

MASTER THESIS PROPOSAL

The effect of artificial light on aquatic macroinvertebrates and invasive crayfish: artificial light at night (ALAN) modifies the behaviour of signal crayfish

PRESENTED TO

DR. VERENA C. SCHREINER (schreiner-verena@uni-landau.de)

DR. GEMMA BURGAZZI (burgazzi@uni-landau.de)

DR. MARINA ARIAS (arias@uni-landau.de)

BY

MD. DIDER HOSSAIN (hoss8144@uni-landau.de)

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Introduction:

Below 1% of the surface of the planet is taken up by freshwater ecosystems, yet 10% of the total species are found herein (Friberg, 2014). Various channel types that set streams apart from most ecosystems are produced by the unidirectional circulation of water across places with diverse topography, lithology, drainage, and enormous woody debris or waste (Brussock et al. 1985). According to Frissell et al. (1986), these distinct channel shapes provide a variety of habitats that are highly constrained in terms of both the kind and form of the resources that organisms can use as food. This structural diversity of streams is important because it impacts a number of ecosystem function such as biological variation of organism, functional feeding groups, dynamics of nutrient, interaction of predator-prey, retention and transportation of organic matter, flora (Wallace and Webster, 1996). It has been demonstrated that invertebrates may change the resource quantity of organic materials in streams through a variety of methods, including predation on macrophytes, grazing on periphyton communities, production of woody debris and leaf litter shredding. By removing standing crops and altering the composition of communities containing both primary producers and heterotrophs, these actions have the potential to alter the structure of an ecosystem. (Wallace & Hutchens, 2000). According to studies by Dvořák and Best (1982) on the macro-invertebrate assemblages that coexist with macrophytes in Lake Vechten (Netherlands), the maximum daily macrophyte intake by macro-invertebrates was equal to 10% of their own mass. Through their activity and grazing, invertebrate communities in stream affect also the transport or loss of nutrients (Wallace & Hutchens, 2000). At the Coweeta Hydrologic Laboratory (North Carolina, USA), two local streams have been used to thoroughly study the impact of macro-invertebrates on the transport of organic materials. According to the study's findings, there were no obvious differences in the transit of particulate organic matter in a stream before insecticide application, but after treatment, it was significantly less in the treated channel than in the untreated stream. (Wallace et al. 1982). Although other functional groups may also be significant, filter-feeding invertebrates of rivers have a significant impact on the preservation of minerals and organic materials. For instance, nutrient cycling in food webs may be the cause of the deposition of nitrogen in streams. Invertebrates that crawl may also move organic matter from the surface to deeper strata, obstructing downstream movement. (Wallace & Hutchens, 2000). The study also added that arthropod predators in rivers are not an exemption to the general rule that predators may fulfil a variety of varied functions at sizes varying from individuals to ecosystems. Through their impacts on the existing reserves of other functional groups, predators can affect the export and preservation of both nutrients and energy. Other processes include nitrogen immobilization in long-lived predator species as opposed to short-lived prey, which slows the rate of nutrient cycling. Invertebrate predators can increase suspended fine particulate organic matter and invertebrate migration in addition to direct eating, which boosts nutrient export. (Wallace & Hutchens, 2000).

According to Maltby (2013), rivers and streams offer ecological services that are essential to human society, such as controlling aquatic environment, providing recreational opportunities, and producing food for aquatic species. These activities are dependent on ecosystem services carried out by a variety of aquatic microorganisms, which are negatively impacted by several stressors (Voß and Schäfer, 2017). The biodiversity of aquatic habitat has diminished faster compared to all other habitats in the world at the exact time, and this drop is mostly attributed to humans, who are the primary agents of environmental change (Friberg, 2014). Studies of multiple stressors are crucial in conservation biology because of the enormous number of stressors that are active either concurrently or sequentially. A comprehensive methodology to mechanistically comprehend the impacts of numerous stressors has not yet been offered, and such initiatives are not overwhelmingly represented in the literature. (Juvigny-Khenafou et al. 2021). Therefore, collaborators are unable to make effective short-term and long-term organizational choices for the sake of preservation, regeneration, or natural systems because they have lack of understanding of how stressors combine to impact biological processes (Lindenmayer et al. 2010).

