29 900 km^2, length 473.4 km) rises in the Vosges mountains at an altitude of 405 m and falls 245 m along its course, joining the Rhone at Lyon (Fig. 1). For 300 km of its lowest reaches, the river is channelised for navigation, with a low gradient (0.08 m km^{-1}). As the river is prone to heavy flooding, surrounding land is only sparsely urbanised, with industrial zones being located near the main towns. Land use in the catchment is predominantly agricultural (58%), mixed with forestry and other semi-natural environments (36%). Livestock farming is common in the upper river valley, with cereal farming, market gardening and wine growing widespread in the lower valley. River water quality has been degraded by navigation, agricultural inputs and urban and industrial discharges, so concentrations of nitrates, phosphates and various toxic pollutants increase from upstream to downstream (Agence de l'eau, 2005). At all sites, the bed of the river is dominated by carbonate-rich sediments, mainly fine sand and silt. River

Figure 1: Study area and location of the mollusc sampling sites (M) and discharge (Q), water temperature (Tw) and water physico-chemical (PC) recording sites.

water is alkaline (mean pH ± SD ranging from 7.88 ± 0.14 to 8.16 ± 0.07 in the different sampling sites, see Table S1), well oxygenated (mean ranging from 9.62 ± 0.36 to 10.27 ± 0.38 mg L^{-1}) and nutrient-rich (e.g. mean total phosphorus concentration ranging from 0.05 ± 0.02 to 0.17 ± 0.09 mg L^{-1}). Water quality is poorest at Lyon where high concentrations of chloride (40.55 ± 11.07 mg L^{-1}) and sodium (23.90 ± 6.96 mg L^{-1}) reflect industrial activity. The Saone receives *c.* 20 tributaries of which the Ognon and the Doubs are the largest. The river is linked to the basins of the Moselle, Seine and Loire by canals that have probably accelerated colonisation by exotic species (Bij de Vaate et al., 2002).

Environmental variables

Mean daily discharge (1977–2011) for three sites on the Saone River (Ray/Saône, Pagny-la-Ville, Mâcon) and two sites on the Saone's main tributaries at Pesmes (River Ognon) and Neublans (River Doubs) (Fig. 1) was obtained from the Banque Hydro (data available at http://www.hydro.eaufrance.fr). Mean daily water temperature data (1977–2011) recorded automatically at the Saone at lock of Couzon (*c.* 10 km from the confluence with the Rhone) were supplied by EDF (Electricitée de France). Water temperature data collected by Office National de l'Eau et des Milieux Aquatiques (ONEMA) were available for the Saone at Jussey and Tillenay, in the Ognon at Thervay and in the Doubs at Gevry (Table S2). We used mean daily air temperature at these sites (SAFRAN model; Vidal et al., 2010) to estimate missing values from 1 January 1977 to 31 December 2011. Water temperature at date t was regressed on the mean air temperature over the $t-6$ to t period (1-week period, R^2 ranging from 0.91 to 0.97).

Mollusc sampling sites

Mollusc communities were sampled monthly (September 1996–December 2011) from the River Saone at Lyon and annually (in September or October, various years, see Table S3) at a further six sites across the river network (Fig. 1). The Saone at Lyon is *c.* 150 m wide with two-thirds of this width consisting of a navigable channel. The sampling site was *c.* 150 m long, and more than half of its surface area was covered with macrophytes (*Nuphar*, *Ceratophyllum*, *Potamogeton* and filamentous algae) from May to October. The sampling sites at Port/Saône, Gray and Pontailler/Saône were located in bypassed river sections (non-navigable old channels). The site at Allériot was downstream of the confluence of the Doubs. Broye-les-Pesmes and Saunières were in the lower reaches of the Ognon and Doubs, respectively, immediately upstream of their confluence with the Saone (Fig. 1). At each site, one 0.25 m^2 sample was taken at each of four stations, at a depth between 0.50 m (depth at which wake effects lessen) and 1.5 m, using a rectangular hand-net (25 x 18 cm, 315 μm mesh). Samples were kept separately (Lyon) or combined into a single sample (annual sampling sites), fixed on-site in 12% neutralised formaldehyde, and molluscs later separated from sediment, identified to species and counted.

Statistical analysis

Temporal variation in mollusc community structure was evaluated for univariate (species richness and total density) and multivariate (species 9 months, species 9 years matrix of mollusc density) data sets. For each site, a principal components analysis (PCA) was performed on $ln(x + 1)$-transformed mollusc density data. Species with an occurrence <10 in the site-specific data set were excluded from the analysis because PCA can be sensitive to rare taxa. To detect trends in environmental (discharge, temperature) and biological time series at the Lyon site, we used a modified MannâKendall trend test developed by Hamed and Rao (1998). This nonparametric analysis (based on ranks) tested for temporal trends once autocorrelation effects were removed. For the Lyon site data, analysis of variance (ANOVA) was used to test for the effect of the heatwave on (i) factorial site scores from the PCA, (ii) species richness and (iii) density of gastropods and bivalves. Data were allocated to one of two groups depending on their collection date before (September 1996–July 2003) or after (August 2003–December 2011) the heatwave. Autocorrelation consistent (HAC) estimators were used to assess the covariance of the ANOVA model parameters and, in turn, the statistical significance of differences between the two groups (Zeileis, 2004). Temporal change at the other sampling sites was described only qualitatively because of limited data before 2003. To determine the return of the biological variables to an initial state, we used a method based on the maximisation of between-group sample variance. This analysis was performed only on time series which showed a clear post-heatwave shift in n values, that is when the first samples following the heatwave were clearly outside the range of the pre-heatwave values. With the heatwave occurring at time t, values could be divided into groups of $t - 1$ pre-heatwave values and $n - t + 1$ post-heatwave values. Under the hypothesis of a return to the initial state, we evaluated the extent to which the x most recent samples ($n, n - 1...n - x + 1$; $x \in [0, n - t]$) were closer to the pre-heatwave values than to the $(n - t + 1) - x$ post-heatwave values. The procedure consisted of finding the value x which maximised the variance between the two groups: (i) a group of samples where the $t - 1$ pre-heatwave samples were pooled together with the x most recent samples and (ii) the rest of the data. Note that if the variance was maximal for $x = 0$, we considered there was no return to the initial state. All analyses were performed using Statistica package library (version 9.0), R (R Development Core Team, 2013) and sandwich packages (Zeileis, 2004).

