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Optimizing laboratory cultures of *Gammarus fossarum* (Crustacea: Amphipoda) as a study organism in environmental sciences and ecotoxicology



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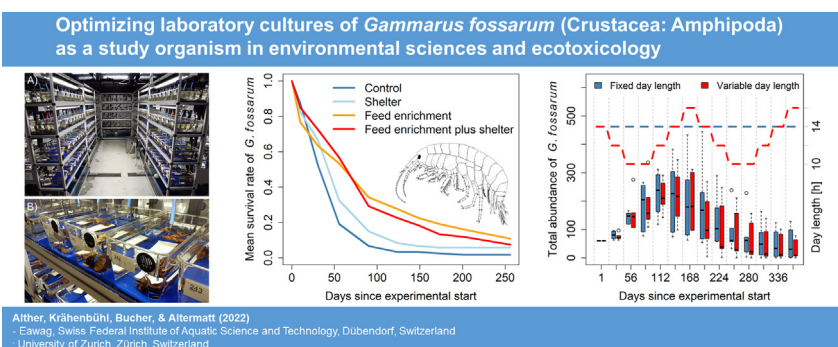
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HIGHLIGHTS

- Keeping and breeding *Gammarus* sp. in the lab proved difficult in previous studies. However, being one of the most widespread freshwater taxon of amphipods it represents a study organism that allows bridging the gap between laboratory and field studies.
- We maintained *Gammarus fossarum* populations successfully under laboratory conditions for more than two years, using them in an experimental assay across one year.
- Supplementing the diet with protein-rich food and providing additional shelter increased survival rate of laboratory-based populations of *G. fossarum* significantly.
- Manipulating day length showed no significant effect on their abundance and reproductive activity.
- We provide detailed husbandry protocols, improving the status of culturing *G. fossarum* as suitable study organism in environmental sciences and ecotoxicology.

GRAPHICAL ABSTRACT



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ABSTRACT

Amphipods are among the most abundant macroinvertebrates in freshwater ecosystems of the Palaearctic and crucial for ecosystem functioning. Furthermore, their high sensitivity to environmental change and pollutants makes them widely used model organisms in environmental sciences and ecotoxicology. In field studies and surveys across Eurasia, species of the genus *Gammarus* are commonly used, yet laboratory-based studies and ecotoxicological tests are often restricted to the in most parts of the world non-native *Hyaella azteca*, as *Gammarus* is much harder to breed and maintain under laboratory conditions. However, for direct comparisons and extrapolations of results of field- vs. laboratory-based studies, the use of the same species would be desirable. Here, we investigated different settings with respect to feeding, shelter and day length to successfully increase survival, juvenile production and their respective growth and survival, and ultimately multi-generation breeding of the amphipod *Gammarus fossarum*. Amphipod populations persisted and reproduced successfully under optimized husbandry conditions for 12 months and were partially

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maintained for another year in populations up to a few hundred individuals. Specifically, supplementing diet with protein-rich food sources as well as the provisioning of shelters improved survival rate of *G. fossarum* significantly. However, we found no significant effect of different day length treatments on the overall relative reproductive activity or on the total amphipod abundance maintained. We conclude that *G. fossarum* can be kept and reared under standardized conditions. Despite the longer generation times of *G. fossarum* and higher effort required for maintenance compared to *H. azteca*, direct ecological relevance and comparability of results to natural systems may justify its future use and development as a study organism for environmental sciences and ecotoxicology.

1. Introduction

Amphipods are a highly diverse group of aquatic invertebrates with globally >10,000 described species (Horton et al., 2022). About 20 % of these are freshwater species (Väinölä et al., 2008), and the West Palearctic harbors about half of this diversity. In freshwater ecosystems, amphipods are widespread and can be the dominating macroinvertebrates (Felten et al., 2008). They represent a highly important link in food webs and substantially contribute to the functioning of aquatic ecosystems (MacNeil et al., 1997) being key-stone shredders in stream ecosystems (Dangles and Malmqvist, 2004).

Due to their high significance in ecological processes and their high sensitivity to environmental perturbations, such as habitat modifications or pollution, amphipods are commonly studied and used in fundamental ecological research (e.g., van den Brink and van der Velde, 1991; Hou et al., 2011; Best et al., 2013; Little and Altermatt, 2019; Little et al., 2020), environmental sciences (MacNeil et al., 2000; MacNeil, 2019) and ecotoxicology (e.g., Kunz et al., 2010). For example, amphipods are good indicators of environmental change with respect to water quality and river morphology (Crane and Maltby, 1991; Coulaud et al., 2011; Chaumot et al., 2015; Eisenring et al., 2016). Further, many species are directly affected and adjust their feeding behaviour in response to changes in the composition of their food source (leaf litter), with cascading effects on the aquatic food web (van Riel et al., 2006; Woodward et al., 2008; Little and Altermatt, 2019; Little et al., 2020). Finally, amphipods are sensitive to a wide range of toxicants, and are thus commonly used by stakeholders and ecotoxicologists for field and laboratory based studies (McCahon and Pascoe, 1988; Felten et al., 2008; Bundschuh and Schulz, 2011; Feckler et al., 2012, 2014; Kosfeld et al., 2020; Fu et al., 2020; Rothe et al., 2022; Švara et al., 2022).

However, for Europe and adjacent regions there is a major discrepancy between field assays, which are commonly performed on a range of (native) species, especially of the genus *Gammarus* (e.g., Bundschuh et al., 2013; Feckler et al., 2014; Eisenring et al., 2016; Burdon et al., 2019; Arlos et al., 2020; Lauper et al., 2022; Švara et al., 2022), and laboratory-based studies, which are most commonly performed on the non-native model organism *Hyalella azteca* (Saussure, 1858) (e.g., Borgmann et al., 2005; Cothran et al., 2013; James and McClintock, 2017; Kosfeld et al., 2020; Fu et al., 2020). The latter is easy to maintain in the lab, as is common for other model organisms such as *Daphnia magna* Straus, 1820, *Caenorhabditis elegans* (Maupas, 1900) or *Danio rerio* (F.Hamilton, 1822), yet may not be necessarily representative of amphipods inhabiting small tributary streams, which are commonly affected by environmental pollutants (e.g., Munz et al., 2017). Of the genus *Gammarus* Fabricius, 1775, the two species complexes *Gammarus fossarum* Koch, 1836 and *Gammarus pulex* (Linnaeus, 1758) are among the most relevant organisms for environmental assessments of streams and small rivers in Europe and adjacent regions, as they are broadly distributed and have a key-stone function in freshwater systems in Eurasia. Consequently, they are frequently used in toxicity tests (Geffard et al., 2010). Neither of these species has been easily cultivated (but see Pöckl, 1995), and thus most past studies and assessments of chemical compounds relied purely on wild-caught individuals (e.g., McCahon and Pascoe, 1988; Bundschuh et al., 2013; Mehennaoui et al., 2016; Arambourou et al., 2017; Straub et al., 2017; Lauper et al., 2022; Švara et al., 2022) or again on more tolerant model organism such as *H. azteca*, representing a species complex native only to North and Central America

(e.g., Wang et al., 2004; Borgmann et al., 2005; Fu et al., 2020). This is problematic for two reasons. Firstly, it is hard to reach a standardization of wild-caught animals with respect to their age, health status (for example, wild-caught animals are often infected by parasites) and nutritional status. Laboratory-cultivated animals allow using native species, to control rearing conditions, and selecting individuals with same life history. Secondly, not using the same species for field assays and laboratory based studies results in a discrepancy between laboratory and field results (Bloor and Banks, 2006). Hence, it is not always clear how these findings can then be extrapolated to natural conditions (e.g., Crane and Maltby, 1991) and a more wider range of species. This is in particular true when the model organisms used in the lab are per se more tolerant (which is often the case for organisms easily cultivatable), and thus may result in inaccurate assessments of environmental states (Wang et al., 2004, but see Ashauer et al., 2011). Running experiments in the lab with the same species than in the field allows to isolate individual factors with and without the natural environmental variation present. Thus, it allows assessing mechanistic dependencies, and how relevant they are (with respect to effect sizes) under (semi-)natural conditions. This is especially relevant for a highly common and widely distributed species complex, such as *G. fossarum*, which is known to have a high intra-species diversity and different lineages across its range (Weiss and Leese, 2016; Wattier et al., 2020), which especially indicates the need to establish different and locally representative stocks of laboratory populations from local field populations. Thus, broadening the set of organisms used for ecological assessments and ecotoxicological studies, and also the ability to establish locally representative lab-populations, is of general interest.

