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# SUCCESSION OF SPECIES DURING THE DEGRADATION OF MAMMAL CARCASSES IN A NORTHERN ITALY FRESHWATER ECOSYSTEM

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PhD Course in

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### RIASSUNTO

La degradazione della sostanza organica morta è uno dei processi che sta alla base del funzionamento degli ecosistemi. I corpi degli animali morti costituisco una fonte di materia ed energia che sostiene la comunità dei detritivori e dei decompositori. Questi organismi, oltre a svolgere un ruolo fondamentale per il mantenimento delle funzioni ecologiche, costituiscono riferimenti utilizzati in campo forense. La degradazione di un animale morto in un qualunque ambiente prevede 5 stadi definiti dalle caratteristiche morfologiche della carcassa che diviene un ecosistema caratterizzato da un'abbondante e differenziata fauna che si modifica nel tempo anche per effetto dei colonizzatori e del ruolo trofico che svolgono. In ambiente terrestre la successione degli organismi che porta alla completa degradazione di un animale morto è stata ampliamente studiata (cfr. per es., (Haglund & Sorg, 1997; Amendt *et al.*, 2010; Myburgh *et al.*, 2013; Sutherland *et al.*, 2013; Megyesi *et al.*, 2005; Gelderman *et al.*, 2018; Payne, 1965; Smith, 1986; Greenberg, 1991; Tomberlin & Adler, 1998)), mentre per gli ambienti acquatici le informazioni, ad oggi, sono molto scarse. In particolare, la Letteratura inerente la decomposizione di carcasse in ambiente dulciacquicolo è limitata allo studio di alcuni mammiferi (ratti, per es. (Tomberlin & Adler, 1998); conigli, per es. (Dalal *et al.*, 2020b); e maiali, per es. (Barrios & Wolff, 2011; Dalal *et al.*, 2020a). In Italia, gli unici studi sono limitati a carcasse di pesci (Fenoglio *et al.*, 2014).

Lo studio affrontato in questa Tesi è incentrato sulla definizione della fauna che si sviluppa su carcasse di maiale (Sus scrofa domesticus) poste in un ambiente dulciacquicolo Italiano e dei ruoli trofici che la caratterizzano, unitamente ad una modellizzazione che tende alla descrizione delle successioni della fauna in funzione dello stadio di degradazione e dei parametri chimico-fisici ambientali. L'utilizzo di Sus scrofa domesticus come modello animale rappresenta un elemento innovativo rispetto a precedenti pubblicazioni riguardanti decomposizioni in ambiente acquatico Italiano. La scelta è motivata dalla somiglianza fisiologica ed anatomica di questo animale con la specie umana che quindi potrebbe permettere una possibile applicazione dei risultati ottenuti in campo forense. È stata studiata la degradazione di 12 carcasse di maiale durante 4 sessioni sperimentali distribuite negli anni 2018 e 2019 (una in Primavera-Estate e una in tarda Estate-Autunno, per ciascun anno). Le carcasse sono state posizionate in 3 punti di campionamento lungo il perimetro di un piccolo stagno artificiale sito in località Piola di Susano (44°21'06.4"N 10°40'51.1"E), Comune di Palagano (MO), nell'Appennino Modenese, Emilia-Romagna, Italia, ad un'altitudine di 865 m s.l.m.. L'area superficiale è di 900 m<sup>2</sup> ca., e la profondità massima di 5 m, soggetta ad una variazione stagionale di 1 m ca.. Prima dell'immersione delle carcasse si è studiata la comunità dello stagno con dei campionamenti pre-operazionali per entrambi gli anni. Le sessioni sperimentali erano parzialmente sovrapposte dal punto di vista temporale.

