www.nature.com/scientificreports

scientific reports

OPEN

Spawning in a threatened freshwater mussel shifts to earlier dates as a result of increasing summer mortality

Tadeusz A. Zając✉ & Katarzyna Zając

Freshwater mussels are among the most imperilled groups of animals on the globe, however, the drivers of mussel declines are still poorly understood. Here we show that in a seasonal environment, *Unio crassus* females can initiate spawning from late winter to late summer, implying a very high phenotypic plasticity of their spawning date. However, they shift their reproductive effort to earlier dates and make greater investments in early broods in order to adapt to high spring temperatures and higher levels of summer mortality. Thus, the reproductive investment during early spring may credit energy to be invested later in self-maintenance, in order to alleviate the impact of mortality. As a result, mussel reproduction is being progressively compressed into earlier and shorter periods. Such constraints must reduce reproductive output and contribute to the decline of this already endangered group. The seasonal pattern of reproductive effort can always serve as an excellent indicator of the conservation status of a given population.

Although large freshwater mussels (Bivalvia, Unionida; hereafter, naiads) can dominate the benthic biomass of inland waterbodies and provide important ecosystem functions and services at a very large scale[1], they continue to be understudied. Moreover, recent mass die-offs have shown that they are in urgent need of conservation action[2]. Their rapid decline is both a worldwide[3] and a European phenomenon[4–8]. The drivers of mussel declines are still poorly understood[9], but the recent increases in the frequency and intensity of such declines point to a relationship with global warming.

The most widespread reaction to the global rise in temperatures has been the shift of phenology in seasonal environments, which is believed to be a simple consequence of higher temperatures[10]. Thus, seasonal environments offer a climate credit[11] to the species inhabiting them, reducing the impact of elevated temperatures, e.g. by their starting reproduction at progressively earlier dates, thereby shifting reproduction to the cooler part of a season. In the case of naiads, it may be suspected that recent temperature rises are coinciding with the mussels' mortality. This has a negative effect on their demography and also exerts very severe selection pressure, the consequences of which are hitherto unknown.

In view of the abrupt nature of global temperature change, the only adaptation mechanism that seems to be keeping pace with the rate of global change is a high phenotypic plasticity, enabling phenotypic adjustment[12]. However, although naiads are threatened at the global scale, hardly anything is known about their phenology, reproductive effort and natural history, not to mention the plasticity of their life history traits. Critically, this knowledge is indispensable in the context of their monitoring and conservation[2].

Life history theory relates to the allocation of energy to time, because the optimally time-distributed allocation of energy may reconcile many conflicting demands (e.g. current reproduction vs. future survival). In life history theory, time is usually understood as a linear lifetime axis along which energy is allocated unevenly in order to maximize lifetime fitness[13]. But since resources fluctuate periodically in the majority of habitats and these fluctuations have been amplified by global warming to levels threatening the survival of many species, it is essential to understand the energy allocations within the course of particular seasons if one is to grasp the impact of phenology shifts on lifetime fitness[14]. Nevertheless, the patterns underlying seasonal allocations of energy and their timing have not yet been explored, although a few important insights have been acquired[15].

To understand the mechanisms underlying the phenology of naiad reproduction, it is essential to consider two reproductive strategies: (1) investment in a single brood, as seen in Anodonta species, and (2) investment in multiple, separate reproductive bouts occurring sequentially throughout the spring and summer period, as

Institute of Nature Conservation, Polish Academy of Sciences, 31-120 Kraków, Al. Adama Mickiewicza 33, Kraków, Poland. ✉email: tzajac@iop.krakow.pl

observed in Unio species[16]. In the latter case, organisms theoretically have the potential to allocate resources differently across reproductive bouts, necessitating an investigation into the extent of phenological plasticity. If such plasticity is absent, the ability to respond rapidly to environmental change is limited, making a rapid shift in reproductive timing unlikely. To assess the possibility of phenological shifts, it is necessary to determine both the full range of dates when reproduction can be initiated and the threshold beyond which further reproduction is no longer possible.

For European naiads, the environmental cues that trigger the onset and cessation of reproduction remain largely unknown. Current knowledge is restricted to general information indicating that reproduction occurs during the spring and summer months[17]. Moreover, data on reproductive investment across the breeding season are scarce and very general[18–20].

A shift in reproductive phenology due to global warming could be advantageous if it results in an extended reproductive period due to earlier initiation. If reproductive output in a given bout is constrained by factors such as body size, as observed in naiads[21], yet available resources allow for further reproduction later in the season, additional breeding bouts will occur to maximise resource utilisation within the seasonal reproductive window. This would enable individuals to produce more broods per season, thereby increasing overall fitness[22], Fig. 1A). Consequently, it can be hypothesised (H1) that an extended reproductive period, due to an earlier onset, is beneficial as it increases the number of reproductive bouts within a season.

However, the fitness benefits of shifting reproductive onset to earlier dates depend not only on the number of bouts but also on the level of investment (i.e., the number of larvae) in each brood. Assuming an *ad libitum* resource supply, the energy allocated to each reproductive bout should be equal, as it is limited primarily by female body size[21]. If, alternatively, reproductive investment is proportional to the availability of organic, mostly algal food throughout the season[23] and to increasing temperatures that accelerate reproductive physiology[24], then investment should be lowest at the beginning of the season, increase to a peak, and then decline (Fig. 1A). If reproductive investment is empirically observed to be skewed towards earlier dates, it suggests the involvement of additional factors that override resource-based allocation strategies.

Life-history theory predicts that when mortality risk is high, organisms should allocate resources to reproduction earlier in life to maximise reproductive output before expected risk of death[14]. The same principle should apply within a given reproductive season: an individual delaying reproduction until the following year faces a high probability of mortality before being able to reproduce again. Consequently, the seasonal pattern of reproductive effort is expected to depend on the mortality risk pattern[15,22].