Here, we aimed to improve the methods to maintain and cultivate *G. fossarum* in the laboratory. The goal was to optimize existing approaches for using this common freshwater amphipod at the intersect of fundamental and applied research (Consolandi et al., 2021). While Pöckl successfully kept *G. fossarum* in the laboratory over extensive time-periods (months to years; Pöckl and Humpesch, 1990, Pöckl, 1992, 1995), this required monthly restocking stock tanks (where reproduction takes place) or keeping individuals separately in smaller tanks. Literally all other studies rely on wild caught individuals directly or run experiments only for a few months the longest (see Consolandi et al., 2021). Therefore, the goal of our study was to find suitable husbandry settings which, firstly, increase the survival of an existing (field caught) cohort of individuals, secondly, increase their reproduction/production of a juvenile cohort as well as of its growth and survival, and, finally, contribute to long-term maintenance and reproduction across multiple generations. To achieve this goal, we tested different breeding protocol improvements, based on existing knowledge (Pöckl, 1992, 1995). *Gammarus fossarum* preferentially feeds on inoculated, naturally senescent leaf litter (Little and Altermatt, 2018), but is often at least partially predatory (Kelly et al., 2002). Therefore, supplementing its diet with a protein-rich food source might increase survival rate (Labaude et al., 2015; Kosfeld et al., 2020). The aim would be to have a standardized medium as it exists for *H. azteca* (Borgmann, 1996). Shelter may reduce stress since *Gammarus* tends to avoid light and hide in macrophytes (e.g. Marchant, 1981; MacNeil et al., 2003) even in the absence of predators where shelter can be crucial (e.g. Kobak et al., 2014). Shelter was previously suggested to increase survival under laboratory conditions in *H. azteca* (Kosfeld et al., 2020) but not systematically tested. Also, some studies suggested that day length (or photoperiod) may have an effect on the reproductive activity of *Gammarus* species (de March, 1982; Steele and Steele, 1986).

Therefore, mimicking natural light conditions might improve reproductive activity and increase total abundance. Specifically, we tested if and how a protein-rich food addition to the diet of *G. fossarum* affects survival rate. Furthermore, we systematically tested how the addition of shelter affects survival rate of *G. fossarum*. Finally, we investigated the effect of different light regimes on the total abundance and reproductive success of *G. fossarum*.

2. Methods

We started our experiment with wild caught *G. fossarum* to study survival rate and reproductive activity under laboratory conditions. Based on initial experience, we did so either by improving diet and shelter conditions (feed enrichment and shelter experiment) or, in a second experiment, manipulating light regimes in order to mimic natural day length cycles (day length experiment). We conducted both experiments at the Aquatikum experimental facility of Eawag, Dübendorf ZH, Switzerland.

2.1. Study organism

Gammarus fossarum is the most widespread and common amphipod in Switzerland (Altermatt et al., 2014, 2019). It inhabits a range of freshwater ecosystems, from streams and creeks to lakes and natural springs. *Gammarus fossarum* comprises a species complex (Wattier et al., 2020), of which at least three species-lineages are known from Switzerland, namely lineages A, B and C (Altermatt et al., 2019; for their distribution see also Alther et al., 2021). These lineages differ in their sensitivity to environmental stressors (Feckler et al., 2012; Eisenring et al., 2016). We caught *G. fossarum* individuals, all belonging to the A-lineage (molecular assignment based on COI; Wattier et al., 2020), from forested sections of two natural streams near Zurich (see below for details). We only used individuals with no obvious acanthocephalan parasite infection (Bojko and Ovcharenko, 2019). For the onset of experiments, we only used individuals larger than 5 mm at natural sex ratios. Reaching sexual maturity is highly temperature dependent in *G. fossarum*. They may reach sexual maturity in roughly 96 days at 20 °C, while the same process can take up to 335 days at 8 °C (Pöckl, 1992; Sutcliffe, 1992). Their offspring then hatches after 347 degree days, again being highly temperature sensitive (Pöckl and Humpesch, 1990 based on individually maintained organisms). Given their usual lifespan of 6 months to 2.5 years under ambient conditions (Welton and Clarke, 1980; Beracko et al., 2012) and given their mean brood size of 9.6 embryos per brood (Beracko et al., 2012), one can estimate the total lifetime fecundity to be 194 eggs (Pöckl, 1993). Welton and Clarke (1980) observed a maximum of six consecutive broods per female under experimental conditions.

2.2. Preconditioning leaves

Throughout all experiments, we fed the amphipods on preconditioned naturally senescent alder (*Alnus glutinosa*) leaves, previously shown to be a preferred food source of *G. fossarum* (Little and Altermatt, 2018). We collected alder leaves in fall 2015 for the feed enrichment and shelter experiment and in fall 2019 for the day length experiment. We carried out leaf litter collections in close vicinity to Eawag and consecutively air-dried them for at least seven days to prepare them for long-term storage in cardboard boxes. Before weighing in leaf material for the experiment, we clipped leaf stems with scissors to maximize the consumable biomass in the experiment. Before the onset of experiments, sufficient amounts of clipped leaves were put in 9 L plastic tanks (Tecniplast, 1284L EUROSTANDARD TYP II L (PC), 365 × 207 × 140 mm, base area: 530 cm²) and covered with fresh water from the local Chriesbach stream (N 47.4044, E 8.6100; Dübendorf, ZH, Switzerland) at room temperature. The tanks were aerated with compressed air using surgical tubing (Sarstedt MPL non-pyrogenic extensions) and two bubble stones per tank (Tetra AS30 Air Stone). After at least five days submerged in stream water, leaves were air-dried again (Leaf conditioning protocol, appendix B).

2.3. Feed enrichment and shelter experiment

We assessed amphipod survival rate in control tanks (hereafter referred to as “control treatment”), tanks with protein-rich food addition (hereafter referred to as “feed enrichment treatment”), tanks with increased shelter availability (hereafter referred to as “shelter treatment”), and tanks with a combination of both (protein-rich food addition and increased shelter availability; hereafter referred to as “feed enrichment plus shelter treatment”). We collected *G. fossarum* amphipods in Sagentobelbach stream, Stettbach ZH (N 47.3927, E 8.5880) on September 27, 2018 using a kicknet approach. We acclimatized the amphipods for two weeks at densities of 500–600 amphipods per 30 L PP plastic tanks (Rotho Clear Box Blanket, 705 × 400 × 165 mm). We carried out the experiment between October 12, 2018 and June 25, 2019 in a flow through system (Flow through protocol, appendix C). We initially put 20 adult *G. fossarum* amphipods in 4 L polycarbonate flow through tank (Tecniplast model 1264C; 268 × 215 × 141 mm; base area: 370 cm²; in two flow through systems, 12 tanks per system, four shelves each with three tanks; flow rate of 11.7 L/h ± 3.68, see Flow through protocol, appendix C) and assigned treatments randomly to tanks. We picked individuals randomly by eye, stirring the stock tanks before every pick, discarding individuals that were obviously harmed or parasitized. We replicated each treatment 6 times, totaling 24 tanks and 480 amphipods. We kept amphipods at ambient temperature of 20 °C since active cooling of the existing flow through system is challenging, especially due to the pumps. We used a 9:1 mix of animal proof tap water (food-safe plastic-only conduit from waterworks, no brass connections (i.e., stainless-steel), avoiding heavy metal contamination of the water) to local Chriesbach stream water to run the flow through system (Supp. Fig. A.1 A). We measured metal concentrations of animal proof tap water and Chriesbach stream water on a regular basis. Additional water quality parameters such as pH or conductivity for Chriesbach stream water were monitored online. All water quality parameters are given in Supp. Table A.2, describing the average conditions during the experiment. All four treatments included two ceramic tile plates (size 200 × 250 mm) and two preconditioned, naturally senescent alder leaves (Supp. Fig. A.1 B). The control treatment received no further treatment. The additional shelter treatment included an additional tile plate (three in total) and three natural stream-collected stones which were boiled for disinfection before usage (Ø ~100 mm; collected in Chämmertenbach stream; N 47.3900, E 8.5990; Stettbach, Zürich, Switzerland). The feed enrichment treatment additionally received a weekly supply with five 1.6 mm juvenile trout feed pellets (Hofmann Nutrition AG Trout Start fish meal complete feed; 48 % raw protein, 20 % raw fat). The feed enrichment plus shelter treatment included an additional tile plate (three in total) and three natural stream-collected stones, and received a weekly supply with five 1.6 mm juvenile trout feed pellets. We fed amphipods ad libitum, replenishing leaves during the course of the experiment, carefully avoiding that leaf availability dominated shelter availability. We surveyed the number of adult and juvenile amphipods roughly every month for 256 days, first in a three-week interval, later in a four-week interval (days 0, 20, 41, 70, 110, 138, 159, 187, 220, and 256). We assigned them visually to adult or juvenile counts based on body size, with adults being larger than 5 mm (Beracko et al., 2012). For cases where the assignment was difficult to judge, we always assigned the individual to juveniles. However, it is experimentally not so easy to tease apart which individuals of which generation (parental, F1, F2, or even mixed) contribute to the maintenance of a viable long-term population. We neither separated nor marked newly hatched individuals and assignment to different generations was not possible.