Gli obiettivi principali di questa Tesi sono: (1) Identificare tassonomicamente la fauna (in particolare, la comunità macroinvertebrata) di uno stagno artificiale; tale finalità si inserisce in un più ampio piano di caratterizzazione, monitoraggio e valorizzazione delle comunità faunistiche dell'Appennino modenese; (2) progettare e verificare modelli per la descrizione qualitativa e quantitativa dei dati relativi ai taxa determinati e al loro ruolo trofico in funzione degli stadi di degradazione e di alcuni parametri ambientali; (3) identificare somiglianze e differenze rispetto ai risultati ottenuti in studi simili condotti in altre regioni del mondo (es. India, Colombia e Canada) al fine di comprendere quali aspetti possono essere generalizzati e quali sono di natura locale; (4) validare un nuovo protocollo di campionamento.

I campionamenti della fauna e la registrazione dei parametri chimico-fisici-ambientali sono stati effettuati ad intervalli regolari di tempo, differenti a seconda dello stadio di degradazione. La fauna campionata (con particolare attenzione posta ai macroinvertebrati acquatici) è stata determinata morfologicamente in laboratorio utilizzando stereomicroscopio e microscopio ottico, sulla base di chiavi dicotomiche. Ai taxa trovati è stato attribuito il ruolo trofico. Le medie dei diversi parametri chimico-fisici (conducibilità, pH, ossigeno disciolto e temperature di acqua e aria) calcolate sugli stadi sono state valutate in funzione del progredire della degradazione (quantificabile con il numero dello stadio). La relazione tra velocità di degradazione e parametri chimico-fisici dell'acqua è stata analizzata mediante regressione lineare. Sono stati calcolati gli indici di Shannon, Margalef e Pielou per i diversi stadi di degradazione delle carcasse. Al fine di determinare le possibili relazioni esistenti fra stadio di degradazione e abbondanze, ruoli trofici, numero di taxa e parametri chimico-fisici, i dati sono stati sottoposti ad analisi statistica, inclusiva di regressioni lineari, test di Shapiro (allo scopo di valutare l'andamento normale o non-normale dei dati), PCA (allo scopo di valutare ed ottenere una rappresentazione grafica della varianza dei due sets di dati) e MANOVA (allo scopo

di determinare la possibile presenza di differenze significative fra i dati raccolti, considerando come parametro da correlare anno e sessione sperimentale, punto di campionamento e stato di degradazione della carcassa).

Una ricca e diversificata fauna rappresentata per la maggior parte da artropodi è stata campionata sulle carcasse. Considerando i ruoli trofici in relazione al numero di taxa e degli individui campionati si apprezza in generale che la presenza dei detritivori (i collettori e i frammentatori) è influenzata dallo stadio. Questi infatti variano in numero di individui e taxa sulla base della disponibilità trofica che è influenzata a sua volta dallo stadio di degradazione e dalla competizione con la fauna terrestre, quando presente. Tali analisi mostrano che, sulla base dei taxa rinvenuti, vi è una differenza statisticamente significativa se si considerano le sessioni sperimentali, gli anni di campionamento e le repliche di campionamento. Inoltre emerge che 24 taxa mostravano una relazione statisticamente significativa con gli stadi di degradazione. Ponendo in relazione la velocità relativa media di degradazione delle carcasse con la temperatura media dell'acqua, si osserva che all'aumentare della temperatura la degradazione avviene più velocemente; una relazione quantitativa fra detta velocità e temperatura è determinata mediante regressione lineare. Fra i parametri chimico-fisici misurati, la temperatura sembra essere quella che maggiormente influenzava la velocità di degradazione, specialmente durante i primi stati di degradazione. Inoltre anche la stagionalità e le condizioni meteorologiche, ad esempio, le forti piogge che portano alla creazione di condizioni limite tra l'ambiente lotico e lentico, influenzano la presenza/assenza di alcuni taxa. Infatti le diverse stagioni e anni mostrano tendenze molto diverse in termini di taxa, mentre gli stati non sembrano mostrare differenze significative tra loro. I ruoli trofici sembrano essere correlati allo stadio della carcassa sulla base di come la sorgente trofica viene resa disponibile durante l'evoluzione della degradazione. La competizione da parte di organismi non acquatici (in particolare, larve di ditteri calliforidi e sarcofagidi) per la sorgente trofica rappresentata dal substrato cadaverico sembra giocare un ruolo determinante.