The most fundamental pattern is dictated by background mortality— the constant, unavoidable risk of death due to predation, disease, or environmental hazards, irrespective of an individual's condition or behaviour. Over time, these risks accumulate, leading to an increasing probability of mortality as exposure duration lengthens. Assuming a linear relationship between mortality risk and time (i.e., decreasing survival probability over the season), selection should favour immediate reproduction once resources and environmental conditions permit, as delaying reproduction increases the risk of pre-reproductive mortality[22]. Given that mortality risk accumulates

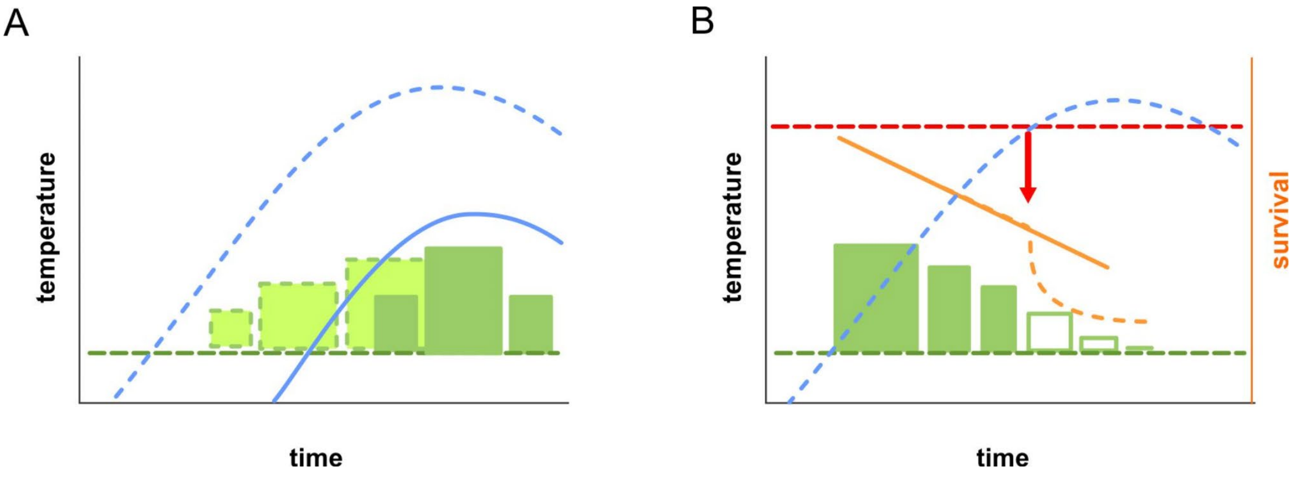

Fig. 1. Hypothetical patterns of energy allocation in relation to temperature (**A**) and survival (**B**) over the course of a reproductive season. The green dashed line in both panels represents the minimum temperature required to initiate reproduction. The solid blue line depicts the seasonal fluctuation in water temperature, while the dashed blue line represents a hypothetical scenario of increased water temperature. The dashed red line indicates the threshold temperature above which mortality rises sharply. In (**A**) dark green boxes illustrate reproductive investment in individual spawning events under typical temperature conditions (solid blue line), while light green boxes represent additional reproductive bouts that could occur under an increased water temperature scenario (dashed blue line). In (**B**), the orange line depicts the cumulative decline in survival due to background mortality, while the dashed orange line represents a scenario in which survival decreases more rapidly following an increase in water temperature beyond the critical mortality threshold (red arrow). The empty green boxes indicate potential reproductive bouts that are not undertaken due to high mortality risk or the elevated physiological costs of self-maintenance.

over time, individuals should allocate the majority of their energy at the beginning of the reproductive window, progressively reducing investment in each subsequent breeding attempt as cumulative mortality risk increases.

If the relationship between mortality and time increases sharply later in the season, then reproduction should not only be concentrated at the onset of the reproductive period but should also terminate early when mortality risk becomes prohibitive. Under high mortality pressure, individuals should prioritise self-maintenance over reproduction, ceasing reproductive activity before dangerous period (Fig. 1B). Thus, it can be hypothesised (H2) that the overall pattern of reproductive investment across sequential reproductive bouts should be skewed towards earlier bouts and dates, while at high mortality rates, the reproductive period should be both early and shortened.

To test these hypotheses, the present study examines progeny production across consecutive spawning events and mortality rates in individually marked females of the thick-shelled river mussel, *Unio crassus* (Unionida, Bivalvia; Fig. 2A). This large freshwater mussel (reaching up to 7 cm in shell length) is long-lived, with a maximum lifespan of approximately 15–20 years, and inhabits lotic environments across Europe[17]. *Unio crassus* reproduces during the spring and summer months, although many aspects of its reproductive biology remain poorly understood. Like other unionids, *U. crassus* produces thousands of eggs, which are initially stored in modified demibranchs forming specialised brooding structures known as marsupia. The eggs develop into larvae (glochidia), which remain in the marsupia for 10–35 days, with developmental rates dependent on ambient water temperature[24]. Once mature, the glochidia are released into the water, where they attach to the