2.4. Day length experiment

We studied the effect of light regime on amphipod survival rate and reproductive activity conducting a long-term laboratory husbandry approach under static conditions. We collected *Gammarus fossarum* (lineage A) amphipods in Chämmertenbach stream, Stettbach ZH (N 47.3900, E 8.5990) on May 18, 2020 and acclimatized them in the lab for one week at densities of 500–600 amphipods per 30 L food-safe PE plastic trays (Kaiserkraft,

Kunststoff-Stapelbehälter, 750 × 445 × 210 mm; base area: 3338 cm²). We set up the experiment on May 26, 2020 and let it run until May 25, 2021 in stagnant water tray cultures (Supp. Fig. A.3; Tray culture protocol, appendix D). We filled twelve food-safe PE plastic trays like the ones previously used for the acclimatization with a 1:3 mix of animal-safe tap water and local Chriesbach stream water. When setting up the experiment, we stocked the trays with 60 randomly chosen adult amphipods (undetermined, natural sex ratio). We placed the trays in four metal racks that were fully covered by black pond foil to prevent any unwanted light source affecting the treatments (Supp. Fig. A.4). Each metal rack had four shelving units. In order to cool the water, we installed a passive cooling system (Cooling system protocol, appendix E). In order to minimize potential contamination, only stainless steel tube fragments (length 20 cm; outer Ø 12 mm) were exposed to the water inside the tanks (see Cooling system protocol, appendix E for details). We constantly kept water temperatures between 12.5 and 15.0 °C, having sensors installed in every second tray and controlled weekly. Water temperature was lower than in the feed enrichment and shelter experiment in order to match the optimal temperature range to increase reproductive activity and juvenile survival of *G. fossarum* (8–12 °C, 70–80 % survival rate; Pöckl and Humpesch, 1990). Water temperatures increased slightly over the course of the day when the light was on (max. +1 °C). We simulated daylight by one LED fluorescent lamp per tray (Philips MAS LEDtube HF 600 mm HO 8W865 T8). We kept day length constant for six of the twelve trays at 14:10 light:dark ratio (L:D ratio, in hours) (fixed day length treatment). For the other six trays we started with a 14:10 L:D ratio and consecutively adapted day length biweekly by 1 h, first increasing day length up to 16 h of light before decreasing day length down to 9 h and back to initial conditions (variable day length treatment). This resulted in the amphipods experiencing the course of a year twice in 365 days (Supp. Table A.5). We provided shelter by adding five tile plate fragments and three natural stones placed in each tray. Two bubble stones, connected to a central aeration system installed in the building, aerated each tray. We fed amphipods ad libitum with preconditioned naturally senescent alder leaves and supplied them weekly with five trout pellets per tray (Hofmann Nutrition AG Trout start; same as in previous experiment). We consecutively surveyed amphipod abundance every 28 days for one year by removing each individual from the tray using plastic disposable pipettes. After we removed all individuals, we additionally changed one third of the water (for water quality parameters see Supp. Table A.2). We removed dead individuals regularly to prevent decay and unwanted side effects. After the counting and water-change routine, we put amphipods back into the corresponding trays. We cleaned the trays bimonthly (removal of sediment) during water change but only in extreme cases set them up freshly.

Since we observed considerable biofilm formation over the course of the experiment, we additionally rated algal growth in the trays visually for each tray during the counting. We rated the algal growth qualitatively on a scale from 0 to 2 (low, medium, and high algal growth; Supp. Fig. A.6 A–C). We started this rating only after five months since initially all trays had no biofilm. We did not assess the taxonomic diversity of algae due to time constraints. We visually assessed the maturity of amphipods (classified as juvenile or adult) with a body-size threshold of smaller or larger than 5 mm (see Beracko et al., 2012). We assigned amphipods as being reproductively active either if they were forming praecopula pairs (male attaching to a female prior to mating) or if we could observe developing eggs in the brood pouch of females. Whereas absolute reproductive activity was the raw number of reproductively active amphipods, we calculated the relative reproductive activity as the proportion of absolute reproductive activity in relation to total amphipod abundance in each tray.

2.5. Analyses

We compared amphipod survival rate among the different treatments implementing a survival analysis using the “survival” package (v. 3.2-13; Therneau and Grambsch, 2000). We tested for differences between survival curves using the G-rho family of tests (Harrington and Fleming, 1982). We

did pairwise comparisons between group levels using a log-rank test, implemented in the “survminer” package (v. 0.4.9; Kassambara et al., 2021), correcting for multiple comparisons after Benjamini and Hochberg (1995).

For the day length experiment, we tested for normal distribution of the response variables using a Shapiro-Wilk test (Shapiro and Wilk, 1965). Because the Shapiro-Wilk test was significant for total abundance and for reproductive activity, we used a nonparametric test to compare the treatments. We used an unpaired two-sample Wilcoxon rank sum test to assess differences in total abundance and relative reproductive activity between the two treatments (Bauer, 1972; Hollander et al., 2015). We inferred effect size for test results by differences in locations for groups since there is a lack of size measurements for non-parametric tests (e.g., Leech and Onwuegbuzie, 2002). To test for rank-based correlations, we used Kendall's tau statistic (Kendall, 1938).

We carried out all statistical analyses in R 4.1.1 (R Core Team, 2021) and R Studio version 2021.09.0 (RStudio Team, 2021). Data and analysis script are available in the appendix file F and on Zenodo (<https://doi.org/10.5281/zenodo.7069017>).

3. Results

3.1. Feed enrichment and shelter experiment

This experiment was conducted in small flow-through tanks, which were ideal to maintain adult amphipods yet too small to support efficient reproduction and survival to adulthood (e.g. due to cannibalism, own observation and experiments by Pöckl with 5 L stock tanks and recurrent restocking, e.g. Pöckl and Humpesch, 1990). Thus, the focus is on survival rates of adults in long(er) term studies. The initial abundance of 20 individuals per tank corresponded to a density of 541 amphipods/m². The overall survival rate was 6.5 % ± 7.6 after 256 days, with the mean survival rate dropping below 50 % after 70 days and all tanks dropping below 50 % survival after 110 days. The different treatments resulted in significant differences in survival of *Gammarus fossarum* over time, reflected in the different survival curves (Fig. 1; $\chi^2 = 34.9$, degrees of freedom = 3; P -value < 0.0001; Supp. Fig. A.7). Tanks with the shelter treatment had significantly higher survival rates compared to the control treatment (log-rank test, P -value = 0.02; Supp. Fig. A.7; Supp. Table A.8). Tanks with the feed enrichment treatment had significantly higher survival rates compared to the control treatment (log-rank test, P -value < 0.0001; Supp. Table A.8). *Gammarus*

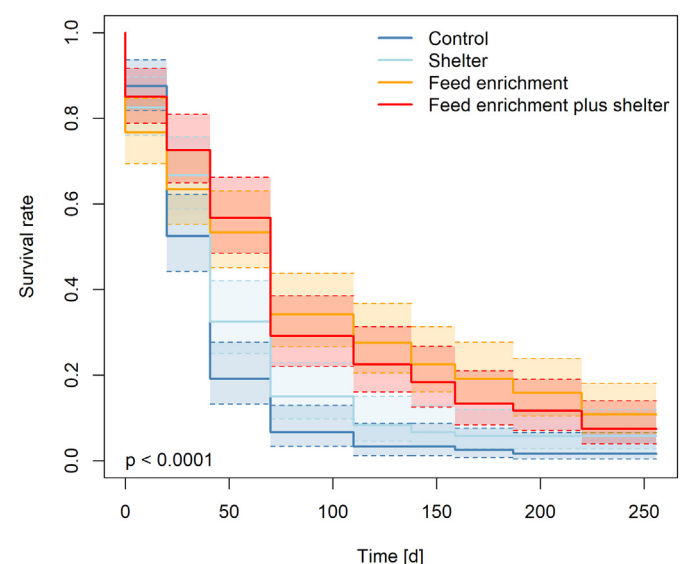


Fig. 1. Survival curves of adult *G. fossarum* under different feed enrichment and shelter treatments. Solid lines are mean survival rates while dashed lines and shading indicate 95 % confidence intervals. We monitored survival rate of *G. fossarum* roughly every month for 256 days in total. See Supp. Fig. A.7 for trajectories of the replicates.

fossarum in the feed enrichment plus shelter treatment showed significantly higher survival rates than the control treatment (log-rank test, P -value < 0.0001; Supp. Table A.8) but did not differ from the feed enrichment treatment (log-rank test, P -value = 0.7; Supp. Table A.8) while being significantly higher than the shelter treatment (log-rank test, P -value = 0.01; Supp. Table A.8). Higher survival in enriched treatments (shelter and/or food) compared to control treatment thus was mainly explained by higher food quality rather than additional shelter. Offspring was neglected in the data analysis since survival to adulthood of offspring in the small tanks was overall very low (maximum was three individuals).