### ABSTRACT

The degradation of dead organic matter is one of the fundamental processes regarding the functioning of ecosystems. The bodies of dead animals constitute a source of matter and energy supporting the community of detritivores and decomposers. In addition to playing an essential role in maintaining ecological functions, these organisms are references used in the forensic field. The degradation of a dead animal in any environment involves 5 stages defined by the physical characteristics of the carcass, which becomes an ecosystem characterised by an abundant and differentiated fauna evolving over time also due to the effect of colonisers and their trophic roles. In a terrestrial environment, the succession of organisms leading to the complete degradation of a dead animal has been extensively studied (see *e.g.*, (Haglund & Sorg, 1997; Amendt *et al.*, 2010; Myburgh *et al.*, 2013; Sutherland *et al.*, 2013; Megyesi *et al.*, 2005; Gelderman *et al.*, 2018; Payne, 1965; Smith, 1986; Greenberg, 1991; Tomberlin & Adler, 1998)), whereas for aquatic environments the information, to date, is very scarce. In particular, the literature concerning the decomposition of carcasses in the freshwater environment is limited to the study of some mammals (rats, *e.g.* (Tomberlin & Adler, 1998); rabbits, e.g. (Dalal *et al.*, 2020b); and pigs, *e.g.* (Barrios & Wolff, 2011; Dalal *et al.*, 2020a). In Italy, the only studies available were conducted on fish carcasses (Fenoglio *et al.*, 2014).

The study conducted in this Thesis focuses on the definition of the fauna developing on pig carcasses (*Sus scrofa domesticus*) placed in an Italian freshwater environment and of the trophic roles that characterise it; a modelling attempting the description of the successions of the fauna as a function of the stage of degradation and of the chemical-physical environmental parameters is also proposed. The use of *Sus scrofa domesticus* as an animal model represents an innovation compared to previous publications concerning decompositions in Italian aquatic environments. The choice is motivated by the physiological and anatomical similarities between this animal and humans, which may therefore allow for a possible forensic application. The degradation of 12 pig carcasses was studied during 4 experimental sessions distributed over the years 2018 and 2019 (one in Spring-Summer and one in late Summer-Autumn, for each year). The carcasses were positioned in 3 sampling points along the perimeter of a small artificial pond located in Piola di Susano (44°21'06.4"N 10°40'51.1"E), Municipality of Palagano (MO), in the Modenese Apennines, Emilia-Romagna, Italy, at an altitude of 865 m a.s.l.. The surface area is approx. 900 m<sup>2</sup>, and the maximum depth is 5 m, undergoing a seasonal variation of approx. 1 m. Before soaking the carcasses, the pond community had been studied with pre-operational sampling both of the years. The experimental sessions were partially overlapped from a temporal point of view.

The main objectives of this Thesis are: (1) the taxonomical identification of the fauna (in particular, the macroinvertebrate community) of an artificial pond; this purpose is part of a broader plan of characterisation, monitoring and enhancement of the fauna communities of the Modena Apennines; (2) the design and testing of models for the qualitative and quantitative description of the data relating to the determined taxa and their trophic roles as a function of the degradation stages and the environmental parameters; (3) the identification of similarities and differences with respect to the results obtained in previous studies conducted in other areas of the world (*e.g.* India, Colombia and Canada), with the aim to highlight which aspects can be generalised and which are local in nature; and (4) the validation of a new sampling protocol.