As major stressors for aquatic habitats, depletion of resources, water contamination, habitat destruction, flow manipulation, species encroachment, artificial light have all been documented (Voß and Schäfer,

2017; Martin et al. 2021). As a result of all of these stressors, the largest rivers often contain the freshwater ecosystems that have been most severely impacted, making biological populations less adaptive (Leitner et al. 2021). Lotic ecosystems are under additional stress because to the growing consequences of global warming and exotic species. Environmental or biological indicators are crucial in monitoring and measuring these impacts of stresses on the lotic ecosystem because we require being capable of assessing how lotic ecosystems react to both natural and human-made stresses in order to conserve and improve them. (Friberg, 2014).

By altering the physico-chemical characteristics of streams, such as nutrient concentration and its molar ratio, the amount of accumulated and dispersed sediment particles, velocity distribution, and water viscosity, multiple-stressors have increased the extinction of species and the reduction of ecological functions. (Juvigny-Khenafou et al. 2021). In order to establish an assessment approach for quite big rivers by defining appropriate biological measures as the foundation for multi-metric bioassessment, Leitner et al. (2021) analysed 1197 arthropod specimens from 94 relatively large European waterways. According to this study, dam building was also included to the list of anthropogenic stressors that affect aquatic habitat. The construction of a dam in a river alters the water flow, and the storage of river water alters the physical and chemical characteristics of the water structure. The population of macroinvertebrates is also altered by these sorts of building activities. (Ogbeibu and Oribhabor, 2002). The first sort of water contamination that was broadly acknowledged by society was contamination with readily biodegradable organic materials from land-based sources (Friberg, 2014). By lowering the amount of oxygen contained in the water, microbial degradation of organic materials has a severe detrimental effect on the bioaccumulation (Hynes, 1960).

Urbanization expansion has an effect on the physical characteristics of aquatic habitats including water quality or quantity, stream size, and photoperiod (Feminella and Walsh, 2005). The complicated process of urbanization has abruptly changed the type and amount of flora present, while altered the rhythms of biological elements and created new landscapes of noise, temperature and light (McKinney, 2002). Sky glow, skyscrapers, car lights, streetlights, fishing boat lights, and illuminated structures are all examples of ecological light pollution that can affect ecosystems in different ways (Longcore and Rich, 2004). This is remarkable because 60% of invertebrates and 30% of vertebrates are nocturnal and therefore could be severely affected by artificial light (Hölker et al. 2010). Ecological light pollution negatively impacts the wide range of habitats by affecting the migration, reproduction, and drift patterns of aquatic macroinvertebrates, which may have an impact on benthic communities and members of the underwater community through alterations in productivity and predator-prey interactions (Longcore and Rich, 2004; Hölker et al. 2010; Perkin et al. 2011). There is proof that light sources placed closure to streams alter the way adult aquatic insects move around the terrestrial environment (Perkin et al. 2011). By causing direct fatalities, fecundity problems, or shifts in sex ratios, artificial light at night (ALAN) may lower overall population numbers. Aquatic insects are most likely to experience direct death, either as a result of adults being drawn to lights or increased mortality due to better predator vision. (Scheibe 2003, Eisenbeis 2006, Perkin et al. 2011). The existence of macroinvertebrate predators suggested that the supplementation of the artificial light had an impact on the predator-prey interaction, maybe by tempting and gathering animals. In comparison to consumers who rely on smell and other physiological stimuli, predators that are visually stimulated may profit more from light pollution. (Martin et al. 2021).

The dispersion of freshwater benthos is hindered by artificial lighting (Perkin et al. 2011). Numerous freshwater invertebrates disperse by downstream flow, which can be disrupted by ALAN to alter community structure (Smith et al. 2009). Many studies of a circadian regularity in the movement of aquatic invertebrates clearly demonstrate a relationship between light intensity and this rhythm (Holt and Waters, 1967). Earlier research has found that ALAN can alter normal inland dispersion patterns, draw aquatic insects to terrestrial environments, and increase death due to fatigue (Horváth et al. 2009, Perkin et al. 2014a). According to research by Garratt et al. (2019), increasing illumination dramatically improved species diversity, total community biomass, behavioural change, interspecific interaction, and organic material availability in a tidal sandy beach environment, although individual taxa had distinct