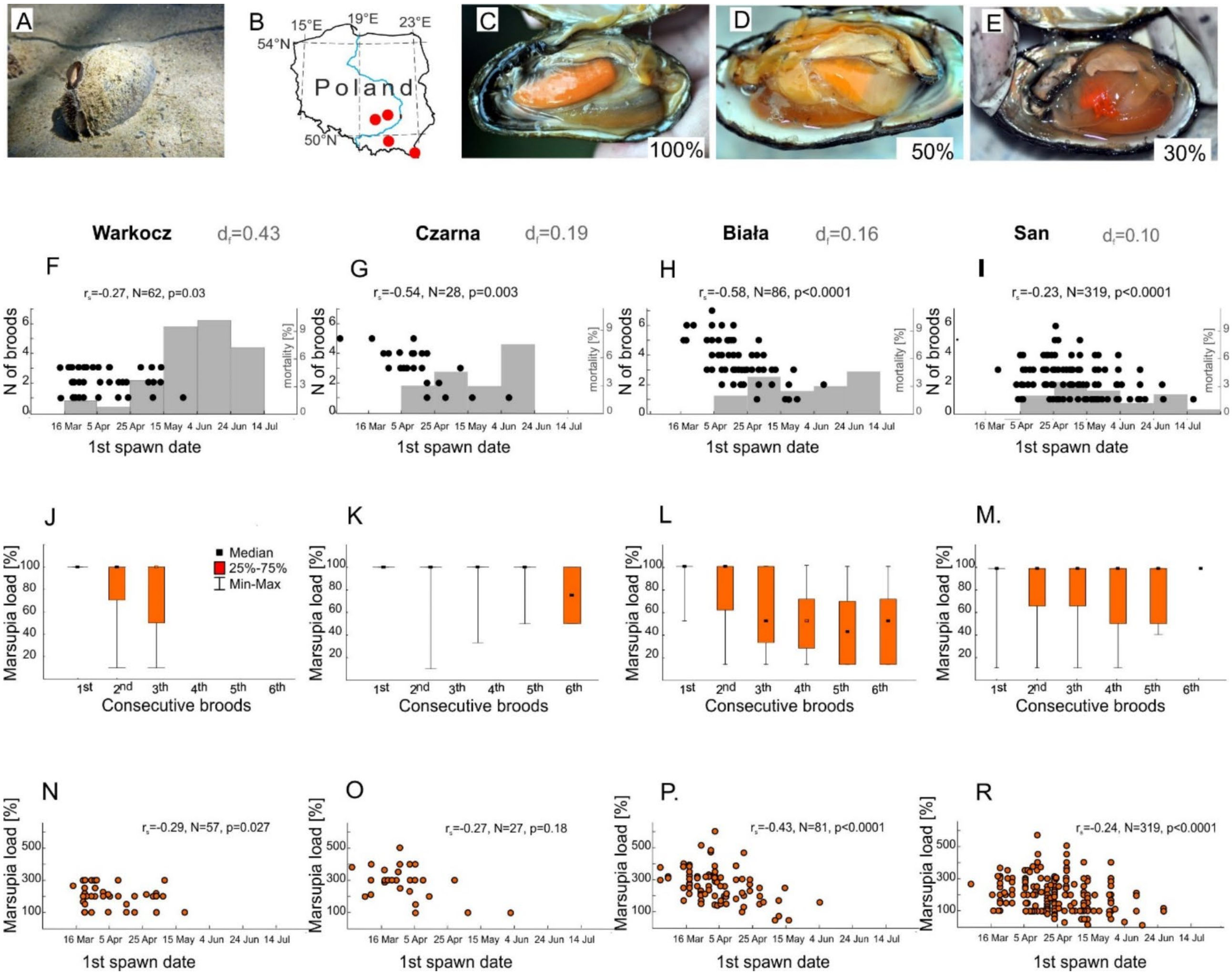

Fig. 2. Reproductive output in *Unio crassus*. (**A**) *Unio crassus* individual. (**B**) Study sites. (**C**–**E**) Examples of marsupia filled with glochidia, with an evaluation of the percentage of marsupial volume occupied by glochidia. (**F**–**I**) Number of broods raised per season (dots; left axis) and mortality intensity over 20-day intervals (grey bars; right axis) in relation to the timing of the first spawning event within the season (x-axis). d_f = mean mortality of females (see col. 11 in the tab. 2). Data are pooled across all study years for each river. (**J**–**M**) Median percentage of marsupia filled in consecutive spawning events of individual females during the reproductive season (dots: median; boxes: 25th–75th percentiles; whiskers: minimum–maximum). (**N**–**R**) Each dot represents the total percentage of marsupial filling, summed across all spawning events for a given individual, shown in relation to the timing of its first spawning event within a given season.

gills and fins of host fish and after a period of encystment, they detach from the host and settle into the benthic environment[25]. Due to its rapid population decline in recent decades, *Unio crassus* is classified as Endangered (EN) on the IUCN Red List and is protected under EU regulations and national conservation laws[26].

Results

In the four rivers (Fig. 2B), spawning was initiated by different females during almost the entire breeding season: in different years the spawning initiation period lasted from 53 to as many as 104 days (70 days on average, col. 5 in Table 1). If all the subsequent spawning events are included, the total breeding period lasted from as early as mid-March (after the ice melt) to as late as early August, i.e. from 61 to 132 days (105 days on average; col. 7 in Table 1).

The maximum number of spawning events of an individual during one reproductive season varied between the rivers from no more than 3 in the Warkocz to 7 in the Biała (average 2.28, SD = 3.635). Among the 551 females that were examined in all rivers and in all seasons, just 49 were found which had not spawned during a given season. Each particular breeding attempt of the same female could differ in the volume of glochidia filling the marsupium (Fig. 2C-E).

The earlier the first spawning event, the more broods raised during the season (Fig. 2F-I; Table 1, col. 8, except the Warkocz in 2019, when spawning ended very early). In the early spawning events, the marsupia were completely filled with larvae (Fig. 2C), whereas later in the season, the proportion of the marsupium volume filled with larvae (Fig. 2D-E) decreased (Fig. 2J-M). The general trend in marsupial filling across all rivers was negative over the course of the season. With each subsequent brood, the log-transformed percentage of marsupium filling decreased significantly (GLMM Model no. 1: estimate = – 0.088, SE = 0.014, $p < 0.001$; for details of statistical models, see Methods), corresponding to an approximate decrease of 8.4% per brood. Similarly, in relation to time within the reproductive season, the log-transformed marsupium filling decreased significantly with each passing day following the first brood (GLMM Model no. 1: est. = −0.0024, SE = 0.00052, $p < 0.001$), corresponding to an estimated 0.24% decline per day. The combined effect of the number of spawning events and their total volume also decreased significantly during the season (Fig. 2N-R for data pooled for given river, Table 1., col. 9 for particular seasons), although an exception was found in the lowland river Czarna (Table 1, see also Fig. 2K and O).

Every season and in all the rivers, spells of adult mortality occurred during late spring and early summer (grey bars in Fig. 2F-I). Of 76 females that died, 60 (79%) had not spawned at all during the season when they died, 8 had spawned once, 1 had spawned twice and 7 had spawned three times. Female mortality was the highest in the Warkocz (39%), then the Biała (27%) and the Czarna (26%), whereas it was noticeably lower in the San (21%; see also Table 2). Background mortality ceased at the end of the breeding season (late June, Fig. 2F-I), which however, do not exclude massive die-offs during mid-August (2019, the San – unpubl. data).

1st spawn date of an individual mussel [day/month]					Total spawning period			
River	Year	The earliest date	The latest date	1st spawn period [days]	The very last spawning date	Total rep. period [days]	No. of broods per season vs. 1st spawn date r_s (*N* ind.) *P*	Marsupium volume filled with larvae r_s (*N* ind.) *P*
1.	2.	3.	4.	5.	6.	7.	8.	9.
San	2015	11 Apr	15 Jun	65	7 Jul	87	-0.45 (21) 0.07	-0.62 (15) 0.01
	2016	7 Apr	10 Jun	64	1 Aug	116	-0.39 (58) 0.004	-0.51 (47) < 0.001
	2017	5 Apr	18 Jul	104	19 Jul	105	-0.79 (85) < 0.001	-0.59 (80) < 0.001
	2018	10 Apr	2 Jun	53	4 Jul	85	-0.44 (126) < 0.001	-0.44 (107) < 0.001
	2019	23 Mar	19 Jun	88	12 Jul	111	-0.27 (68) 0.03	-0.32 (61) 0.01
	mean	*5 Apr*	*18 Jun*	*75*	*14 Jul*	*101*		
Biała	2015	1 Apr	10 Jun	70	18 Jul	108	-0.60 (47) < 0.001	-0.45 (40) 0.003
	2016	20 Mar	23 May	64	30 Jul	132	-0.52 (44) < 0.001	-0.34 (41) 0.030
	mean	*26 Mar*	*1 Jun*	*67*	*24 Jul*	*120*		
Czarna	2019	18 Mar	8 Jun	82	9 Jul	119	-0.54 (34) 0.003	-0.27 (27) 0.18
Warkocz	2019	14 Mar	8 May	55	18 Jun	96	-0.08 (31) 0.76	-0.09 (21) 0.69
	2021	20 Mar	20 May	61	20 May	61	-0.46 (37) 0.005	-0.46 (36) 0.005
	mean	*17 Mar*	*14 Jun*	*58*	*2 Jul*	*78*		