The sampling of the fauna and the recording of the chemical-physical environmental parameters were carried out at regular intervals of time, different according to the stage of degradation. The sampled fauna (with particular attention paid to aquatic macroinvertebrates) was morphologically determined in the laboratory using stereomicroscopes and optical microscopes, on the basis of dichotomous keys. The trophic role was attributed to the taxa. The averages of the different chemical-physical parameters (*viz.*, conductivity, pH, dissolved oxygen and water and air temperatures) calculated for each stage were evaluated according to the progress of the degradation (quantifiable with the stage number). The relationship between degradation rate and chemical-physical parameters of water was analysed by linear regression. Shannon, Margalef and Pielou indices were calculated for the different stages of carcass degradation. In order to determine the possible relationships between the degradation stage and abundances, trophic roles, number of taxa and chemical-physical parameters, the data underwent statistical analyses, *viz.* linear regressions, Shapiro test (in order to evaluate the normal or non-normal data trend), PCA (in order to evaluate and obtain a graphical representation of the variance of the two sets of data) and MANOVA (in order to determine the possible presence of significant differences between the data collected, considering how parameter to be correlated year and experimental session, sampling point and state of degradation of the carcass).

A rich and diverse fauna, mostly represented by arthropods, was sampled on the carcasses. Considering the trophic roles in relation to the number of taxa and individuals sampled, the presence of detritivores (collectors and shredders) is generally influenced by the stage. These in fact vary in number of individuals and taxa on the basis of trophic availability, which is in turn influenced by the stage of degradation and by competition with terrestrial fauna, when present. These analyses show that, on the basis of the taxa found, there is a statistically significant difference if the experimental sessions, the sampling years and the sampling replicas are considered. Furthermore, 24 taxa show a statistically significant relationship with the degradation stages. By relating the average relative rate of degradation of carcasses with the average water temperature, as the temperature increases, degradation occurs faster; a quantitative relationship between the rate of degradation and temperature was determined by linear regression. Among the chemical-physical parameters measured, the temperature seems to be the one that most influences the rate of degradation, especially during the first states of degradation. In addition, seasonality and weather conditions, for example, heavy rains that lead to the creation of boundary conditions between the lotic and lentic environment, influence the presence / absence of some taxa. In fact, the seasons and years show very different trends in terms of taxa; the stages do not seem to show significant differences between them. The trophic roles appear to be related to the carcass stage based on the way the trophic source is provided during the evolution of degradation. The competition originated by non-aquatic organisms (in particular, calliphorid and sarcophagid dipteran larvae) for the trophic source (*i.e.* the cadaveric substrate) seems to play a determining role.

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## Chapter 1

## Introduction

All life depends on water for existence. Although 71% of our planet is covered by water, only a very small proportion is associated with the continental areas to which humans are primarily confined. Over 99% of the water associated with continents is in the form of groundwater or ice and is therefore unusable. Humans mostly depend on freshwater streams, rivers, marshes, lakes, and shallow groundwaters; we rely heavily on a rare commodity (Dodds & Whiles, 2020). Freshwater ecosystems occur on all continents and have been described and classified by a diversity of terms and definitions (Finlayson & Van der Valk, 1995; Mitsch & Gosselink, 2015; Gerbeaux et al., 2018). Among the Earth ecosystems, aquatic habitats are comprised of three types: (1) freshwater including standing waters (lentic), for example, natural container habitats such as tree holes, ponds and lakes, as well as moving water systems (lotic), for example, streams and rivers; (2) transitional communities such as estuaries or embayments and other wetlands, for example, temporary ponds; and (3) marine systems that include shorelines, inland saline lakes, and open ocean (Wallace, 2015). As Heinrich (2012) stated, carrion decomposition in aqueous environments may follow similar patterns to what has been observed in terrestrial ecosystems, but there are major differences in these processes that are fundamentally constrained to aquatic ecosystems. What is learned about such differences can be gathered from comparisons of lentic and lotic freshwater systems as well as the largest of aquatic systems, oceans, which appear to exhibit biogeochemical processes of both lentic and lotic water bodies. Thus, with water bodies of all size and type, it is a series of complex physical, chemical, and biological interactions that allow biogeochemical cycles and biotic communities to function (Schindler, 1991a), which in turn ultimately influences the role of decomposition in these systems (Wallace, 2015).