3.2. Day length experiment

This experiment was conducted in large tanks that allowed reproduction and growth of juveniles. Also, it included the provisioning of shelter and addition of proteins to improve survival. The initial abundance of 60 individuals per tray corresponded to a density of 180 amphipods/m². Abundances were rising during the first half of the experiment and decreased toward the end of the experiment across both treatments (Fig. 2). Mean abundances over the course of the experiment corresponded to 125.3 (min: 0; max: 445) and 111.2 (min: 0; max: 346) amphipods for fixed and variable day lengths, respectively. This translates to mean densities of 376 and 333 amphipods/m². Day length showed no significant effect on the amphipod abundance (Supp. Fig. A.9; Wilcoxon rank sum test; W = 3787; P -value = 0.412). Abundance distributions of the two treatments overlapped for all counting events during the experiment. The initial increase was mostly driven by juvenile individuals (Supp. Fig. A.10; left panels), of which some made it to adulthood, increasing the number of adults after some months (Supp. Fig. A.10; right panels). Although we here focus on the quantitative analyses of population dynamics in twelve experimental tanks during the first year (duration of detailed monitoring), we observed viable populations for up to 21 months and longer. In February 2022, five tanks that originated from the described experiment (started in May 2020) and additional three tanks that were not part of the described experiment (started mid 2019) contained 93 individuals (mean \pm SD = 11.6 \pm 11.8 individuals per tank). We transferred individuals from these tanks to a single new tank with an annual temperature cycle (range 4–18 °C) in February 2022. Several hundred individuals were alive in June 2022.

Relative reproductive activity was slightly higher in the variable day length treatment (Fig. 3 A; Wilcoxon rank sum test; W = 1605; P -value =

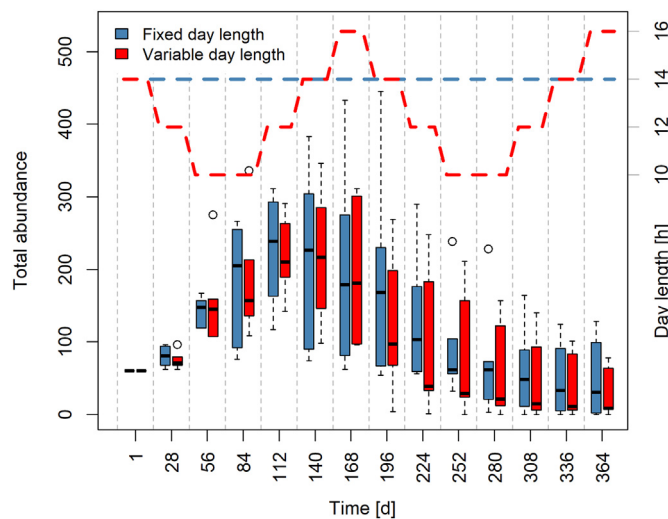


Fig. 2. Total abundance across replicates at different time points for the different day length treatments. Abundances across replicates per treatment are given as boxplots, depicting median (solid line in the box), interquartile range IQR (hinges; 25th and 75th percentiles) and most extreme values within 1.5 x IQR (whiskers, points outside this range are plotted separately). The day length treatments (right-hand y-axis) are given by dashed red and blue lines, respectively.

0.006), but the effect size was very small (median of the differences between fixed and variable day length = 0.07; mean relative reproductive activity was 0.15 vs. 0.22 for fixed and variable day length). When considering the different time points, temporal variability in relative reproductive activity was high in both treatments (Fig. 3 B). No clear pattern emerged beside that relative reproductive activity was negatively correlated with total abundance (Kendall's tau for positive association = 0.11, z = 1.7849; P -value = 0.037).

The proportion of juveniles did not differ significantly between fixed and variable day length treatments (Supp. Fig. A.11; Wilcoxon rank sum test; W = 3463; P -value = 0.369). Algal growth did not differ between day length treatments (Wilcoxon rank sum test; W = 1467; P -value = 0.310). High algal growth was positively correlated with amphipod densities in the tray (Fig. 4; Kendall's tau for positive association = 0.11, z = 1.7849; P -value = 0.037).

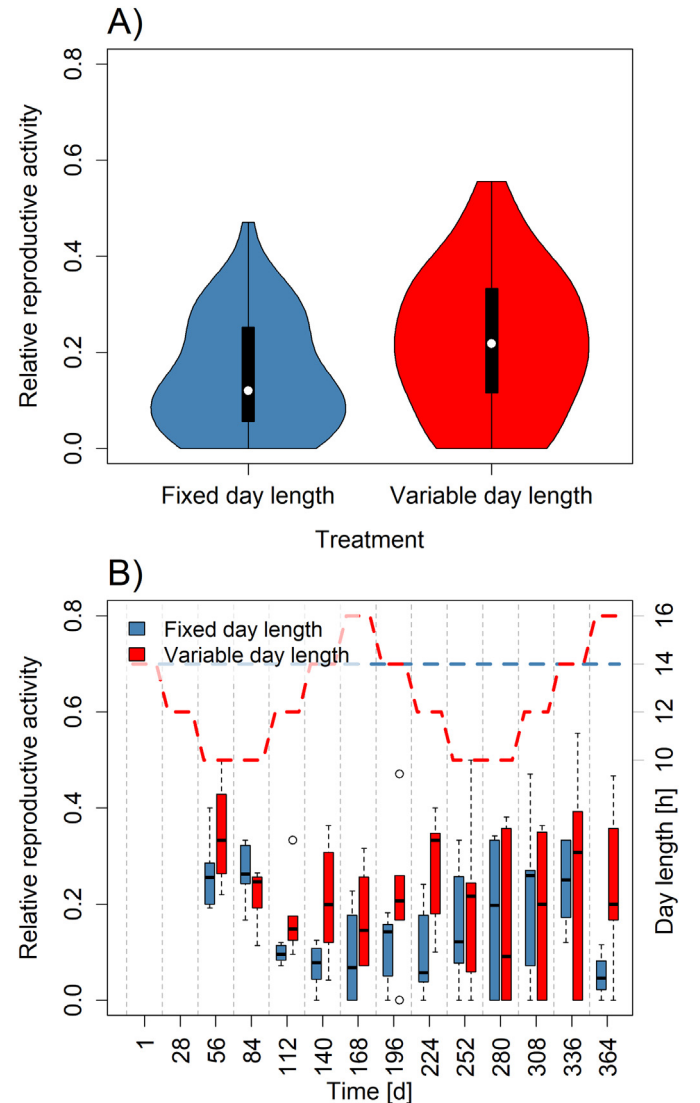


Fig. 3. Relative reproductive activity for fixed and variable day length treatments. A) Violin plot pooled across all replicates (t = 364 d; white dot = median; box = interquartile range IQR, 25th and 75th percentile; whiskers = most extreme data points that are no more than 1.5 times the IQR from the box; violin shape = kernel density of data points). B) Boxplot for all counting events separately. Reproductive activity included pre-copulating pairs and gravid females registered during each counting event. Reproductive activity across replicates per treatment is given as boxplots, depicting median (solid line in the box), interquartile range IQR (hinges; 25th and 75th percentiles) and most extreme values within 1.5 x IQR (whiskers, points outside this range are plotted separately). The day length treatments (right-hand y-axis) are given by dashed red and blue lines, respectively.