Results:

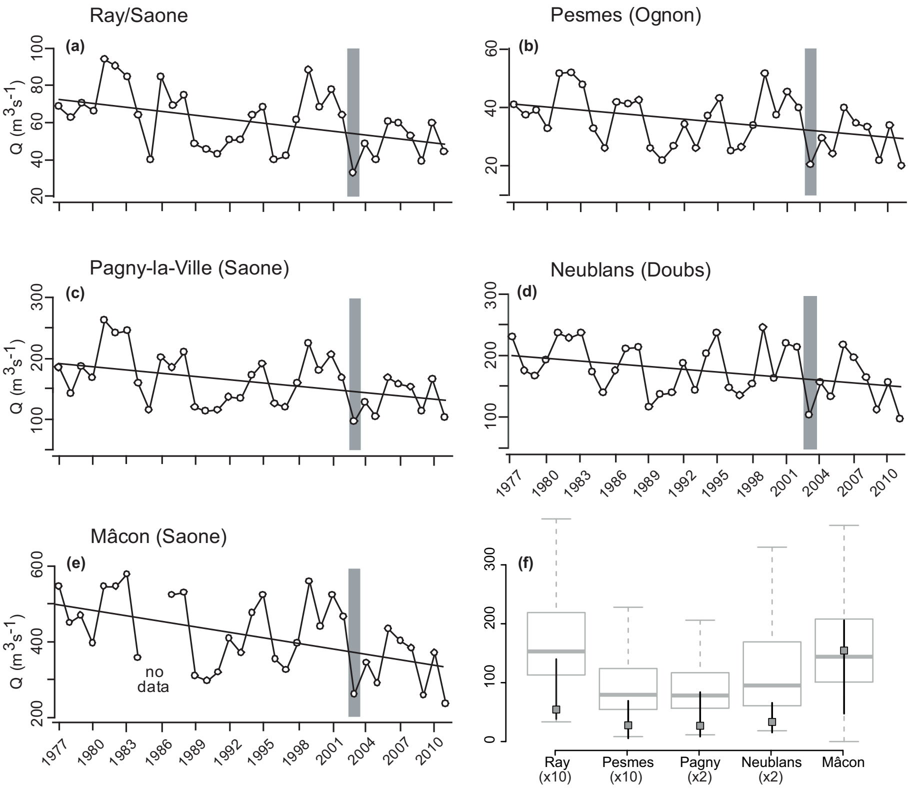

Figure 2: Mean annual discharge in the Saone, Ognon and Doubs rivers (a–e), with linear trends shown as solid lines. Y axes are scaled to data. The 2003 heatwave is highlighted in grey. Boxplot of the summer (July–September) mean daily discharge at the different sites between 1977 and 2012 (f). Lower and upper limits of the boxes represent the first and third quartiles of the data. The thick horizontal grey segments represent the median values. For each boxplot, the whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box. Range of the 2003 values (thick vertical black line) and the 2003 median (large grey squares) is highlighted. To increase readability, some values have been multiplied by a factor f (indicated in the figure as xf).