### **1.1 Physico-Chemical Parameters**

Chemistry controls physiology, biogeochemistry, behavior of pollutants, and many aspects of how organisms interact with their environment and each other. The way chemicals transform and move through the environment is an essential aspect of ecosystem function (Dodds & Whiles, 2020). In general, a variety of physico-chemical attributes influence and sustain ecological systems (EPA, 2008). While decomposition in aquatic systems may be mediated through biological mechanisms, for example, microbial-macroinvertebrate trophic interactions, it is a suite of physico-chemical parameters that influence the dominant pathway of decomposition in aquatic habitats (Merritt & Wallace, 2010). Water contains so many dissolved compounds that almost every river system in the world is unique in its combination of them (Hughes, 2019).

Natural fresh waters can be considered as a complex solution of weak concentrations of a large number of substances whose interactions include chemical dissociations. Acidity/alkalinity are master features that result from intricate chemical processes. They influence all physicochemical and biological processes taking place in aqueous solutions (coagulation, absorption, precipitation, oxidation, reduction, hydrolysis, nitrification, denitrification, aerobic and anaerobic degradation of organic matter etc.). Under natural conditions, the pH value of dilute waters is influenced by atmospheric CO<sub>2</sub> invasion/evasion affecting the ionic balance and causing pH decrease/increase. The pH level of most freshwaters lies between 6 and 9; however natural peatbogs, laden in fulvic and humic acids, tend to reach pH 4.6-5.3; similar values can be measured in anthropogenically acidified lakes. Above pH 11, no photosynthesis is possible (Sommer, 1994); values above pH 10 may result in caustic burns to fish and to invertebrates. As pH increases above 9.5, under high temperature, ammonium (NH<sub>4</sub>) turns into toxic ammonia (NH<sub>3</sub>) gas) causing seasonal fish kills and zooplankton poisoning in eutrophic freshwaters (common in temperate regions in summer and in the tropics) (Hughes, 2019). Other hydro-chemical factors, such as pH and salinity, may influence aquatic carrion decomposition indirectly by impacting the diversity of organisms that initiate the process, specifically primary and secondary decomposers such as bacteria, fungi, and invertebrates, thereby affecting ecosystem functioning in general. Unless impacted from anthropogenic sources, the normal pH for freshwater ecosystems ranges from 6.5 to 8.5 (Berezina, 1999), However, acidification of streams and rivers through anthropogenic activities has been occurring on a global scale and this process has been shown to negatively impact a number of aquatic micro- and macroorganisms essential in the decomposition of aquatic organic material including carrion (Schindler, 1991b).

Electrical conductivity (EC) is a function of the total dissolved concentration; it is the inverse of resistance that can be measured with a Wheatstone bridge. Field EC measurements are easy and reliable and EC is a very useful parameter for characterising changes in water chemistry in time/space. The ionic composition of individual water bodies reflects local geochemical patterns and/or local climatic conditions, such as spatial changes in the amount of precipitation. Understanding the processes driving freshwater ecosystems requires not only insight into the dynamics of the organisms under study, but how those organisms interact with their physical environment. For freshwater ecosystems it is water, and in particular the hydrochemistry, that drives the distribution and composition of aquatic organisms (Moss, 2018; Finlayson et al., 2018; Patten, 1990). Salinity represents the total concentrations of sodium and chloride and other cations and ions in aquatic systems and is an integral part of the biochemistry of both terrestrial and aquatic ecosystems (Dunlop et al., 2005). The impacts of salinity are known to result in significant shifts in the integrity and diversity of aquatic ecosystems (Dunlop et al., 2005). Salinity increases may cause toxic effects on the physiologies of freshwater taxa, thereby resulting in a change in diversity (Nielsen et al., 2003), which may significantly impact decomposition (Gessner et al., 2010). The time required to progress through the various stages of decomposition in aquatic systems may be as much as two times longer in the freshwater systems that have been impacted to be more brackish compared to unimpacted freshwater systems (Haefner et al., 2004; Zimmerman & Wallace, 2008).