associations with ALAN. Martin et al. (2021) studied seagrass ecosystem structure on Seahorse Key, Florida (USA) to observe the effects of artificial light and fluctuations in diurnal rhythm on fish and macroinvertebrates associated with seagrasses. This study found that there were alterations in community structure, increased species diversity at night, and some variations in the species diversity, there was no immense difference in the richness of fish and macroinvertebrates between daylight and night for short time application of ALAN. (Martin et al. 2021). However, ecosystems that have been continuously exposed to ALAN may have adapted to it, leaving researchers unable to detect the initial effects (Bennie et al. 2015, Hölker et al. 2015, Spoelstra et al. 2015). Gjerløv et al. (2010) investigated the impact of changing the amount of ALAN in streams on periphyton and macroinvertebrates. According to this research, the presence of light boosted macroinvertebrate density, periphyton biomass, and primary production by 31%. In another study, Diptera were found to be more attracted to light-emitting diodes (LED) lamps than to high pressure sodium (HPS) lamps. In addition, Diptera and Lepidoptera were more attracted to the LED lamps compared to Coleoptera, Ephemeroptera, and Trichoptera (Pawson et al. 2014). Many aquatic organisms, especially freshwater macroinvertebrates, control their diurnal activity based on the natural cycles of day and night (Hölker et al. 2010, Perkin et al. 2011, Perkin et al. 2014b). Therefore, the rhythms of diurnal behaviour of these species may change when ALAN disrupts normal light and dark cycles. Throughout the day, numerous benthic organisms in rivers feed on sediment, but at night they separate and migrate to escape the danger of being eaten by fish that feed on the flow (Allan 1987, Brittain and Eikeland 1988). It has been demonstrated that ALAN can interrupt the shredders (*Gammarus jazdzewskii*) behaviour. In addition, wide spectrum LED lighting increases consumption of leaves while reducing shredder growth and activity (Perkin et al. 2014b, Wallace et al. 1997).

One of the main causes of species extinction and ecological change is biological invasion (Simberloff et al. 2013). Ecological light pollution is an example of an anthropogenic stressor that occurs at high levels and can increase the effectiveness of biological invasions (MacDougall & Turkington 2005, Martin et al. 2021), leading to a loss of native species biodiversity (Gutiérrez-Cánovas et al. 2013). Invasive species are considered a serious threat to overall ecosystem functioning because they have significant ecological impacts on native taxonomic communities and affect the biodiversity of individual species (Carbonell et al. 2017, Flood et al. 2020). Functional responses are more commonly tested in research for ecological and biological assessment (Mondy et al. 2012, Belmar et al. 2019). Recently, functional responses have been recognized as a potential method for the evaluation of effects related to biological invasion (Colin et al. 2018, Mathers et al. 2020a). Functional diversity depends on the aspects of biodiversity that affect the functioning of an ecosystem, it measures how diverse and variable biological and ecological features are (Tilman et al. 1997, Schmera et al. 2017). Functional diversity classified as functional evenness, functional richness and functional divergence (Edie et al. 2018, Schmera et al. 2017). Functional richness is characterized as the quantity of trait space inhabited by a species. Functional evenness describes how abundances are distributed over the trait space, while functional divergence demonstrates how the pattern of abundance maximizes variability in the trait space (Mason et al. 2005, Mouillot et al. 2005, Schmera et al. 2017). Through either deliberate introduction or unintentional transfer, aquatic crayfish constitute one of the most extensively dispersed creatures worldwide (Gallardo et al. 2016, Mathers et al. 2020b). The ecological impact of their polytrophic eating habits could therefore spread throughout the food chain in areas where they become successfully established (Mathers et al. 2020b). *Pacifastacus leniusculus* invasion of macroinvertebrate communities was found to have the strongest response in terms of functional richness and divergence metrics, suggesting that *P. leniusculus* can alter the types of functional niches and severe trait values. Also, it is noted that biodiversity benefits observed in control rivers were not replicated in invaded streams, possibly indicating that invasion hindered ecological improvements. (Mathers et al. 2020a). In different research by Guareschi et al. (2021), it was discovered that the invasion of macroinvertebrate populations by *Dikerogammarus haemobaphes* caused substantial decreases in functional richness and redundancy, as well as alterations in macroinvertebrate functional composition linked to voltinism and resilience. It had also found that crayfish consumed more Culicidae, *Chironomus*, Tanytarsini,

Orthocladinae taxa and the variety of macroinvertebrates was severely impacted by crayfish, however the impact was inversely associated with crayfish size (Correia and Anastacio, 2008).

Research question:

How can artificial light directly affect the ecology of a stream to reveal disruption of macroinvertebrate communities? (Perkin et al. 2011; Smith et al. 2009; Holt and Waters, 1967)

How does artificial light affect interactions between macroinvertebrates and crayfish?