Table 1. The timing of reproduction in populations of *U. crassus* and its relationships with the number of broods and the total reproductive output of females.

	Timing			Spawning vs. mortality window				Total	Ratio		
River	Year	1st spawning event [Md]	Death dates [Md]	Length [days]	% of F spawning (all F)	No. of broods per F [mean]	Sum of marsupia contents per F [mean]	Marsupia contents in season [mean]	Marsupia contents in window to total	Prop. of F dying	Rep. effort lost due to mortality
1	2	3	4	5	6	7	8	9	10	11	12
Biała	2015	20 Apr	25 May	35	53 (47)	1.7	144	354	0.41	0.22	0.13
	2016	15 Apr	1 Jun	46	93 (44)	2.3	217	381	0.57	0.09	0.04
	mean	*17 Apr*	*28 May*	*41*	*73*	*2.0*	*180*	*368*	*0.48*	*0.16*	0.08
Czarna	2019	12 Apr	24 May	42	47 (34)	2.1	203	454	0.45	0.19	0.11
San	2015	28 Apr	07 Jul	70	100 (21)	3.5	317	342	0.93	0.00	0.00
	2016	3 May	09 Jul	67	100 (58)	3.3	246	290	0.85	0.04	0.01
	2017	11 May	20 May	9	27 (85)	0.4	39	545	0.07	0.04	0.04
	2018	5 May	14 Aug	101	100 (126)	5.0	315	315	1.00	0.02	0.00
	2019	1 May	10 May	9	3 (68)	0.4	39	353	0.11	0.42	0.37
	mean	*3 May*	*20 May*	*51*	*66*	*2.52*	*191*	*369*	*0.59*	*0.10*	0.05
Warkocz	2019	27 Mar	6 Jun	69	84 (31)	3.4	240	240	1.00	0.38	0
	2021	10 Apr	10 Jun	61	100 (37)	1.81	171	171	1.00	0.47	0
	mean	3 Apr	08 Jun	65	92	2.61	205	205	1.00	0.43	0
All	mean	14 Apr	28 May	50	67	2.49	196	364	0.60	0.15	0.08

Table 2. The lengths of periods between median spawning dates and median mortality dates and their influence on reproductive output. Reproductive effort lost due to mortality was calculated as the difference between the total marsupium load during all spawning events and the marsupium load produced during the reproductive window standardized by the total reproductive output, and multiplied by the proportion of dying females.

The overall difference between spawning and death dates in reference to the first spawning date in given river were highly significant: the Md of 1st spawns occurred on 26th day since the first spawn, whereas the Md date of death date was 42 days later (GLMM no. 2: estimate = -0.85, SE = 0.061, $P << 0.001$). Pairwise comparisons of the results of GLMM model no. 3 showed the following log-transformed differences between spawning and death: the Biała river (est. = −0.93, SE = 0.140, $p < 0.001$), the Czarna (est. = −0.72, SE = 0.236, $p = 0.0022$), the San (est. = −0.66, SE = 0.078, $p < 0.001$), and the Warkocz (est. = −1.36, SE = 0.131, $p < 0.001$). The largest difference was observed in the Warkocz, indicating a longer temporal separation between spawning and death in this river compared to others. Significant differences in events timing between rivers were also observed: events occurred significantly later in the San compared to the Biała (est. = 0.29, SE = 0.080, $p < 0.001$) while events in the Warkocz were marginally earlier (est. = −0.18, SE = 0.109, $p = 0.089$) compared to the Biała. The interaction between event type and river was significant in the Warkocz (est. = 0.42, SE = 0.191, $p = 0.026$), confirming that the difference between spawning and death timing was greater in this river. The variance of the random effect for river was negligible, suggesting that most variability was captured by the fixed effects.

In 2019, the Warkocz, Czarna and San were monitored in parallel. Separate Generalized Linear Models were used to assess differences in spawning and death timing across these rivers in 2019. In the reference to spawning timing GLMM model no. 4 revealed significant differences between rivers, with spawning occurring later in the San (est. = 0.52, SE = 0.121, $p = 0.0001$) and earlier in the Warkocz (est. = −0.45, SE = 0.143, $p = 0.0055$) compared to the Czarna. The largest difference was observed between the San river and the Warkocz (est. = 0.97, SE = 0.125, $p < 0.0001$).

In contrast, the death timing model (GLMM model no. 5) showed no significant differences between rivers ($p > 0.05$). Post-hoc comparisons confirmed that death timing was consistent across the Czarna, San, and Warkocz, with no significant pairwise differences observed ($p > 0.49$). The death timing appears to be more consistent, in comparison to spawn dates (Fig. 3A).

A GLMM model no. 6 revealed significant differences in the timing of spawning and death events in the San River. Overall, death occurred significantly later than spawning (est. = 0.39, SE = 0.170, $p = 0.022$), with significant interactions between event type and season (Fig. 3B): spawn vs. death dates differed significantly in 2016 (est. = -1.35, SE = 0.599, $p = 0.024$), in 2018 (est. = -1.39, SE = 0.369, $p = 0.0002$) and in 2019 (est. = 0.39, SE = 0.170, $p = 0.022$), however no significant differences were found for 2017. The largest temporal separation between spawning and mortality was found in 2018, and in 2016, although then the number of dead mussels was low; while in 2017 events were more synchronous, also showing this tendency in 2019. The disproportionately high mortality in the San river in 2019 coincided with temperature extremum (Fig. 2C) in comparison to other years.

The differences between the median date of the first spawning events (Table 2, col. 3) and the median date of mussel deaths (col. 4) found in particular seasons and rivers yielded time windows of very different lengths (col. 5).