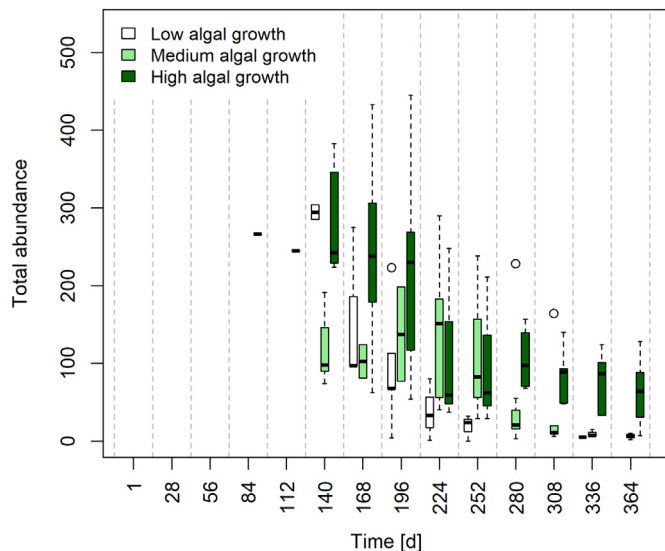


Fig. 4. Correlation of algal growth and total abundance of amphipods during the day length experiment. Replicates of the different day length treatments were pooled. We visually assigned observations to one of the three groups of algal growth (low, medium, high; see Supp. Fig. A.6).

4. Discussion

4.1. Feed enrichment and shelter experiment

Our experiments showed that maintenance of *G. fossarum* in experimental tanks is feasible over longer time (also see Pöckl, 1995). While many previous studies kept *Gammarus* over a few days to weeks only (Xuereb et al., 2011; Coulaud et al., 2011; Abo-Taleb et al., 2020; Chaumot et al., 2020), our experiment ran for almost 37 weeks (close to 9 months). This experiment was conducted in relatively small tanks, that can be highly replicated, and allow easy quantification of animals over time, yet are likely suboptimal for successful reproduction (own observation and e.g. Pöckl and Humpesch, 1990). For example, cannibalism is a well-known phenomenon among *Gammarus* species, reducing survival rates in juveniles, also in comparably large tanks (Dick, 1995). Up to a fifth of the juveniles may be preyed upon by conspecifics (Dick, 1995). Indeed, we mostly observed survival of initially stocked populations, and only minimal successful reproduction. Overall survival rate was slightly lower than in comparable studies (e.g. Fig. 1a in Pöckl, 1995), but we kept the animals in groups rather than individually (data on groups are not given in detail in Pöckl, 1995 but are lower as well). While abundances decreased considerably throughout the experiment (Fig. 1), most of the tanks, and especially tanks with shelter and food supplementation, still had surviving amphipods after 256 days. Given that the animals used to start the experiment were of unknown age/age structure, it is likely that much of the mortality is due to natural senescence, and that the up to nine months of survival of animals that were already (sub)adults when the experiment started show the feasibility of long-term maintenance. It has previously been shown that amphipods require shelter to increase survival rate (Duffy and Hay, 1991; Kosfeld et al., 2020). Survival rate was indeed higher when providing additional shelter (Supp. Table A.8). However, providing additional shelter simultaneously increases handling efforts during experimental studies, rendering it unfeasible for certain experimental needs, e.g., when involving extensive handling or quantification of individuals.

The second breeding protocol improvement that we tested, namely the protein-rich food addition, requires only minimal additional handling effort, yet is effective for increasing survival. We therefore would recommend this for long-term maintenance in general. Several studies show the benefits of providing scarce nutrients to amphipod cultures compared to rearing them purely on preconditioned leaves (Rollin et al., 2018; Trochine et al.,

2021). Indeed, also in our study, survival rate significantly increased in all treatments with protein-rich food addition (Fig. 1). This suggests that naturally senescent leaves should be supplemented with an additional protein source for successful maintenance and breeding of *G. fossarum*. While the protein supply might minimally increase nutrient level in the medium (which could influence the water quality, even when circulating the water through a biofilter as in our first experiment), this potential drawback was clearly outweighed by the overall increase in survival. However, depending on the foreseen application this aspect needs to be considered. Possibly, this increased survival due to addition of protein supplements is twofold: firstly, these protein pellets are a nutritious food supplement, and may provide additional resources and give a more balanced diet than leave litter only. Secondly, the provisioning with animal proteins may reduce cannibalism. Together, this increases survival of amphipods, and is especially recommended for studies that are looking at long-term effects on *Gammarus* survival and maintenance.

Trial runs (data not shown) showed that the trout feed pellets quickly start molding when not consumed, therefore one needs to establish a balance of how many to add in what time intervals. We used about six pellets per 20 individuals per week (see Flow through protocol). Trout feed pellets were only added in quantities that were consumed within a short time (2–3h), such that no left-overs or waste products were accumulating. Using the flow through systems (incl. a circulation through a biofilter) and regular water exchanges, we could prohibit long-term accumulation of waste products. Preliminary results indicate that using flakes instead of pellets lowered the occurrence of mold in the tanks. Our feed enrichment and shelter experiment showed that there was a need to improve further aspects of the *Gammarus* husbandry, which is why we conducted the second experiment, manipulating day length and using larger tanks with lower water temperature.

4.2. Day length experiment

The day length experiment lasted over one year and aimed at improving maintenance and survival of *G. fossarum*, and allowing reproduction and successful generational turnover. To do so, we conducted the experiment in larger experimental containers, which we generally recommend when it comes to including reproduction in the maintenance. Larger containers might reduce cannibalism on juveniles (Dick, 1995). Also, we actively cooled the water to match optimal temperatures for *G. fossarum* reproduction (Pöckl and Humpesch, 1990). Contrary to our expectation, the day length experiment sustained relatively high amphipod densities over time, independent of the treatment. There was no significant effect of fixed vs. variable day length treatments (Fig. 2), contradicting a major influence of photoperiod on breeding success in *G. fossarum*. Both experimental treatments would have allowed starting an experiment with lab-bred *G. fossarum* individuals after a few months, fulfilling one of the initial goals of the study.

However, looking at total abundance only might mask some effects on reproductive activity or juvenile survival. *Gammarus fossarum* reproduced continuously over the course of the whole experiment under fixed and variable day length. As in previous studies (de March, 1982; Steele and Steele, 1986), day length showed an effect on the relative reproductive activity of *G. fossarum* (Fig. 3). Relative reproductive activity was slightly higher under variable day length treatments, but the effect was small (Fig. 3 A). This matches with observational data in natural streams, where *G. fossarum* reproduced most time of the year, but ovigerous females were absent in some sites in October and November (Pöckl, 1993), suggesting some seasonality in reproduction. Sutcliffe (1992) showed that day length controlled ovarian diapause in marine *Gammarus lawrencianus* Bousfield, 1956, providing experimental evidence for seasonality in reproduction. Steele and Steele (1986) worked with marine amphipods, namely *Gammarus setosus* Dementieva, 1931 and *G. lawrencianus*, and showed a clear correlation of day length on reproductive activity. Unlike in our experiment, this effect may be pronounced in marine species (Davenport et al., 2005; Kaartvedt, 2008), since water temperature should be buffered in marine ecosystems

by the high thermal mass of the oceans (Thompson et al., 2013). In an experiment by de March (1982), he found that short day lengths were necessary to induce reproduction in the phylogenetically closely-related freshwater *Gammarus lacustris* G.O. Sars, 1863. In our experiments, this effect was not present. However, the animals were kept over a shorter time and collected during different seasons, rendering conclusions about long-term success of breeding *Gammarus* under different light regimes inconclusive.

Even though relative reproductive activity was slightly higher under variable day length treatments, this did not translate into higher proportion of juveniles observed (Supp. Fig. A.11). While this was not among our initial goals, increasing the proportion of juveniles by increasing their survival is crucial for successful breeding of *G. fossarum*. It has been shown that there is a clear effect of temperature on juvenile survival and growth (Pöckl and Humpesch, 1990; Pöckl, 1992). Future breeding protocol improvements should consider lower and seasonally variable temperatures.

Finally, we observed that there was different growth of algae across the different replicates (probably due to minor differences in the inoculum). The algae data was insufficient to detect eventual significant differences between fixed and variable day length treatments. However, there was an effect of qualitative differences in algal growth on amphipod abundance (Fig. 4) and relative reproductive activity (data not shown). It has been shown before that algal growth can be crucial for amphipod growth and performance by providing essential nutrients (Rollin et al., 2018, Trochine et al., 2021). Our feed enrichment and shelter experiment supports the finding that nutrient availability is key for amphipod growth, with protein-rich food addition having had the most pronounced effect on survival rate (and subsequently on total abundance). Both, algal biofilm (as in previous studies) or protein pellets as in our experiment provide scarce nutrients when only providing preconditioned, naturally senescent alder leaves (Rollin et al., 2018).