How does night-time illumination alter macroinvertebrate behaviour and growth? (Perkin et al. 2014b, Wallace et al. 1997)

To what extent is the functional composition of macroinvertebrates altered by *Pacifastacus leniusculus* invasion? (Guareschi et al. 2021)

Hypothesis:

I hypothesized that

- a) ALAN will cause taxon-specific responses (dispersion pattern) in macroinvertebrate communities (Garratt et al. 2019).
- b) ALAN attracts benthic communities, changing the distribution of these insects over time.
- c) Consumption of aquatic prey by secondary consumers will increase due to ALAN impacts and changes in their diet composition (Martin et al. 2021).
- d) The morphological and functional diversity of benthic communities would respond to interactions between the presence of *Pacifastacus leniusculus* and ALAN (Mathers et al. 2020b).
- e) The combined effects of ALAN and *Pacifastacus leniusculus* will result in a reduction in the functional diversity of macroinvertebrates (Guareschi et al. 2021).

Work plan:

Following Campaioli et al. (1994), Lechthaler and Stockinger (2005), all macroinvertebrates with organic material were filtered through a sieve and macroinvertebrates were determined at the species or genus level (e.g., Ephemeroptera, Trichoptera) and at the family level (e.g., Chironomidae). Following Usseglio-Polatera et al. (2000) and Tachet et al. (2000), macroinvertebrates will be assigned to one of six diet types (depositional feeder, shredder, scraper, filter feeder, piercer, and predator). For species like Ephemeroptera trait data will be gathered and for other species like Chironomidae trait data will be gathered at the family level. The affiliation of each taxon to each group will be determined using a fuzzy coding method, which took into consideration intra-genus and intra-family variance (Chevene et al. 1994). According to Dolédec et al. (2006), affinity scores, which might vary between 0 and 3 or 0 and 5, indicated how strongly a taxon was associated with a particular characteristic category. The relative abundance of each taxon inside each stream will be multiplied by affinity score. We will be able to determine the relative abundance of each feeding group in each stream using a trait-by-stream matrix (Larsen and Ormerod 2010, Manfrin et al. 2013, Manfrin et al. 2016).

Statistical analysis:

Each statistical analysis will be based on a repeated BACI (before-after, control-impact) design and employ a variety of statistical methods, to evaluate the impact of ALAN and crayfish invasion on populations of benthic communities (Stewart-Oaten et al. 1986). A preliminary analysis will be conducted for control portions to determine the effects of regular light-dark cycles on macroinvertebrate populations. The second statistical analysis will be performed to look at sections where ALAN had exposed to benthic communities. The third statistical analysis will be conducted to determine the interaction between crayfish and benthic communities without applying ALAN. And the final analysis

will be carried out to determine the combined effect of ALAN and crayfish invasion on benthic populations. Communities in the treated and control sections will compare after permitting fresh colonization to occur during the second to sixth week. A linear mixed effects model will be created using the "lme4" package to examine how ALAN and crayfish invasion affects benthic communities (Bates et al. 2014). Likelihood ratio tests will be used to examine factors such as control, treatment, pre-application of ALAN/pre-crayfish invasion to benthic communities, post-application of ALAN/post-crayfish invasion to benthic communities, the presence of both ALAN and crayfish invasion, and compared to a simplified model (without factors) for a fully linear mixed model (Pinheiro and Bates, 1995). By using MuMIn package, the model's marginal (R^2_m) explanation of variance will be calculated (Nakagawa and Schielzeth, 2013; Barton 2009). Least squares mean will be used from the lsmeans package to perform a comparison score as relative importance for each linear mixed model with a strong interaction (Lenth, 2016). For each linear mixed model, evaluations will establish between the control and treatment streams over the same time period, as well as comparisons between the same experimental section (control/treatment) at two distinct time points (before the start of the experiment and at the end of the experiment). To facilitate comparability of potential changes due to ALAN and crayfish invasion, all functional metrics will be standardized (Guareschi et al. 2021). Visual inspection of the graphical residual distribution for normality and homogeneity of variance will be served as the basis for the validation of all models (Zuur et al. 2009). Fuzzy principal component analysis will perform on the community-level weighted mean matrix (CWM) to investigate possible variations in the functional diversity of benthic communities (Chevene et al. 1994, Bruno et al. 2019). Trying to cross the "taxon x trait" and "taxon x site" matrices will be yielded the CWM matrix, which will be contained the percentage for every trait feature from each sample location. This made it possible to detect possible changes in the functional structure of the community as well as alterations in the size or form of the functional area. (Guareschi et al. 2021).

Time schedule:

Vacation: 29th November – 17th January

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