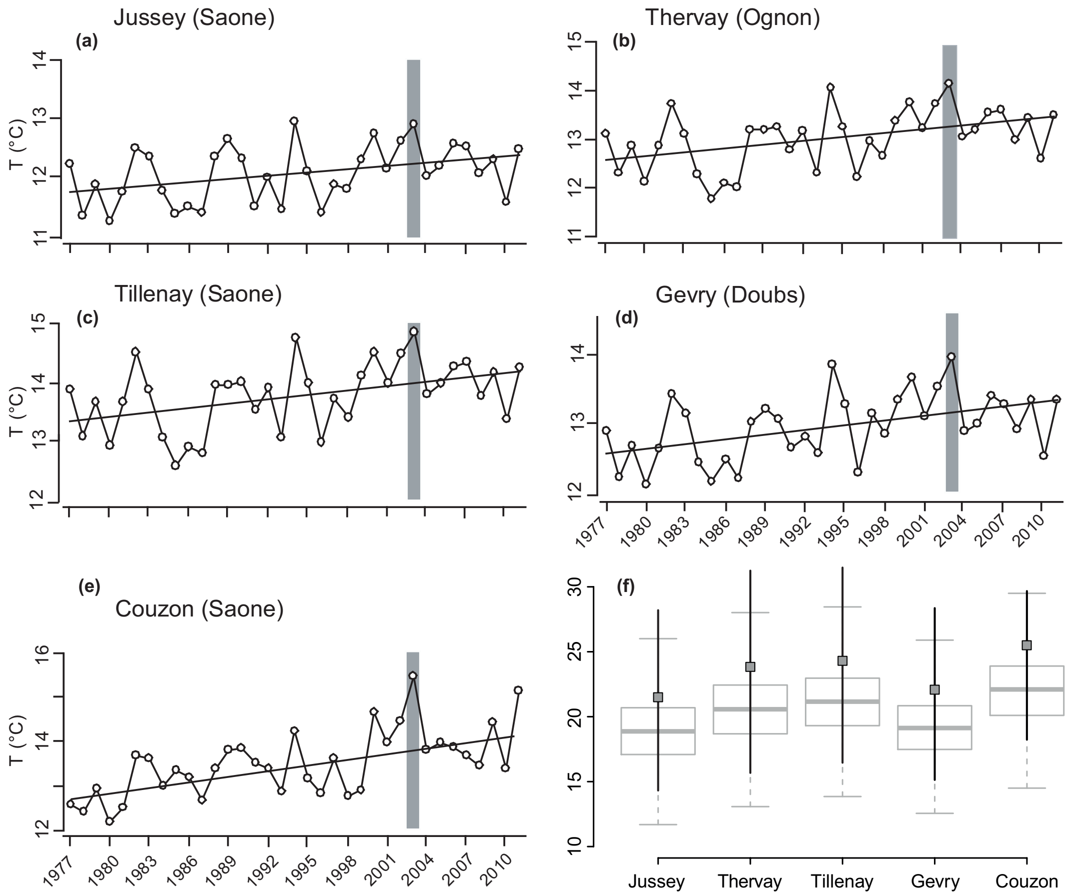

Figure 3: Mean annual water temperature in the Saone River at Jussey, Tillenay and Couzon, in the Ognon at Thervay and in the Doubs at Gevry (a–e), with linear trends shown as solid lines. Y axes are scaled to data. The 2003 heatwave is highlighted in grey. Boxplot of the summer (July–September) mean water temperature at the different sites between 1977 and 2011 (f). Lower and upper limits of the boxes represent the first and third quartiles of the data. The thick horizontal grey segments represent the median values. For each boxplot, the whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box. Range of the 2003 values (thick vertical black line) and the 2003 median (large grey squares) is highlighted.

Discharge and temperature data

Mean annual discharge varied among wet and dry years and declined significantly at all the sites from 1977 to 2011 (Mann–Kendall trend test, $P < 0.0001$ at Ray/S. and Pagny-la-Ville, $P < 0.01$ at Mâcon and Pesmes, $P < 0.05$ at Neublans) (Fig. 2). The post-heatwave years, especially 2005, 2009 and 2011, caused this negative trend, as no obvious tendency was observed between 1977 and 2003 (Mann–Kendall trend test, $P > 0.05$ at all the sites). The lowest mean daily discharge in summer (July–September) was in 2003 (for Neublans, Pagny-la-Ville, Pesmes and Ray/S.), with flows 3.1–4.4 m^3 s^{-1} lower than the mean summer dis-

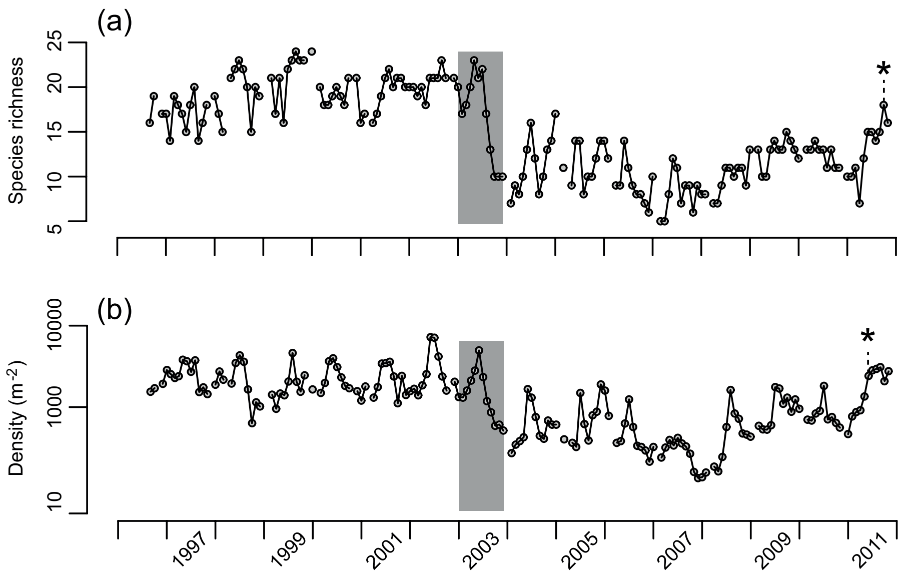

Figure 4: Monthly variation of species richness (a) and density (b) of molluscs in the Saone River at Lyon. The 2003 heatwave is highlighted in grey. Dates of recovery are highlighted by stars.

charge for the period 1977–2011 (Fig. 2f). Conversely, the 2003 summer discharge at Mâcon did not deviate markedly from conditions in 1977–2011 (Fig. 2f). Mean annual water temperature at Couzon (*c.* 10 km from Lyon) increased significantly ($P = 0.0003$) by 0.05 °C year^{-1} from 1977 to 2011. However, this warming mainly occurred before the heatwave and no significant trend could be distinguished from 2003 to 2011 ($P = 1$). We observed the same pattern for water temperature at Jussey and Tillenay (Saone River), Thervay (Ognon) and Gevry (Doubs River) for the period 1977–2003 ($P < 0.05$ at all sites) and 2003–11 ($P > 0.6$ at all sites). The mean summer (July–September) water temperature was lowest in 1977 at Gevry and Couzon and in 1996 at the other sites. The mean daily temperature was highest in 2003 for all the sites. For instance, at Couzon in 2003, the maximum temperature was 29.5 °C (versus 24.3–26.4 °C from 1996 to 2002) and exceeded 25 °C for 75 days (versus 0–17 days from 1996 to 2002). The median summer water temperature of 2003 was particularly high at all sites (21.5

Table 1: Time (year or year and month according to sampling frequency) at which we observed a return to initial conditions for mollusc density, species richness and community structure. Community structure is summarised by scores of the samples on the two-first axes (F1 and F2) of principal components analysis (PCAs) performed on $ln(x+1)$-transformed densities of species

			Community structure	
Site (river)	Density	Species richness	F1	F2
Port/Saône	2008	No return	2008	No shift/gradual change
Gray (Saone)	2008	2009	2008	No return
Pontailler/Saône	2009	No return	2008	No return
Allériot (Saone)	2011*	2010	2006*	No return
Lyon (Saone)	June 2011	October 2011	June 2011	No shift/gradual change
Broye-les-Pesme (Ognon)	2008	2008	2008	No return
Saunière (Doubs)	2005	2004	2004	No shift/gradual change

*No clear shift (the first post-heatwave samples correspond to extreme values of the pre-heatwave period).