The measurement of temperature, in or near to the water, is essential for the study of freshwater ecosystems as it controls the rate of chemical reactions; (therefore) the rate of biological processes; the equilibria of many physico-chemical processes such as the amount of dissolved oxygen; and other variables which are affected by temperature (e.g., pH and electrical conductivity), and thus need temperature correction (Hughes, 2019). Temperatures are subject to the daily schedule of sampling, season, and altitude. Usually, it is preferable to refer to historic mean temperature of the sampling area. Aquatic organisms are exposed to a thermal diversity that varies both spatially and temporally in all types of aquatic systems, and such variation can be influenced both naturally and from anthropogenic alteration (Ward & Stanford, 1982). Air temperatures can have significant effects on streams, ponds, and littoral zones of lakes (Macan & Maudsley, 1966; Brown, 1969; Smith & Lavis, 1974; Dale & Gillespie, 1977). However, morphometry (i.e., the size and shape) as well as continentality are major determinants of thermal regimes in lentic systems from lakes to oceans (Hutchinson, 1957). Temperature is a major factor affecting the life histories of those aquatic organisms intimately involved in all

stages of the decomposition of organic material from aquatic bacteria (White et al., 1991; Pomeroy & Wiebe, 2001), invertebrates (Sweeney & Vannote, 1978; Ward & Stanford, 1982; Burkepile et al., 2006) to freshwater and marine fish (Pepin, 1991). Therefore, it should be no surprise that the rate of decomposition of organic matter in lotic and lentic systems tends to increase as a function of temperature, regardless of the system or seasonal fluctuations in temperate water bodies or in more stable climates such as in tropical seas. The effect of temperature on poikilothermic organisms involved with decomposition activities is considered a dominant factor in aquatic organic matter processing (Webster & Benfield, 1986; Sorg et al., 1997; Merritt & Wallace, 2010; Tank et al., 2010). Dissolved oxygen and temperature are two of the fundamental variables that affect lake and pond ecology (Addy & Green, 1997). Dissolved oxygen is the amount of oxygen in solution and can fluctuate daily depending on the temperature, salinity, altitude, groundwater inflow, and anthropogenic activities or accidents (Addy & Green, 1997). Most aquatic organisms are intrinsically linked to respiring oxygen in solution; their survival and subsequent role in decomposition activities require oxygen. Because oxygen solubility in water is negatively correlated with temperature, certain groups of aquatic invertebrates that play substantial roles as shredding of carrion tissue may be excluded from such low oxygen environments (Merritt & Wallace, 2010). Thus, depending on the oxygen concentration in a specific habitat, the faunal community available to colonize carrion may be quite different (Hobischak, 1997).

Humidity is the amount of gaseous water (water vapour) in the atmosphere. In freshwater ecosystems it affects plant evapotranspiration; temperature by evaporative cooling; and is one of the controlling factors in the evaporation of water from water surfaces, influencing catchment water budgets. Humidity can be reported either as absolute humidity (mass per volume), specific humidity (dimensionless ratio), or relative humidity (percentage). Relative humidity is the most common measurement and should always be reported with temperature so that the dewpoint temperature (temperature at which air is fully saturated and liquid water starts to condense) can be calculated (Hughes, 2019).

### **1.2 Ecological Succession**

Ecological succession is defined as the non-seasonal, directional and continuum way of colonisation and extinction of a site on the behalf of the species populations (Begon *et al.*, 2006).