4.3. Conclusions and future directions

Our study demonstrated that wild caught *G. fossarum* can be maintained under laboratory conditions for extended time-periods of at least 9–12 months, if shelter and protein rich food supplementation are provided which help to increase survival of the animals. We showed that manipulating day-length is of minor relevance for long-term reproduction. However, while the observed effects of a more natural day length cycle were small, we still recommend their implementation as its small effect may still contribute to the breeding activity of *G. fossarum* under laboratory conditions. Improved survival in the second experiment may be largely attributed to switch from smaller and warmer tanks to bigger and cooler tanks. While we could keep *G. fossarum* for several months in the first experiment, numbers even increased in the first few months of the second experiment. While we improved laboratory culturing of *G. fossarum* (increased reproduction and survival), the rearing across several generations was not yet completely reached, yet will also require longer time periods (with the generation times given).

Amphipod reproductive activity may be regulated by temperature even though day length is thought to be more consistent in temperate ecosystems (de March, 1982). Some laboratory experiments by Pöckl and Humpesch (1990) and Pöckl (1992, 1995) support the importance of temperature for successful breeding in *G. fossarum*. For example, reproductive success was highest at 11.8 °C (Pöckl and Humpesch, 1990). Hence, implementing variable (and lower) temperature regimes may maintain reproductive activity over a longer time. Preliminary results from the transferred individuals support this direction. While feeding rates increase with temperature, i.e. they double when increasing water temperature from 10 to 18 °C, mortality rates also increase at elevated temperatures (83 % after 26 days; Schmidlin et al., 2015). Another promising improvement to breed freshwater *Gammarus* species is the implementation of flow regimes (Marchant, 1981), mimicking natural stream flow conditions more realistically (e.g., see a possible set-up in Supp. Fig. A.12), but require active cooling, since larger amounts of water are pumped. Finally, more focus should be given on different algal diets and their effects on individual amphipod survival rate (specifically juvenile survival), reproductive activity, and consequently total abundance (Pöckl,

1995; Trochine et al., 2021). While a standardized medium exists for *H. azteca* (Borgmann, 1996), we refrained from a standard medium for *G. fossarum*, yet relied on local water. For future work, possible next steps would be to get continuous water quality data throughout the experiment, to assess the stability of the husbandry system. The main issue in the second experiment was the low survival of juveniles when kept with adult individuals and future husbandry optimizations should focus on this aspect. Reducing cannibalism (Dick, 1996; Otto, 1998) and providing a specific diet for juvenile animals (Pöckl, 1995) are very promising directions to further improve the husbandry protocol. Since laboratory applications would require *G. fossarum* individuals raised under standardized conditions with known age distribution, separating freshly hatched juveniles without manually picking them would be a major improvement.

Overall, our results and the proposed further steps will improve the use of *G. fossarum* to become a more standard and more manageable study organism for experimental applications in ecology and ecotoxicology. We could show that keeping field caught *G. fossarum* under optimized laboratory conditions may produce a high amount of juveniles of standardized age and condition (Supp. Fig. A.10), especially during the first six months after establishment of the cultures. These animals could further be raised to the target size required for laboratory studies. Applications would range from using them for toxicity tests (comparable to GamTox, Gerhardt, 2011, or HYBIT, Kosfeld et al., 2020), experiments on ecosystem functioning (Little et al., 2019 as example), to behavioural studies.

CRedit authorship contribution statement

Roman Alther: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Andrin Krähenbühl:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft. **Pascal Bucher:** Investigation, Methodology, Writing – review & editing. **Florian Altermatt:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Data availability

Data are available in appendix F and on Zenodo (<https://doi.org/10.5281/zenodo.7069017>).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.158730>. Supplementary tables and figures are available in appendix A. Supplementary protocols are available in appendices B–E. Raw data as text files and the R analysis script are available in appendix F and on Zenodo (<https://doi.org/10.5281/zenodo.7069017>).