°C at Jussey, 23.8 °C at Thervay, 24.3 °C at Tillenay and 25.5 °C at Couzon) and deviated strongly from the distributions of the summer data 1977–2011 (Fig. 3f).

Mollusc data

A total of 156 673 molluscs were collected at Lyon between September 1996 and December 2011, but of these, only 38 613 (24.6%) were found after the heatwave. Thirty-two species (18 gastropods, 14 bivalves) were collected at this site over the study period, of which eight (*Ancylus fluviatilis*, *Stagnicola sp.*, *Galba truncatula*, *Gyraulus laevis*, three unionid species and *Pisidium milium*) were rare (<10 individuals in the full data set). After the heatwave, the formerly dominant species *Valvata piscinalis* was replaced by the exotic *Corbicula fluminea*. Between 1997–2003 and 2004–11, the mean relative abundance of *Valvata* at Lyon declined from 28.6 to 5.4% whereas that of *Corbicula* increased from 13.4 to 39.8%. A total of 90 775 individuals were collected from the other six sites, and the number of mollusc species observed at individual locations was ranged from 27 (Gray) to 32 (Saunières). Twenty species were common to all the sites.

Temporal change in mollusc community at Lyon

The 2003 heatwave had a major impact on the richness (43.1% reduction) and abundance (74.2% reduction) of molluscs (ANOVA $d.f. = 163$, all $P - values < 0.001$). Richness was more strongly reduced for gastropods (52.3%) than bivalves (34.8%). Abundances of both groups were strongly suppressed (gastropods by 85.5% and bivalves by 64.0%). After the heatwave, both species richness and density of molluscs continued to decline until 2007 and then increased progressively until 2011 (Fig. 4). Mollusc density and richness returned to predisturbance levels in June and October 2011, respectively (Fig. 4, Table 1).

The first two axes of the PCA accounted for 57.4% of the inertia in the mollusc data (F1 = 41.5%, F2 = 15.9%, respectively). The sudden change in the structure of the mollusc communities caused by the 2003 heatwave (see Mouthon and Daufresne, 2006) is shown by the clear separation of scores from 1997–2003 and 2004–11 from left to right along axis 1 of the PCA (Fig. 5a). Axis 2 shows the temporal evolution of community structure in the years before (from the bottom of the axis upwards) and after (from the top of the axis downwards) the heatwave. The test performed on the monthly factorial scores on F1 revealed a negative trend before the heat- wave (Mann–Kendall trend test, $P < 0.001$) and no trend after the heatwave (Fig. 5b, c). There was a significant difference in the mean of the monthly factorial scores before and after 2003 on F1 (ANOVA, $d.f. = 163$, $P < 0.0001$), but not on F2 ($P = 0.58$). The heatwave effect explained 76.6% of the variance on F1 and only 2.3% on F2. In fact, the monthly factorial scores on F2 did not show a shift in their values but a positive trend (Mann–Kendall trend test, $P < 0.001$) versus negative trend (Mann–Kendall trend test, $P < 0.001$) before versus after the heatwave (Fig. 5b, c). We observed a recovery in F1 values in June 2011. The situation is more complex for F2, where the 2011 scores are closer those of 1996 than 2003.

Species with low scores on F1 and F2 (e.g. *Pisidium moitessierianum*, *Pisidium henslowanum*, *Pisidium subtruncatum*, Fig. 6) declined in abundance with time, especially after the heatwave. However, some species showed signs of recovery, especially those with higher scores on F1 (e.g. *Potamopyrgus antipodarum*, Fig. 7). Species with high scores on F2 and low scores on F1 (e.g. *Musculium acustre*, *Bithynia tentaculata*, *Pisidium amnicum*, *V. piscinalis*, Fig. 6) increased up to the heatwave. Densities then rapidly declined and remained very low (e.g. *M. lacustre* and *V. piscinalis*, Fig. 7). Species with high scores on F2 and F1 showed similar patterns up to the heatwave, but this temporarily boosted, rather than suppressed, their densities. For these species, the subsequent decrease in density occurred later in the time series (e.g. *D. polymorpha*, Fig. 7). Finally, species with low F2 scores and intermediate F1 scores showed an initial decrease in density and an increase after the heatwave (e.g. *Physa acuta*, Fig. 7). Species with intermediate scores on F1 and F2 did not show marked trends in their densities (e.g. *C. fluminea*). Note that the mean annual density of the invasive *D. polymorpha*, which prefers stony habitats, remained low over the whole study period (maximum 10.8 individuals m^{-2} in 2003).

At Lyon, persistent signs of community recovery from the heatwave were not observed until 2009, when several gastropod species reappeared (*V. piscinalis*, *Valvata cristata* and B. tentaculata). Further recovery was evident by 2011 when Gyraulus albus and M. lacustre returned and the density of sphaeriid bivalves increased. Nonetheless, post-heatwave mollusc communities remained different in structure from pre-heatwave communities (F1 x F2 score combination remains different), with several species common pre-2003 (*Lithoglyphus naticoides*, *Theodoxus fluviatilis*, *Radix balthica* and *P. amnicum*) still absent in 2011.