It is the predictable change in species composition over time following disturbance, ranging from regular harsh conditions associated with seasonality, through stronger disturbances such as flood or drought, to complete obliteration of species or creation of entirely new habitats. The literature differentiates between primary and secondary succession. In primary succession, disturbance removes all or most of the organisms or conditions cause creation of a new habitat. In secondary succession, disturbance removes only some of the species or substantially reduces populations (Dodds & Whiles, 2020).

Like zonation, the process of succession is generally common to all environments, both terrestrial and aquatic. The processes of species colonization and replacement drive succession (Smith & Smith, 2015). Colonization by new species increases local species richness. Species replacement typically results from competition or an inability of a species to tolerate changing environmental conditions. Species replacement over time acts to decrease species richness. During the early phases of succession, diversity increases as new species colonize the site. However, as time progresses, species become displaced, replaced. The peak in diversity during the middle stages of succession corresponds to the transition period, after the arrival of later successional species but before the decline (replacement) of early successional species. The rate of displacement is influenced by the growth rates of species involved in the succession. If growth rates are slow, the displacement process moves slowly; if growth rates are fast, displacement occurs more quickly. This observation led Michael Huston (Smith & Huston, 1987) to conclude that patterns of diversity through succession vary with environmental conditions (particularly resource availability) that directly influence the rates of organisms growth. By slowing the population growth rate of competitors that eventually displace earlier successional species, the period of coexistence is extended, and species diversity can remain high. This hypothesis predicts the highest diversity at low to intermediate levels of resource availability by extending the period of coexistence.

Disturbance can have an effect similar to that of reduced growth rates by extending the period during which species coexist. Disturbance acts reducing or eliminating as new species colonize the site. Diversity declines as autogenically changing environmental conditions and competition result in the displacement of early successional species. If the frequency of disturbance (defined by the time interval between disturbances) is high, then later successional species will never have the opportunity to colonize the site. Under this scenario, diversity remains low. In the absence of disturbance, later successional species displace earlier ones and species diversity declines. At an

intermediate frequency of disturbance, colonization can occur, but competitive displacement is held to a minimum. The pattern of high diversity at intermediate frequencies of disturbance was proposed independently by Michael Huston and by Joseph Connell (Connell & Slatyer, 1977, Connell, 1978, Smith & Huston, 1987) and is referred to as the intermediate disturbance hypothesis.

A class of serial substitutions is that of degradative sequences, which take place on a relatively short time scale, of the order of months or years. Any "package" of dead organic substance, be it the body of an animal or a plant, the remains of a snake or an arthropod, or a fecal deposit, is exploited by microorganisms or detritivorous animals. Usually different species invade and disappear from time to time, as the degradation of organic matter depletes some resources and makes others available, while the variations in the physical condition of the detritus favour first one species, then another. Ultimately, the degradative sequences end because the resource is completely metabolised and mineralised (Begon *et al.*, 2006).

In the span of less than a day, a once living animal becomes the resource upon which numerous organisms colonize and utilize to rear their offspring. With favorable abiotic conditions, such as warm temperatures and no precipitation, this carrion resource can be colonized, within minutes after death, by those species that are well adapted to locating such patchy resources. Other species arrive and within a few days, the carrion resource may host tens to hundreds of species and thousands of individuals, developing into a rich, albeit ephemeral, community (VanLaerhoven, 2015).

### **1.3 Organic Detritus and Cadaveric Ecosystem**

Detritus, defined as any source of nonliving organic matter (Swift *et al.*, 1979), is considered the basal trophic level of many food webs (Lindeman 1942; Teal 1962; Odum 1969; Moore *et al.*, 2004) and is an important component of recycling energy and nutrients in ecosystems (Swift *et al.*, 1979; Hättenschwiler *et al.*, 2005; Moore & Schindler 2008; Barton *et al.*, 2013a, b). Given this importance, the decomposition of detritus has been intensively studied for many years with reviews and empirical studies providing ample evidence that this process is fundamental to ecosystem properties through complex ecological linkages (Swift *et al.*, 1979; Hättenschwiler & Gasser 2005; Parmenter & MacMahon 2009; Gessner *et al.*, 2010). By far, the most studied portion of the detrital pool has been phototrophically derived sources of nonliving organic matter

(e.g. abscised leaves, grass, or decaying algae). Although it is vital to understand the decomposition of phototrophically derived aspects of ecosystems, there has been relatively limited research attention on the heterotrophically derived component of the detrital pool-carrion (Benbow *et al.*, 2015).