References

- Abo-Taleb, H.A., Zeina, A.F., Ashour, M., Mabrouk, M.M., Sallam, A.E., El-Feky, M.M.M., 2020. Isolation and cultivation of the freshwater amphipod *Gammarus pulex* (Linnaeus, 1758), with an evaluation of its chemical and nutritional content. *Egypt. J. Aquat. Biol. Fish.* 24, 69–82.
- Altermatt, F., Alther, R., Fišer, C., Jokela, J., Konec, M., Küry, D., Mächler, E., Stucki, P., Westram, A.M., 2014. Diversity and distribution of freshwater amphipod species in Switzerland (Crustacea: Amphipoda). *PLOS ONE* 9, e110328.
- Altermatt, F., Alther, R., Fišer, C., Švara, V., 2019. Amphipoda (Flohkrebse) der Schweiz. *Fauna Helvetica*. 32. info fauna CSCF & SEG, Neuchâtel.
- Alther, R., Fronhofer, E.A., Altermatt, F., 2021. Dispersal behaviour and riverine network connectivity shape the genetic diversity of freshwater amphipod metapopulations. *Mol. Ecol.* 30, 6551–6565.
- Arambourou, H., Decamps, A., Quéau, H., Dabrin, A., Neuzerter, D., Chaumot, A., 2017. Use of *gammarus fossarum* (Amphipoda) embryo for toxicity testing: a case study with cadmium. *Environ. Toxicol. Chem.* 36, 2436–2443.
- Arlow, M.J., Focks, A., Hollender, J., Stamm, C., 2020. Improving risk assessment by predicting the survival of field gammarids exposed to dynamic pesticide mixtures. *Environ. Sci. Technol.* 54, 12383–12392.
- Ashauer, R., Hintermeister, A., Potthoff, E., Escher, B.I., 2011. Acute toxicity of organic chemicals to *Gammarus pulex* correlates with sensitivity of *Daphnia magna* across most modes of action. *Aquat. Toxicol.* 103, 38–45.
- Bauer, D.F., 1972. Constructing confidence sets using rank statistics. *J. Am. Stat. Assoc.* 67, 687–690.
- Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B Methodol.* 57, 289–300.
- Beracko, P., Šýkorová, A., Štangler, A., 2012. Life history, secondary production and population dynamics of *Gammarus fossarum* (Koch, 1836) in a constant temperature stream. *Biologia* 67, 164–171.
- Best, R.J., Caulk, N.C., Stachowicz, J.J., 2013. Trait vs. phylogenetic diversity as predictors of competition and community composition in herbivorous marine amphipods. *Ecol. Lett.* 16, 72–80.
- Bloor, M.C., Banks, C.J., 2006. An evaluation of mixed species in-situ and ex-situ feeding assays: the altered response of *Asellus aquaticus* and *Gammarus pulex*. *Environ. Int.* 32, 22–27.
- Bojko, J., Ovcharenko, M., 2019. Pathogens and other symbionts of the amphipoda: taxonomic diversity and pathological significance. *Dis. Aquat. Org.* 136, 3–36.
- Borgmann, U., 1996. Systematic analysis of aqueous ion requirements of *Hyalella azteca*: a standard artificial medium including the essential bromide ion. *Arch. Environ. Contam. Toxicol.* 30, 356–363.
- Borgmann, U., Couillard, Y., Doyle, P., Dixon, D.G., 2005. Toxicity of sixty-three metals and metalloids to *Hyalella azteca* at two levels of water hardness. *Environ. Toxicol. Chem.* 24, 641–652.
- van den Brink, F.W.B., van der Velde, G., 1991. Amphipod invasion on the Rhine. *Nature* 352, 576.
- Bundschuh, M., Schulz, R., 2011. Population response to ozone application in wastewater: an on-site microcosm study with *Gammarus fossarum* (Crustacea: Amphipoda). *Ecotoxicology* 20, 466–473.
- Bundschuh, M., Zubrod, J.P., Klemm, P., Elsaesser, D., Stang, C., Schulz, R., 2013. Effects of peak exposure scenarios on *gammarus fossarum* using field relevant pesticide mixtures. *Ecotoxicol. Environ. Saf.* 95, 137–143.
- Burdon, F.J., Munz, N.A., Reyes, M., Focks, A., Joss, A., Räsänen, K., Altermatt, F., Eggen, R.L.L., Stamm, C., 2019. Agriculture versus wastewater pollution as drivers of macroinvertebrate community structure in streams. *Sci. Total Environ.* 659, 1256–1265.
- Chaumot, A., Geffard, O., Armengaud, J., Maltby, L., 2015. Chapter 11 - gammarids as reference species for freshwater monitoring. In: Amiard-Triquet, C., Amiard, J.-C., Mouneyrac, C. (Eds.), *Aquatic Ecotoxicology*. Academic Press, pp. 253–280.
- Chaumot, A., Coulaud, R., Adam, O., Quéau, H., Lopes, C., Geffard, O., 2020. In situ reproductive bioassay with caged *gammarus fossarum* (Crustacea): part 1 – gauging the confounding influence of temperature and water hardness. *Environ. Toxicol. Chem.* 39, 667–677.
- Consolandi, G., Ford, A.T., Bloor, M.C., 2021. Feeding behavioural studies with freshwater *gammarus* spp.: the importance of a standardised methodology. In: de Voogt, P. (Ed.) *Reviews of Environmental Contamination and Toxicology* Volume 253. Springer International Publishing, Cham, pp. 1–41.
- Cothran, R.D., Henderson, K.A., Schmidberg, D., Relyea, R.A., 2013. Phenotypically similar but ecologically distinct: differences in competitive ability and predation risk among amphipods. *Oikos* 122, 1429–1440.
- Coulaud, R., Geffard, O., Xuereb, B., Lacaze, E., Quéau, H., Garric, J., Charles, S., Chaumot, A., 2011. In situ feeding assay with *gammarus fossarum* (Crustacea): modelling the influence of confounding factors to improve water quality biomonitoring. *Water Res.* 45, 6417–6429.
- Crane, M., Maltby, L., 1991. The lethal and sublethal responses of *Gammarus pulex* to stress: sensitivity and sources of variation in an in situ bioassay. *Environ. Toxicol. Chem.* 10, 1331–1339.
- Dangles, O., Malmqvist, B., 2004. Species richness–decomposition relationships depend on species dominance. *Ecol. Lett.* 7, 395–402.
- Davenport, J., Berggren, M.S., Brattegard, T., Brattenborg, N., Burrows, M., Jenkins, S., McGrath, D., MacNamara, R., Sneli, J.-A., Walker, G., Wilson, S., 2005. Doses of darkness control latitudinal differences in breeding date in the barnacle *Semibalanus balanoides*. *J. Mar. Biol. Assoc. U. K.* 85, 59–63.
- Dick, J.T.A., 1995. The cannibalistic behaviour of two *gammarus* species (Crustacea: Amphipoda). *J. Zool.* 236, 697–706.
- Dick, J.T.A., 1996. Post-invasion amphipod communities of Lough Neagh, Northern Ireland: influences of habitat selection and mutual predation. *J. Anim. Ecol.* 65, 756.
- Duffy, J.E., Hay, M.E., 1991. Food and shelter as determinants of food choice by an herbivorous marine amphipod. *Ecology* 72, 1286–1298.
- Eisenring, M., Altermatt, F., Westram, A.M., Jokela, J., 2016. Habitat requirements and ecological niche of two cryptic amphipod species at landscape and local scales. *Ecosphere* 7, e01319.
- Feckler, A., Thielsch, A., Schwenk, K., Schulz, R., Bundschuh, M., 2012. Differences in the sensitivity among cryptic lineages of the *Gammarus fossarum* complex. *Sci. Total Environ.* 439, 158–164.
- Feckler, A., Zubrod, J.P., Thielsch, A., Schwenk, K., Schulz, R., Bundschuh, M., 2014. Cryptic species diversity: an overlooked factor in environmental management? *J. Appl. Ecol.* 51, 958–967.
- Felten, V., Charmantier, G., Mons, R., Geffard, A., Rousselle, P., Coquery, M., Garric, J., Geffard, O., 2008. Physiological and behavioural responses of *Gammarus pulex* (Crustacea: Amphipoda) exposed to cadmium. *Aquat. Toxicol.* 86, 413–425.
- Fu, Q., Fedrizzi, D., Kosfeld, V., Schlechtriem, C., Ganz, V., Derrer, S., Rentsch, D., Hollender, J., 2020. Biotransformation changes bioaccumulation and toxicity of diclofenac in aquatic organisms. *Environ. Sci. Technol.* 54, 4400–4408.
- Geffard, O., Xuereb, B., Chaumot, A., Geffard, A., Biagiatti, S., Noël, C., Abbaci, K., Garric, J., Charmantier, G., Charmantier-Daures, M., 2010. Ovarian cycle and embryonic development in *gammarus fossarum*: application for reproductive toxicity assessment. *Environ. Toxicol. Chem.* 29, 2249–2259.
- Gerhardt, A., 2011. GamTox: a low-cost multimetric ecotoxicity test with *Gammarus* spp. For in and ex situ application. *Int. J. Zool.* 2011, 1–7.
- Harrington, D.P., Fleming, T.R., 1982. A class of rank test procedures for censored survival data. *Biometrika* 69, 553–566.
- Hollander, W., Wolfe, D.A., Chicken, E., 2015. *Nonparametric Statistical Methods*. Third edition. John Wiley & Sons Ltd, Hoboken, New Jersey.
- Horton, T., Lowry, J., De Broyer, C., Bellan-Santini, D., Coleman, C.O., Corbari, L., Costello, M.J., Daneliya, M., Dauvin, J.-C., Fišer, C., Gasca, R., Grabowski, M., Guerra-García, J.M., Hendrycks, E., Hughes, L., Jaume, D., Jazdzewski, K., Kim, Y.-H., King, R., Krapp-Schickel, T., LeCroy, S., Lörz, A.-N., Mamos, T., Senna, A.R., Serejo, C., Sket, B., Souza-Filho, J.F., Tandberg, A.H., Thomas, J., Thurston, M., Vader, W., Väinölä, R., Vonk, R., White, K., Zeidler, W., 2022. World Amphipoda Database. <http://www.marinespecies.org/amphipoda>.
- Hou, Z., Sket, B., Fišer, C., Li, S., 2011. Eocene habitat shift from saline to freshwater promoted tethyan amphipod diversification. *Proc. Natl. Acad. Sci. U. S. A.* 108, 14533–14538.
- James, W.R., McClintock, J.B., 2017. Anti-predator responses of amphipods are more effective in the presence of conspecific chemical cues. *Hydrobiologia* 797, 277–288.
- Kaartvedt, S., 2008. Photoperiod may constrain the effect of global warming in arctic marine systems. *J. Plankton Res.* 30, 1203–1206.
- Kassambara, A., Kosinski, M., Biecek, P., 2021. *survminer: Drawing Survival Curves Using “ggplot2.” R Package*.
- Kelly, D.W., Dick, J.T.A., Montgomery, W.I., 2002. The functional role of *gammarus* (Crustacea, Amphipoda): shredders, predators, or both? *Hydrobiologia* 485, 199–203.
- Kendall, M.G., 1938. A new measure of rank correlation. *Biometrika* 30, 81–93.
- Kobak, J., Jermacz, Ł., Płachocki, D., 2014. Effectiveness of zebra mussels to act as shelters from fish predators differs between native and invasive amphipod prey. *Aquat. Ecol.* 48, 397–408.
- Kosfeld, V., Fu, Q., Ebersbach, I., Esser, D., Schauer, A., Bischof, I., Hollender, J., Schlechtriem, C., 2020. Comparison of alternative methods for bioaccumulation assessment: scope and limitations of in vitro depletion assays with rainbow trout and bioconcentration tests in the freshwater amphipod *Hyalella azteca*. *Environ. Toxicol. Chem.* 39, 1813–1825.
- Kunz, P.Y., Kienle, C., Gerhardt, A., 2010. *Gammarus* spp. In aquatic ecotoxicology and water quality assessment: toward integrated multilevel tests. In: Whitacre, D.M. (Ed.), *Reviews of Environmental Contamination and Toxicology*. Springer, New York, pp. 1–76.
- Labauze, S., Cézilly, F., Tercier, X., Rigaud, T., 2015. Influence of host nutritional condition on post-infection traits in the association between the manipulative acanthocephalan *Pomphorhynchus laevis* and the amphipod *Gammarus pulex*. *Parasit. Vectors* 8, 1–12.
- Lauper, B.B., Anthamatten, E., Raths, J., Arlow, M., Hollender, J., 2022. Systematic underestimation of pesticide burden for invertebrates under field conditions: comparing the influence of dietary uptake and aquatic exposure dynamics. *ACS Environ. Au* 2, 166–175.
- Leech, N.L., Onwuegbuzie, A.J., 2002. *A Call for Greater Use of Nonparametric Statistics*. Chantanooga, TN.
- Little, C.J., Altermatt, F., 2018. Species turnover and invasion of dominant freshwater invertebrates alter biodiversity-ecosystem function relationship. *Ecol. Monogr.* 88, 461–480.
- Little, C.J., Altermatt, F., 2019. Differential resource consumption in leaf litter mixtures by native and non-native amphipods. *Aquat. Ecol.* 53, 151–162.
- Little, C.J., Fronhofer, E.A., Altermatt, F., 2019. Dispersal syndromes can impact ecosystem functioning in spatially structured freshwater populations. *Biol. Lett.* 15, 20180865.
- Little, C.J., Fronhofer, E.A., Altermatt, F., 2020. Nonlinear effects of intraspecific competition alter landscape-wide upscaling of ecosystem function. *Am. Nat.* 195, 432–444.
- MacNeil, C., 2019. Differences in the abilities of native and invasive amphipods to tolerate poor water quality and recolonise degraded habitats. *Hydrobiologia* 834, 119–129.
- MacNeil, C., Dick, J.T.A., Elwood, R.W., 1997. The trophic ecology of freshwater *gammarus* spp. (Crustacea: Amphipoda): problems and perspectives concerning the functional feeding group concept. *Biol. Rev.* 72, 349–364.
- MacNeil, C., Dick, J.T.A., Elwood, R.W., 2000. Differential physico-chemical tolerances of amphipod species revealed by field transplantations. *Oecologia* 124, 1–7.
- MacNeil, C., Fielding, N.J., Hume, K.D., Dick, J.T.A., Elwood, R.W., Hatcher, M.J., Dunn, A.M., 2003. Parasite altered micro-distribution of *Gammarus pulex* (Crustacea: Amphipoda). *Int. J. Parasitol.* 33, 57–64.
- de March, B.G.E., 1982. Decreased day length and light intensity as factors inducing reproduction in *Gammarus lacustris lacustris* sars. *Can. J. Zool.* 60, 2962–2965.