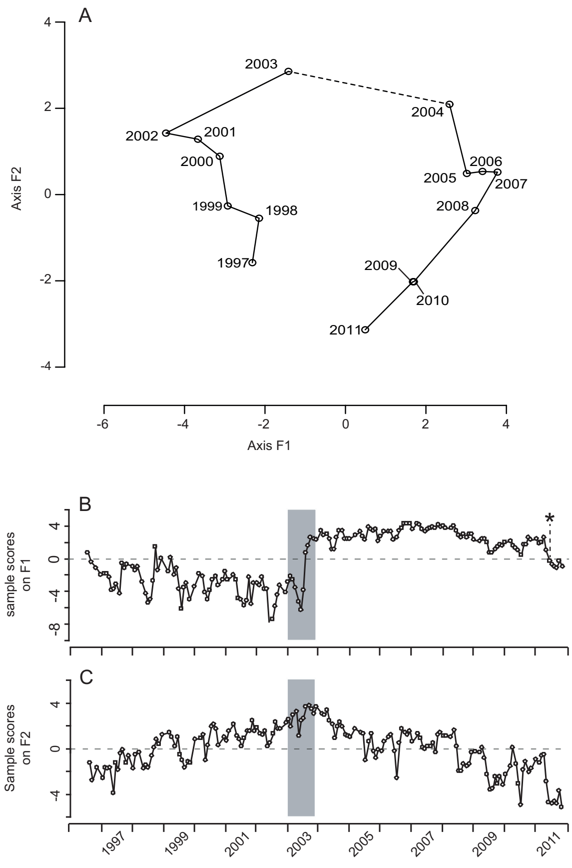

Figure 5: Results of the principal component analysis performed on mollusc quantitative data in the Saone River at Lyon. (a) Mean yearly (black line) factorial scores of the monthly samples on the two-first axes of the principal components analysis (PCA) (F1, F2). (b, c) Times series of scores of the monthly samples on F1 and F2. Horizontal dashed lines represent overall means. The 2003 heatwave is highlighted in grey. Dates of recovery are highlighted by stars.

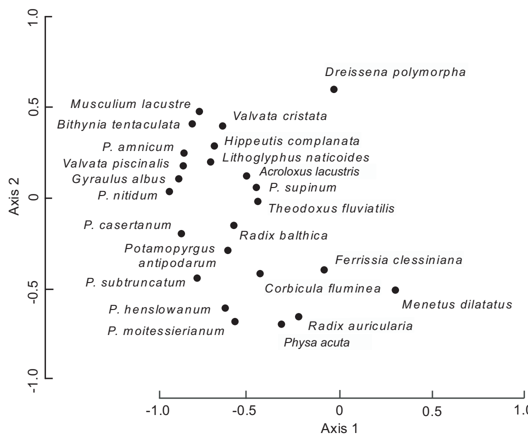

Figure 6: Results of the principal component analysis performed on mollusc quantitative data in the Saone River at Lyon, scores of the species on the two-first axes (P = *Pisidium*).

Temporal change across the Saone catchment area

We observed a decline in species richness and mollusc density after the heatwave at the six sites (Fig. 8, see also Mouthon and Daufresne, 2006), although the change in density at Allériot was less clear than in the other sites (the first post-heatwave values are close to those of 1997–98). The rate of recolonisation varied among sites (Fig. 8, Table 1). Mollusc densities increased rapidly (from 2005) at Saunières, but recovery was slower elsewhere. Complete recovery of mollusc densities (to pre-heatwave levels) was observed at Port/S., Gray and Broye in 2008 and at Pontailler/S. in 2009 (Fig. 8, Table 1). In contrast, sphaeriid bivalves never returned to pre-heatwave densities at Broye and Saunières. At Allériot, a site degraded by chemical discharges, recovery time is difficult to identify due to marked variation before the heatwave. At this site, low densities recorded in 1997 and 1998 were attained in 2004, whereas higher densities observed in 1999 (after the high water levels of the Doubs in June and July), were not exceeded until 2011 (Fig. 8). Gastropods (mainly the numerically dominant P. antipodarum, 55–83% total numbers) were the main contributors of community recovery at all sites except Gray, where sphaeriid bivalves were more common (53.3% total numbers). Trends in species richness were similar to those observed in density, except at Port/S. and Pontailler/S. where the number of species present did not recover (Fig. 8, Table 1).