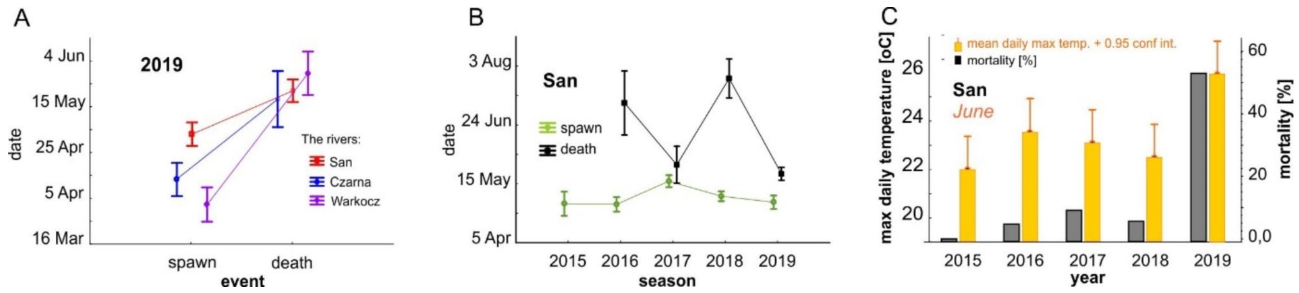

Fig. 3. Factors related to mortality in the studied populations of *U. crassus*: **A** – comparison of mean spawning dates and death dates in the different rivers (the Czarna, San and Warkocz; mean with whiskers denoting 0.95 confidence intervals) during the same season (2019); **B** – comparison of mean spawning and death dates in different years (2015–2019) in the same river (the San; mean with whiskers denoting 0.95 conf. interv.); **C** – comparison of mortality (grey bars) with the maximum daily temperature in June in the River San (orange bars; mean with whiskers denoting 0.95 conf. interv.)

The window length correlates with the proportion of spawning females (col. 6, $r_s = 0.88$, N = 10, P = 0.002) and the number of their broods not influenced by mortality before glochidia release (col. 7, $r_s = 0.96$, N = 10, P < 0.001). It is clear that in many years, all or almost all the glochidia could have been released before the onset of the mortality period (e.g. the Warkocz river). On the other hand, the ratio of the reproductive effort within a window to the total reproductive effort (col. 10) indicates that if spawning is postponed, there is an inversely proportional risk of the spawn being lost if the female dies. The length of the time window (col. 5) correlates negatively with the relative decrease in reproductive investment (col. 12) due to female mortality in a given year (r_s= -0.86, $N = 10$, $P = 0.001$). In other words, the more intense the selection related to lost reproduction investment in late spawning events, the shorter the time window for safe spawning. If one assumes that an average female spawns 364% of marsupium volumes during a season (mean for col. 9) and has a mean probability of death of 15% (mean for col. 11), then 8% of glochidia, on average, will be affected by the female mortality period (mean of col. 12), which reflects current phenotypic selection against late spawning.

Discussion

In the four *Unio crassus* populations studied between 2015 and 2021, females initiated spawning from late winter to late summer, demonstrating a high degree of phenotypic plasticity in spawning timing. Instances of both late initiation of the first spawning event and very late subsequent spawnings indicate that neither low winter nor high summer temperatures entirely prevent reproduction. This suggests that spawning is physiologically feasible throughout the season, even when the marsupium is filled to its maximum capacity with larvae (see Fig. 2K).

These findings support Hypothesis 1, which predicts that earlier breeding facilitates a greater number of reproductive bouts within a season. However, the data also indicate that mussels do not follow the theoretically advantageous pattern of increasing reproductive investment in response to improving environmental conditions over the season (Fig. 1A). Instead, investment per reproductive bout declines significantly both across sequential broods of individual females and over time within the season.

This declining investment may be explained by background mortality, which was recorded in each river during every breeding season. Notably, mortality never occurred prior to the spawning period. While, at the level of an individual, death obviously cannot preclude reproduction, at the population level, it is plausible that mortality could be particularly intense after winter, preventing early reproductive investment. However, this was not the case in the studied populations. Instead, mortality occurred during the breeding season, meaning its probability accumulated over time. Consequently, to minimise the risk of mortality before reproducing, most individuals should allocate the majority of their reproductive energy at the beginning of the reproductive window, reducing investment in each subsequent reproductive bout. This pattern is consistent with Hypothesis 2, although further, long term research is needed both to confirm and clarify the underlying mechanisms.

Studies on reproductive effort in Unio species remain scarce, with most literature providing only general descriptions of reproductive timing (e.g. [17,20]). The only exception is the work of Hohwald[19], who first reported that *U. crassus* females do not always produce sufficient glochidia to completely fill their marsupia. Hohwald also documented that *U. crassus* produces multiple broods per season, reporting "up to five spawning events".

Theoretically, repeated handling of mussels could artificially increase the apparent number of reproductive bouts, for instance, by inducing brood loss through stress or disturbance. However, experimental tests of handling effects conducted in the Biała and San rivers[24], under different disturbance regimes (biweekly, weekly, and every other day), showed that only the highest disturbance frequency (every other day) induced miscarriage of broods. Even under these conditions, miscarried orange conglutinates were clearly visible in the water, and brood loss was evident from disruptions in glochidial developmental stages. Since no such patterns were observed in the present study, handling effects are unlikely to have influenced our results.

From a theoretical perspective[22], the observed decline in reproductive investment was attributed to increasing mortality risk. However, alternative explanations remain possible. One particularly plausible factor is the capital vs. income breeding strategy[27]. A recent study[20] on related species *U. tumidus* reported that individuals resorb resources from the gonads in December, likely as an adaptation for somatic maintenance during winter. This may explain how these mussels can initiate spawning very early in the season, even in the absence of food availability. However, such capital-stored resources may be insufficient to sustain late-season breeding, leading to a progressive decline in reproductive investment as pre-winter energy reserves are depleted. Nevertheless, *U. tumidus* in the studied population maintained continuous gametogenesis throughout the year (a pattern also observed in *U. crassus*, based on our own unpublished data). Despite this, *U. tumidus* does not reproduce early; instead, spawning and glochidial release occur in June–July, which supports an income breeding strategy rather than capital breeding[20].

We examined only four rivers, which limited our ability to directly test the relationship between individual reproductive investment and background mortality. However, even within these four populations, clear patterns emerged. In the Warkocz river, which exhibited the highest mortality, spawning was significantly advanced compared to the other rivers, with reproduction initiated well before major mortality events, suggesting stronger selection for early reproduction. Additionally, reproductive investment was highly restricted or even ceased entirely in later broods. By contrast, in the San River, where mortality was lowest, the breeding season began later, spawning frequently overlapped with mortality events, and reproductive investment in late broods remained high.