- Marchant, R., 1981. The ecology of gammarus in running water. In: Lock, M.A., Williams, D.D. (Eds.), *Perspectives in Running Water Ecology*. Springer, US, Boston, MA, pp. 225–249.
- McCahon, C.P., Pascoe, D., 1988. Use of *Gammarus pulex* (L.) in safety evaluation tests: culture and selection of a sensitive life stage. *Ecotoxicol. Environ. Saf.* 15, 245–252.
- Mehennaoui, K., Georgantzopoulou, A., Felten, V., Andrei, J., Garaud, M., Cambier, S., Serchi, T., Pain-Devin, S., Guérol, F., Audinot, J.-N., Giambérini, L., Gutleb, A.C., 2016. *Gammarus fossarum* (Crustacea, Amphipoda) as a model organism to study the effects of silver nanoparticles. *Sci. Total Environ.* 566–567, 1649–1659.
- Munz, N.A., Burdon, F.J., de Zwart, D., Junghans, M., Melo, L., Reyes, M., Schönenberger, U., Singer, H.P., Spycher, B., Hollender, J., Stamm, C., 2017. Pesticides drive risk of micropollutants in wastewater-impacted streams during low flow conditions. *Water Res.* 110, 366–377.
- Otto, C., 1998. Factors affecting the disjunct distribution of amphipods along a north Swedish River. *Oikos* 83, 21–28.
- Pöckl, M., 1992. Effects of temperature, age and body size on moulting and growth in the freshwater amphipods *Gammarus fossarum* and *G. roeseli*. *Freshw. Biol.* 27, 211–225.
- Pöckl, M., 1993. Reproductive potential and lifetime potential fecundity of the freshwater amphipods *Gammarus fossarum* and *G. roeseli* in Austrian streams and rivers. *Freshw. Biol.* 30, 73–91.
- Pöckl, M., 1995. Laboratory studies on growth, feeding, moulting and mortality in the freshwater amphipods *Gammarus fossarum* and *G. roeseli*. *Arch. Hydrobiol.* 134, 223–253.
- Pöckl, M., Humpesch, U.H., 1990. Intra- and inter-specific variations in egg survival and brood development time for Austrian populations of *Gammarus fossarum* and *G. roeseli* (Crustacea: Amphipoda). *Freshw. Biol.* 23, 441–455.
- R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- van Riel, M.C., van der Velde, G., Rajagopal, S., Marguillier, S., Dehairs, F., bij de Vaate, A., 2006. Trophic relationships in the rhine food web during invasion and after establishment of the Ponto-Caspian invader *Dikerogammarus villosus*. *Hydrobiologia* 565, 39–58.
- Rollin, M., Coulaud, R., Danger, M., Sohm, B., Flayac, J., Bec, A., Chaumot, A., Geffard, O., Felten, V., 2018. Additive effect of calcium depletion and low resource quality on *gammarus fossarum* (Crustacea, Amphipoda) life history traits. *Environ. Sci. Pollut. Res.* 25, 11264–11280.
- Rothe, L.E., Loeffler, F., Gerhardt, A., Feld, C.K., Stift, R., Weyand, M., Grabner, D., Sures, B., 2022. Parasite infection influences the biomarker response and locomotor activity of *gammarus fossarum* exposed to conventionally-treated wastewater. *Ecotoxicol. Environ. Saf.* 236, 113474.
- RStudio Team, 2021. RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA.
- Schmidlin, L., von Fumetti, S., Nagel, P., 2015. Temperature effects on the feeding and electron transport system (ETS) activity of *gammarus fossarum*. *Aquat. Ecol.* 49, 71–80.
- Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (Complete Samples). *Biometrika* 52, 591–611.
- Steele, V.J., Steele, D.H., 1986. The influence of photoperiod on the timing of reproductive cycles in *gammarus* species (Crustacea, Amphipoda). *Am. Zool.* 26, 459–467.
- Straub, S., Hirsch, P.E., Burkhardt-Holm, P., 2017. Biodegradable and petroleum-based microplastics do not differ in their ingestion and excretion but in their biological effects in a freshwater invertebrate *gammarus fossarum*. *Int. J. Environ. Res. Public Health* 14, 1–16.
- Sutcliffe, D.W., 1992. Reproduction in *gammarus* (Crustacea, Amphipoda): basic processes. *Freshw. Forum* 2, 102–128.
- Švara, V., Michalski, S.G., Krauss, M., Schulze, T., Geuchen, S., Brack, W., Luckenbach, T., 2022. Reduced genetic diversity of freshwater amphipods in rivers with increased levels of anthropogenic organic micropollutants. *Evol. Appl.* 15, 976–991.
- Therneau, T.M., Grambsch, P.M., 2000. *Modeling Survival Data: Extending the Cox Model*. Springer, New York, NY.
- Thompson, R.M., Beardall, J., Beringer, J., Grace, M., Sardina, P., 2013. Means and extremes: building variability into community-level climate change experiments. *Ecol. Lett.* 16, 799–806.
- Trochine, C., Díaz Villanueva, V., Brett, M.T., 2021. The ultimate peanut butter on crackers for hyalella: diatoms on macrophytes rather than bacteria and fungi on conditioned terrestrial leaf litter. *Freshw. Biol.* 66, 599–614.
- Väinölä, R., Witt, J.D.S., Grabowski, M., Bradbury, J.H., Jazdzewski, K., Sket, B., 2008. Global diversity of amphipods (Amphipoda; Crustacea) in freshwater. *Hydrobiologia* 595, 241–255.
- Wang, F., Goulet, R.R., Chapman, P.M., 2004. Testing sediment biological effects with the freshwater amphipod *Hyalella azteca*: the gap between laboratory and nature. *Chemosphere* 57, 1713–1724.
- Wattier, R., Mamos, T., Copilaș-Ciocianu, D., Jelić, M., Ollivier, A., Chaumot, A., Danger, M., Felten, V., Piscart, C., Žganec, K., Rewicz, T., Wysocka, A., Rigaud, T., Grabowski, M., 2020. Continental-scale patterns of hyper-cryptic diversity within the freshwater model taxon *gammarus fossarum* (Crustacea, Amphipoda). *Sci. Rep.* 10, 16536.
- Weiss, M., Leese, F., 2016. Widely distributed and regionally isolated! Drivers of genetic structure in *gammarus fossarum* in a human-impacted landscape. *BMC Evol. Biol.* 16, 153.
- Welton, J.S., Clarke, R.T., 1980. Laboratory studies on the reproduction and growth of the amphipod, *Gammarus pulex* (L.). *J. Anim. Ecol.* 49, 581–592.
- Woodward, G., Papantoniou, G., Edwards, F., Lauridsen, R.B., 2008. Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. *Oikos* 117, 683–692.
- Xuereb, B., Bezin, L., Chaumot, A., Budzinski, H., Augagneur, S., Tutundjian, R., Garric, J., Geffard, O., 2011. Vitellogenin-like gene expression in freshwater amphipod *gammarus fossarum* (Koch, 1835): functional characterization in females and potential for use as an endocrine disruption biomarker in males. *Ecotoxicology* 20, 1286–1299.