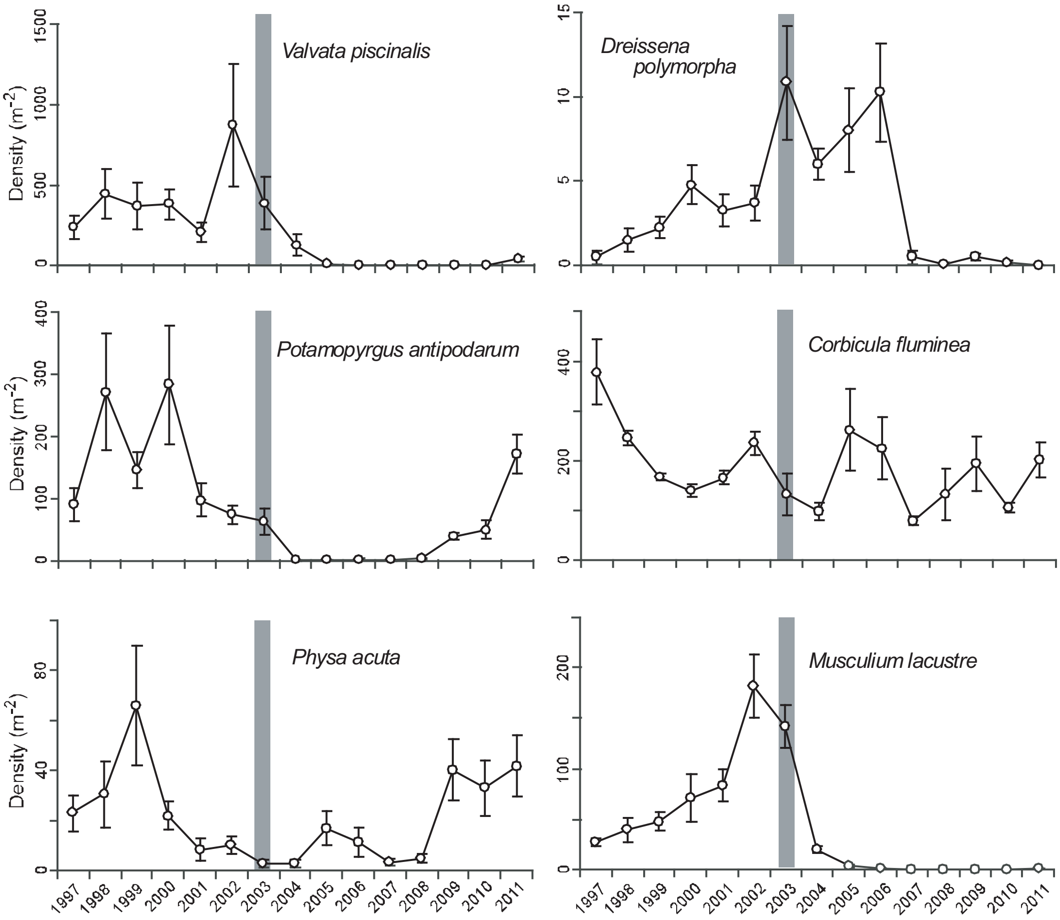

Figure 7: Time series of mean annual density (±SD) of six key species: *Valvata piscinalis*, *Potamopyrgus antipodarum*, *Physa acuta*, *Dreissena polymorpha*, *Corbicula fluminea* and *Musculium lacustre* in the Saone River at Lyon. *Y* axes are scaled to data. The 2003 heatwave is highlighted in grey.

The PCA revealed how the heatwave of 2003 modified the structure of mollusc communities at all the sites (Fig. 9). For four sites (Port/S, Gray, Broye and Saunières), marked shifts in community structure along the first axis suggest pronounced impacts of the heatwave. For two sites (Pontailler/S and Allériot), change along the second axis (accounting for less variation) was more prominent, revealing less severe heatwave effects. Mollusc communities were also affected by low temperature during summer (all sites in 2007), floods in summer (all sites in 2007, Saunières in 1997, 2010 and 2011) and autumn (Saone River in 1998 and in August 2006), and dredging (Gray and Allériot in 2009). The timing of recovery from the heatwave differed among the sites, ranging from 2004 (Saunières) to 2008 (Pontailler/S. and Broye, Table 1). Recovery of mollusc populations was faster in the Doubs than in the Saone and the Ognon, and overall, the years 2008–11 proved to be a favourable period for recolonisation. Nonetheless, scores on the second axis (F2) remained very different from the initial state at all the sites, and no complete returns to the pre-heatwave community structures were observed.

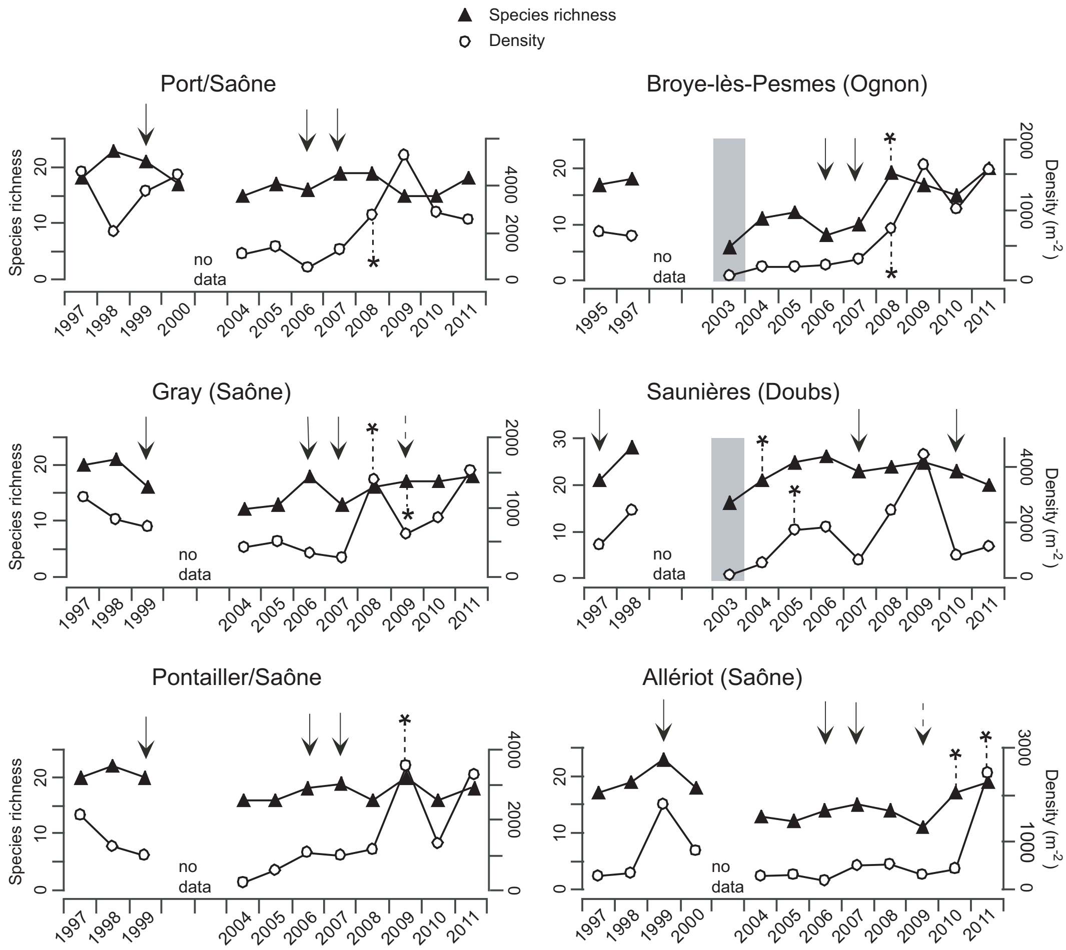

Figure 8: Annual variation of species richness and density of mollusc communities at the annual sampling sites in the Saone, Ognon and Doubs rivers. Sampling dates (solid arrow) preceded by summer floods and dredging of river bed (dotted arrow) are shown. Y axes are scaled to data. The 2003 heatwave is highlighted in grey. Dates of recovery are highlighted by stars.