According to the theory of energy allocation[13,14,28,29], energy investment in self-maintenance has absolute priority over investment in reproduction and growth. This principle is particularly important in long-lived organisms such as freshwater mussels. Producing one or two additional broods in a given summer may significantly increase short-term reproductive success, but it also exposes individuals to a higher mortality risk, potentially jeopardising dozens of future broods over a 20-year lifespan. This trade-off explains why reproductive investment is often divided into multiple smaller broods, particularly in warm conditions where rapid larval development allows individuals to fine-tune their reproductive timing before mortality risk escalates. This self-maintenance may also explain why 49 females in the study completely refrained from reproduction and died later in the season.

The primary driver of mortality in 2019 appears to have been a large-scale environmental factor, as spawning dates varied significantly between rivers, whereas mortality dates were nearly identical across all sites. Although formal statistical testing was not possible, the coincidence of mortality events with the unprecedented June 2019 heatwave (which had no analogue in the 70-year European temperature record [30]) suggests that elevated temperatures exerted a large-scale impact. The effect of temperature on mortality may be direct (e.g. via reduced oxygen availability) or indirect, such as by increasing susceptibility to disease[31].

A physiological threshold may also limit early-season spawning shifts. In high-mortality rivers, spawning began immediately after ice melt, when water temperatures exceeded 5 °C[24]. However, at such low temperatures, larval development takes at least three times longer than in late spring. In extreme cases, development may fail to reach completion[32]. Thus, even if selection favours earlier breeding, physiological constraints may prevent a corresponding shift in the first spawning event. The predicted rate of climate warming may not alter this mechanism: a 10 °C increase in winter temperatures (e.g. from – 10 °C to 0 °C) will not reduce the 5 °C developmental threshold, whereas a 10 °C increase in spring or summer temperatures (e.g. from 15 °C to 25 °C) could be lethal for mussels[33]. This progressive shortening of the reproductive window may explain high interannual variation in recruitment in unionids[34], a hypothesis that requires further testing.

In conclusion, in the studied populations *Unio crassus* exhibited high flexibility in spawning timing, allowing for earlier initiation, increased spawn number, and thus enhanced reproductive success. However, energy investment per brood declined progressively over the season, likely due to high late-spring mortality selecting for earlier reproduction. Further research is needed to directly test the trade-off between reproductive investment and self-maintenance costs to assess its demographic consequences for *U. crassus* populations.

Methods

Data were collected in 2015–2021 in four rivers (southern and central Poland; Table 1, Fig. 2B): two mountain rivers, i.e. the San (studied in 2015–2019, 49°11’50.1”N 22°40’ 56.8E, 544 m asl) and the Biała (2015–2016; 49°51’37.9 N, 21°02’3.3E, 228 m asl), and two lowland ones, i.e. the Warkocz (2019 and 2021; 50°50’25.0”N, 20°45’27.0”E, 258 m asl) and the Czarna (2019; 50°56’50.0”N, 19°50”59.0E, 200 m asl). All four rivers harbour big populations of *U. crassus*, with recent recruitment and large densities. All four rivers are protected by the Natura 2000 network and are included in the state system of monitoring in accordance with the EU Habitats Directive. All were evaluated as FV (favourable).

The San River is a perennial mountain river located in the Eastern Carpathians, southeastern Poland. It is one of the major tributaries of the Wisła (Vistula) River, flowing for 457 km and draining a catchment area of approximately 16,861 km^2. At the study site near Procisne village, the river has a relatively straight channel, approximately 50 m wide, with shallow waters (0.3–0.7 m depth). The discharge at low water levels is approximately 5–6 m^3/s, with seasonal variations influenced by snowmelt and rainfall. The river channel along the study reach remains entirely natural, with high water quality, low nutrient levels, and minimal pollution. This is due to the river drains the Bieszczady National Park, a strictly protected area with almost no anthropogenic impacts. The riverbed is predominantly composed of gravel and exposed rock, while fine sediments accumulate along the banks, supporting herbaceous vegetation, mainly *Carex* species. The surrounding catchment is dominated by mixed forests, with minor contributions from grasslands.

The Biała River is a perennial river in the Western Carpathians (southern Poland) and forms part of the Vistula River basin. It flows for 96 km from the Beskid Mountains to its confluence with the Dunajec River, draining a catchment of approximately 940 km^2, which is predominantly covered by mixed forests, meadows, and agricultural fields. At the study site, the channel remains natural, featuring a gravel bed approximately 14 m wide, incised 2–3 m into a fine sediment floodplain. The river follows a pool-riffle sequence, with low water depths ranging from 0.1 to 1.5 m and a discharge of approximately 2–2.5 m^3/s at low water levels. The banks are overgrown with Salix species, while the floodplain supports degraded riparian forests near the riverbed, with meadows dominating the remaining landscape. Water quality is mesotrophic.

The Warkocz River is a perennial stream in the Świętokrzyskie Mountains, a highland region in central Poland. It flows for 17.5 km, draining a 54 km² catchment of forested and agricultural landscapes before joining the Lubrzanka River, which ultimately flows into the Vistula River. At the study site, the river has a naturally meandering channel, 5–10 m wide, incised approximately 2 m into fine sediments. The riverbed is composed of sandy substrate with gravel patches in riffles. The depth varies considerably, ranging from 10 cm in riffles to 1–1.5 m in pools. The banks are overgrown with alder (Alnus species), while the floodplain supports alder-dominated forest near the riverbanks, with meadows covering the remaining area. No data are available on water quality or discharge.

The Czarna River is a perennial, lowland, meandering river in central Poland, flowing for approximately 47.5 km and draining a 637 km² catchment before joining the Pilica River, a left-bank tributary of the Vistula. The river flows through a flat lowland landscape, incising approximately 2 m into fine sediments. At the study site, the river retains a natural channel, with a width of approximately 10 m and a depth of 0.7–1 m at low water levels. The riverbed consists primarily of sand, with the presence of large woody debris and marginal vegetation dominated by *Sparganium* species. The floodplain is covered with meadows and pastures. Approximately 4 km upstream, a large fish pond influences local hydrology, though its exact impact on the study site remains unquantified.