The rate of recovery accelerated late in the time series, with the cold and wet year of 2007 being apparently particularly favourable for recolonisation (the mean discharge in summer in 2007 was 2.0–2.2 times higher than the mean summer value of the period 1977–2011 for each of these sites, and summer 2007 was the second coldest in terms of mean values at Tillenay, Thervay and Couzon and the fourth coldest at Gevry and Jussey). Eight biological variables among the 28 studied showed signs of recovery in 2008 (Table 1).

Discussion:

Long-term monitoring in the River Saone and its tributaries provided evidence for slow and incomplete recovery of mollusc community structure from the severe impacts of the 2003 heatwave (see Mouthon and Daufresne, 2006). Nevertheless, recovery of specific community descriptors (PCA scores, species richness, density) was observed and progressed at different rates across the river network. Typically, the recovery time (e.g. of total density and species richness) of aquatic invertebrates to a pulse disturbance is <3 years (Resh et al., 1988; Niemi et al., 1990; Yount and Niemi, 1990; Lake, 2000;

Watanabe et al., 2005). In this study, complete recovery to pre-heatwave densities required 2–8 years, whereas recovery of species richness was incomplete at some sites but occurred rapidly (1 year) at one site (Saunières). A complete return to the initial community structure was not observed at any site, although there were clear signs of partial recovery after 1 (at Saunières) to 8 years (at Lyon).

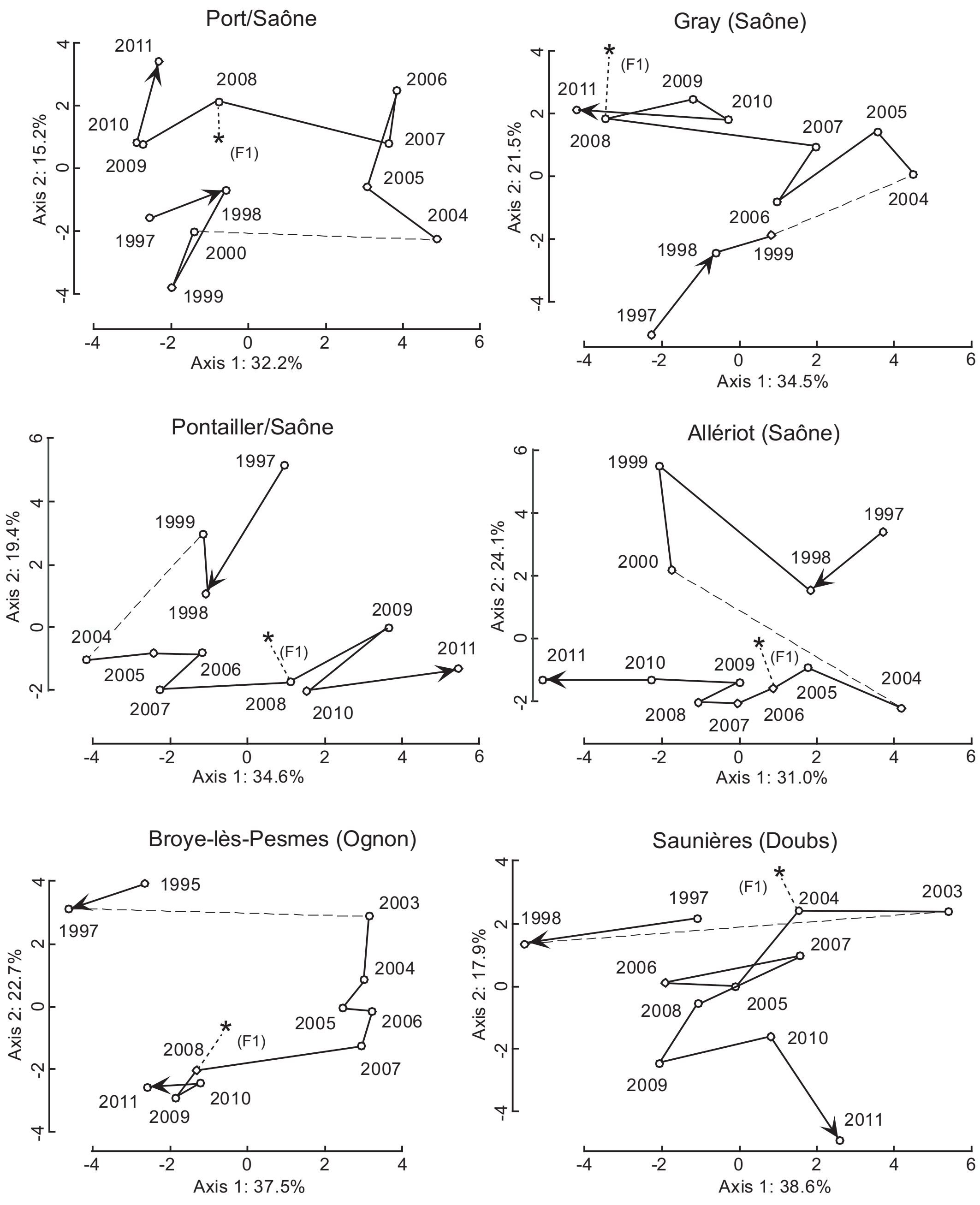

Figure 9: Factorial scores of the yearly samples on two-first axes (F1, F2) of the principal component analysis performed on mollusc quantitative data at the annual sampling sites in the Saone, Ognon and Doubs rivers. Dates of recovery are highlighted by stars.

The magnitude and duration of responses to major pulse disturbances, such as a heatwaves, can depend on the presence or absence of an underlying press (Collier Quinn, 2003) or ramp disturbance. Both the magni- tude of change and the recovery time were the lowest at Saunières on the Doubs (Table 1), the site least impacted by non-climatic anthropogenic pressures. The species-rich assemblages at Saunières suggest that the lower reaches of the Doubs are more suitable for molluscs than the middle (bypassed sections) and lower reaches of the Saone and Ognon rivers. Rapid recovery at Saunières may be explained by the presence of donor sites like backwaters permanently connected with the main stream, and tributaries with good water quality, that favour mollusc recolonisation by drift (Niemi et al., 1990; Yount and Niemi, 1990; Mackay, 1992; Collier and Quinn, 2003; Watanabe et al., 2005). This capacity of mollusc populations for rapid recovery suggests that the resilience of the Doubs is greater than that of the Saone and the Ognon. Nevertheless, there is a clear underlying trend in the structure of the mollusc community of the Doubs which is a likely consequence of gradual environmental change, probably global warming. Such changes in community structure may modify resilience to disturbance, and the question remains as to whether the present community will recover as quickly following future heatwaves. Surprisingly, in the lower Saone where the impact of these press and ramp disturbances was greatest, the time to complete return of mollusc density to pre-heatwave levels was highly variable. Recovery was especially rapid at Allériot (<1 year; Table 1, Fig. 9), a degraded site where mollusc density is generally low. Again, this site located right downstream of the confluence with the Doubs probably benefited from high-quality donor sites upstream. At the other sites, recovery from the heatwave may have been delayed and/or diverted by gradual warming and/or navigation and pollution of the river over the sampling period.

A progressive increase in water temperature from 1977 to 2011 affected the ecology of the Saone, Doubs and Ognon rivers (Mouthon and Daufresne, 2006, 2010, ; Fig. 3), while the decade 2001–11 was the warmest recorded in France since 1900 (http://climat.meteofrance.com). At Lyon, between the September 1996–July 2003 and August 2003–04 periods, the decline in mean density was >50% for 19 species (12 gastropods and seven bivalves) and <50% for three species (the gastropod *Ferrissia clessiniana* and two bivalves *M. lacustre* and *Pisidium supinu*m). The species colonising both the upper and lower part of rivers or reaching their maximal den- sity in the lower part are eurythermic (Meier-Brook, 1975; Mouthon, 1999). Nevertheless, most of them were strongly affected by the heatwave. Besides potential direct thermal effects, a fall in dissolved oxygen concentrations at the end of the night may also have affected molluscs during the heatwave. A better knowledge of the thermal niches of the species and their relative ability to face anoxia would further understanding of underlying mechanisms. However, the increase in the density of the exotic *Menetus dilatatus* (gastropod) and *D. polymorpha* (bivalve) tends to show that the most eurytolerant species were favoured, although their populations remained very low (Mouthon and Daufresne, 2006). Regarding climatic drivers, moderate floods in summer 2007 appeared to have initiated (Saone and Ognon) or reactivated (Doubs) the recolonisation process and favoured the return of density to pre-heatwave levels, possibly by increasing drift. In addition, the summer water temperatures after the heatwave of 2003 were generally close to mean values for 1996–2011, while the low discharges after the heatwave undoubtedly promoted the recovery of mollusc densities. Lower variation in discharge (absence of strong floods) and temperature favouring good oxygenation of the water during the summer period ensured conditions beneficial to the development of these quiet-water organisms (Boycott, 1936; Dillon, 2000).

Boat-generated wakes and resuspension of bed sediment by boat propellers negatively affect the growth and distribution of aquatic macrophytes (Murphy and Eaton, 1983; Vermaat and De Bruyne, 1993) which constitute an important habitat for molluscs (especially gastropods). More than 5000 boats (3/4 carrying goods and 1/4 river cruises or sailing) transit the Couzon lock each year, so navigation activities may have slowed recovery in the most downstream sites. Chemical pollution has also been identified as a problem in the river and could potentially prevent the recolonisation of sensitive recolonists. However, while toxic substances (e.g. pesticides, metals, PAHs) were particularly prevalent in the downstream part of the Saone (Agence de l'eau, 2005), there has been no significant reduction in water quality since the heatwave (SEQ-Eau, available at http://sierm.eaurmc.fr/eaux-superficielles/fichiers-telechargeables/grilles-seq-eau-v2.pdf), and hence, influences on recovery are uncertain. Nevertheless, unusually low chl-a concentrations in the Saone downstream of the Doubs confluence since at least 1987, the cause of which remains unknown but possibly involved pollution (Fruget and Persat, 2000; Agence de l'eau, 2005), may have affected the recovery of molluscs in these reaches. In fact, molluscs that feed on phytoplankton (*C. fluminea*, *D. polymorpha* and the Unionidae) or periphyton (mainly gastropods) may be food-limited (Mouthon, 2001; Mouthon and Daufresne, 2008). However, pedal feeding on organic matter by *C. fluminea* (Way et al., 1990; Hakenkamp and Palmer, 1999) could explain the persistence of this species (Fig. 7), although its growth is generally slower in such instances (Mouthon, 2001). Finally, recent introductions of invasive species may have influenced recovery. New mollusc species such as the bivalve *C. fluminea* and other seston and deposit feeders, such as *Hypania invalida*, *Chelicorophium curvispinum* and mysids, may compete for food with native molluscs. Invasive non-mollusc predators such as *Dikerogammarus villosus* (Van der Velde et al., 2000; Dick et al., 2002; Devin et al., 2005) may also modify the structure of mollusc communities (Strayer, 2010). In conclusion, mollusc densities did recover across the river network. However, we did not observe complete recovery of mollusc community state (defined as the combination of structure 9 density 9 species richness) at any site, even though 8 years have elapsed since the heatwave. The arrival of exotic species (Strayer, 2010), and an increase in the frequency of extreme events possibly linked to global warming in future years, could impede complete recovery of the mollusc community in the Saone and its major tributaries (Beniston et al., 2007; IPCC, 2007; Planton et al., 2008). It could be that the abrupt shift of mollusc community structure caused by the heatwave and underlying warming trend represents a new stable state, as outlined by theory (Scheffer et al., 2001; Scheffer and Carpenter, 2003; van Nes and Scheffer, 2004). Our study suggests that global warming could shape how biotic communities respond to future pulse disturbances, and may lead to the formation of communities consisting of species that are extant today, but in new combinations not found at present (Williams Jackson, 2007).

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