In each of the four rivers, a random selection of mussels was collected in very early spring, just after the ice melt. Then, ca. 130 individuals of the dominant size class (ca. 5 cm long, except in the river Czarna, where the dominant size was ca. 6–7 cm), were individually marked with an alphanumeric code engraved on the periostracum, sexed by gonad puncturing to find sperms or oocytes, then released back into the river at the same site from which they had been taken, but grouped together to facilitate finding them again.

The marsupia of female mussels were inspected every 10–14 days (weekly in the Biała River in 2015–2016). The percentage of marsupium volume filled with larvae (Fig. 2C–E) was estimated visually. To assess the developmental stage of the larvae, the marsupia were punctured using a thin needle, and the contents were examined in situ under a field microscope[24]. During each inspection, females were typically sampled twice to verify that the developmental stages of the glochidia in consecutive samples were consistent with the established developmental sequence of brooding glochidia (morula, blastula, …, D-shaped, …, snapping-open glochidium with a protruded larval thread). This method enabled precise determination of the laying date in early-season broods, when larval development proceeds slowly enough to allow for age estimation in days[24]. After examination, mussels were immediately returned to the water. The total number of broods raised per season was then determined for each female, and the cumulative volume of marsupium filled with larvae was estimated across all broods. The number of individuals for which the total marsupium volume was estimated was lower than the number of individuals analysed for brood count (Table 1, columns 8 and 9). Individuals were excluded from volume estimation if there was any uncertainty regarding the completeness of their broods, for example suspected miscarriage, partial glochidia release, or any inconsistencies in glochidial developmental stages between consecutive inspections, which could indicate that larvae developed and were released within a period shorter than the inspection interval.

For analysing the impact of temperature on mortality in the San (Fig. 3C.), temperature data for the Solina reservoir, in the upper course of the San, were used. These data were obtained from the State Institute of Meteorology (SIM) and are publicly available at https://danepubliczne.imgw.pl/data/dane_pomiarowo_obserwacyjne/dane_hydrologiczne/miesieczne/. The reservoir water temperature was selected as it effectively averages the temperatures of the San's numerous tributaries, including that of the study site, and is less susceptible to local fluctuations or short-term disturbances. Thus, it provides a representative measure of large-scale thermal conditions, responding only to significant climatic or hydrological changes.

Statistics

To account for differences in study years across rivers, if not stated otherwise, in all GLMM models dates of spawn and death were standardized as the number of days since the first female spawn in a given year and river. Because data were collected in different years in different rivers the coding enabled the use of data from all rivers and all years in the GLMM analyses. A Gamma distribution with a log link function was used to model the skewed distribution of coded dates. The results of GLMM models were reported on the log scale and transformed to the original scale where necessary for interpretation. All statistical modelling was performed in R4.4.2., simple statistics were computed using Statistica 13.1.

We applied a generalized linear mixed-effects model (GLMM, model no 1.) to analyse the factors influencing reproductive investment in mussels, measured as the percentage of marsupium fullness. The model used a Tweedie distribution with a log-link function to accommodate the positively skewed distribution of the data and the high frequency of values near the upper bound (100%). The fixed effects included brood sequence number and the number of days since the first brood in a given river in given year to assess sequential and temporal patterns in reproductive investment. Random effects were specified to account for hierarchical data structure, including variation between rivers and between individual mussels nested within rivers. Model parameters were estimated using the glmmTMB package, which is optimized for Tweedie-distributed responses. Model diagnostics were performed using the DHARMa package to assess residual patterns and model fit.

To evaluate the general difference in timing between spawning and death events across all rivers, a Generalized Linear Mixed Model (GLMM, model no 2.) was employed, including event type (spawning or death) as a fixed effect and river as a random intercept to account for river-level variability. This approach allowed for the estimation of an overall difference between spawning and death dates. To analyse differences in the timing of spawning and death within each of the rivers, a model GLMM No 3. included fixed effects for event type (spawning or death), river, and their interaction (type × river). River was also included as a random effect to account for variability between rivers. Due to the limited number of repeated measures across individual mussels within rivers, the inclusion of individual ID as a random effect led to issues with model convergence and overparameterization. Excluding individual ID ensured a parsimonious model that could be reliably estimated, while river-level random effects accounted for variability between rivers. Post-hoc pairwise comparisons of spawning and death timing within each river were conducted using the Tukey adjustment for multiple comparisons. Estimated marginal means (EMMs) were derived from the model to assess these contrasts. All above analyses were performed using the lme4 package for GLMMs and emmeans for post-hoc comparisons.

Because the table of data is not orthogonal (Table 1), whereas all three rivers (the Czarna, San, Warkocz) were studied in 2019, this allows to compare the differences between rivers without influence of different seasons. Moreover, 2019 was a year with exceptional mortality (Fig. 3C), which specifically predestine this year to be studied. To analyse the differences in spawning and death timing between these rivers, we applied separate Generalized Linear Models (models No 4 and 5) for each event type (spawning and death). The response variable was the date of the event, transformed into the number of days since the earliest recorded event in the dataset. The fixed effect in both models was river, modelled as a categorical variable. Pairwise comparisons between rivers were performed using Tukey-adjusted post-hoc tests. Statistical analyses were conducted using the glm function for model fitting and the emmeans package for post-hoc comparisons.

To analyse differences in the timing of spawning and death events across seasons in the San river, a Generalized Linear Mixed Model (GLMM, model No 6) was applied. The response variable was the relative timing of events within each season, calculated as the number of days since the first observed event in that season. The fixed effects included event type (Spawn or Death), season, and their interaction. Individual ID was included as a random effect to account for repeated measures within individuals. Post-hoc pairwise comparisons between Spawn and Death were conducted within each season using the Tukey adjustment for multiple comparisons. Analyses were performed using the lme4 and emmeans packages.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Received: 21 October 2024; Accepted: 24 February 2025

References

1. Zieritz, A. et al. A global synthesis of ecosystem services provided and disrupted by fresh-water bivalve molluscs. *Biol. Rev.* **97**, 1967–1998 (2022).
2. Sousa, R. et al. A roadmap for the conservation of freshwater mussels in Europe. *Conserv. Biol.* **37**, e13994 (2023).
3. Lopes-Lima, M. et al. Conservation of freshwater bivalves at the global scale: diversity, threats and research needs. *Hydrobiologia* **810**, 1–14 (2018).
4. da Silva, J. P. et al. Streams in the mediterranean region are not for mussels: predicting extinctions and range contractions under future climate change. *Sci. Total Environ.* **883**, 163689 (2023).
5. Mouthon, J. & Daufresne, M. Effects of the 2003 heatwave and Climatic warming on mollusc communities of the Saône: a large lowland river and of its two main tributaries (France). *Glob Chang. Biol.* **12**, 441–449 (2006).
6. Nakamura, K., Sousa, R. & Mesquita-Joanes, F. Collapse of native freshwater mussel populations: prospects of a long-term study. *Biol. Conserv.* **279**, 109931 (2023).
7. Ollard, I. & Aldridge, D. C. Declines in freshwater mussel density, size and productivity in the river Thames over the past half century. *J. Anim. Ecol.* **92**, 112–123 (2023).
8. Sousa, R. et al. Die-offs of the endangered Pearl mussel *Margaritifera margaritifera* during an extreme drought. *Aquat. Conserv.* **28**, 2144–1248 (2018).
9. Aldridge, D. C. et al. Freshwater mussel conservation: A global horizon scan of emerging threats and opportunities. *Glob Chang. Biol.* **29**, 575–589 (2023).
10. Parmesan, C. Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst.* **37**, 637–669 (2006).
11. Vaughan, I. P. & Gotelli, N. J. Water quality improvements offset the Climatic debt for stream macroinvertebrates over Twenty years. *Nat. Commun.* **10**, 1956 (2019).
12. Merilä, J. & Hendry, A. P. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol. Appl.* **7**, 1–14 (2014).
13. Stearns, S. C. *The Evolution of Life History Traits* (Oxford Univ. Press, 1992).
14. Kozłowski, J. Optimal allocation of resources to growth and reproduction: implications for age and size at maturity. *Trends Ecol. Evol.* **7**, 15–18 (1992).
15. Varpe, Ø. Life history adaptations to seasonality. *Integr. Comp. Biol.* **57**, 943–960 (2017).
16. Ćmiel, A. M., Zając, T., Zając, K., Lipińska, A. M. & Najberek, K. Single or multiple spawning? Comparison of breeding strategies of freshwater Unionidae mussels under stochastic environmental conditions. *Hydrobiologia* **848**, 3067–3075 (2021).
17. Lopes-Lima, M. et al. Conservation status of freshwater mussels in Europe: state of the Art and future challenges. *Biol. Rev.* **92**, 572–607 (2017).
18. Aldridge, D. C. The morphology, growth and reproduction of Unionidae (Bivalvia) in a Fenland waterway. *J. Molluscan Stud.* **65**, 47–60 (1999).
19. Hochwald, S. Plasticity of life-history traits in *Unio crassus. Ecology and evolution of the freshwater mussels Unionoida*, pp. 127–141 (2001).
20. Abdelsaleheen, O., Taskinen, J. & Kortet, R. Reproductive cycle, fecundity and growth of the freshwater mussel Unio tumidus (Bivalvia: Unionidae) from lake Viinijärvi, Finland. *J. Molluscan Stud.* **90**, eyae024 (2024).
21. Ćmiel, A. M. et al. The size and shape of parasitic larvae of naiads (Unionidae) are not dependent on female size. *Sci. Rep.* **11**, 1–10 (2021).
22. McNamara, J. M., Welham, R. K., Houston, A. I., Daan, S. & Tinbergen, J. M. The effects of background mortality on optimal reproduction in a seasonal environment. *Theor. Popul. Biol.* **65**, 361–372 (2004).
23. Maggard, I. J. et al. Freshwater mussels prefer a diet of stramenopiles and fungi over bacteria. *Sci. Rep.* **14**, 11958 (2024).

24. Zając, K. & Zając, T. A. Seasonal patterns in the developmental rate of glochidia in the endangered thick-shelled river mussel. *Unio crassus Philipsson 1788 Hydrobiologia*. **848**, 3077–3091 (2021).
25. Aldridge, D. C. et al. Fishing for hosts: larval spurting by the endangered thick-shelled river mussel. *Unio crassus Ecol.* **104**, e4026 (2023).
26. Lopes-Lima, M., Prié, V., Österling, M. & Zając, T. A. *Unio crassus. The IUCN Red List of Threatened Species*: e.T210291828A215467836. (2024). Available at: https://www.iucnredlist.org/fr/species/210291828/215467836 (2024.).
27. Bonnet, X., Bradshaw, D. & Shine, R. Capital versus income breeding: an ectothermic perspective. *Oikos* **83**, 333–342 (1998).
28. Jokela, J. Within-season reproductive and somatic energy allocation in a freshwater clam. *Anodonta piscinalis Oecologia*. **105**, 167–174 (1996).
29. Jokela, J. & Mutikainen, P. Phenotypic plasticity and priority rules for energy allocation in a freshwater Clam: a field experiment. *Oecologia* **104**, 122–132 (1995).
30. Sulikowska, A. & Wypych, A. How unusual were June 2019 temperatures in the context of European climatology? *Atmosphere* **11**, 697 (2020).
31. Karvonen, A., Rintamäki, P., Jokela, J. & Valtonen, E. T. Increasing water temperature and disease risks in aquatic systems: climate change increases the risk of some, but not all, diseases. *Int. J. Parasitol.* **40**, 1483–1488 (2010).
32. Heinricher, J. R. & Layzer, J. B. Reproduction by individuals of a nonreproducing population of *Megalonaias nervosa* (Mollusca: Unionidae) following translocation. *Am. Midl. Nat.* **141**, 140–148 (1999).
33. DuBose, T. P., Atkinson, C. L., Vaughn, C. C. & Golladay, S. W. Drought-induced, punctuated loss of freshwater mussels alters ecosystem function across Temporal scales. *Front. Ecol. Evol.* **7**, 274 (2019).
34. Ries, P. R., Newton, T. J., Haro, R. J., Zigler, S. J. & Davis, M. Annual variation in recruitment of freshwater mussels and its relationship with river discharge. *Aquat. Conserv.* **26**, 703–714 (2016).

Acknowledgements

The study was supported partly by LIFE+ project "LIFE4delta" LIFE17-NAT_PL_000018, funded by the European Union and partly by statutory funds of the Institute of Nature Conservation, Polish Academy of Sciences. The study was conducted on the basis of permits Nos. WNP.6401.190.2014.RN-2, WNP.6401.1.117.2016.ŁL.2, WNP.6401.88.2019.JSz.2, WNP.I.6401.30.2019.AD, OP-I.6401.168.2014.MMr, OP-I.6401.163.2016.MMr and DZP-WG.6401.78.2021.TŁ, granted to study a protected species (*U. crassus*).). Kacper Zając gave some useful tips about R.

Author contributions

while both K.Z. and T.Z. contributed equally to this work, T.Z. took the leading part in data analysis and writing the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to T.A.Z.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.