In Swift et al. (1979) carrion was considered detritus and was placed into the same compartment as any type of decaying organic matter, mostly represented by plant biomass. Others have given more ecologically relevant conceptual definitions to carrion, either as a "cadaver decomposition island" (Carter et al., 2007) or as an "ephemeral resource patch" (Doube, 1987; Finn, 2001). Carrion has historically been defined as dead and decaying flesh or as a carcass of an animal (Merriam-Webster, 2014) and has by far been most often considered as vertebrate animals. Carrion as related to its use in this thesis includes a broader representation of decomposing animal tissue (heterotrophically derived biomass) in ecology and evolution, including the decaying carcass of any once living, heterotrophic organism. Although there have been advances in understanding carrion in ecosystems, studies of vertebrate carrion in general, and ungulate carrion in particular, have provided a basis for uncovering the important but largely overlooked role of carrion in complex ecosystem structure and function (Hobbs, 1996, 2006; Melis et al., 2007; Bump et al., 2009a, b). Some of this research has been developed to understand the basic ecological and evolutionary relevance of carrion (Fuller, 1934; Payne, 1965; Schoenly & Reid, 1987; Tomberlin et al., 2011a; Barton et al., 2013a); however, a substantial portion of what is understood about these processes has come from studies with forensic (Mégnin 1894; Carter et al., 2007; Tomberlin et al., 2011b; Pechal et al., 2014) or disease significance (Miller et al., 2004; Hugh-Jones & Blackburn, 2009; Villet, 2011).

The importance of carrion to the detrital energy and nutrient foundation in ecosystems has historically been considered minimal; however, recent research has demonstrated that carrion can be significant to ecosystem processes often through indirect interactions among several trophic groups (Towne, 2000; Yang, 2004; Yang *et al.*, 2008; Bump *et al.*, 2009a; Parmenter & MacMahon 2009; Beasley *et al.*, 2012; Hawlena *et al.*, 2012; Barton *et al.*, 2013a). Barton *et al.* (2013a) provided an excellent review of carrion ecology that introduces how this discipline can be developed from a broader empirical basis from other ephemeral resources. The authors also argued effectively that carrion has significant impacts on terrestrial biodiversity and ecosystem properties through influence on soil nutrients, belowground microbial communities, plants, arthropods, and other invertebrates and vertebrates.

The majority of the published literature referring to carrion ecology focuses on vertebrate decomposition as it is these larger organism's carcasses that typically survive long enough for the processes and mechanisms to be studied. Vertebrate bodies comprise various soft and hard tissues. The soft tissues consist of organs, muscles, ligaments, tendons, and hide, whereas the hard tissues make up the skeleton and include bone, teeth, cartilage, horn, and antler (Lyman, 1994). Soft tissues will typically decay faster than hard tissues, and it is the dead and decaying tissues of the animal that are referred to as carrion. The hard tissues, especially bone, can be preserved for extended periods of time. Cartilage is harder than most soft tissues, but softer than bone, and occupies internal areas of the body that are not close to the skin surface. Horn is primarily keratin, the same protein which makes up hair, hoof sheaths, and feathers while antler resembles bone (Lyman, 1994). Teeth are a different material and are largely composed of enamel and dentine, two of the hardest skeletal tissues in the body. The chemical and macrostructural properties of hard tissues make them resilient against the normal decomposition processes that impact soft tissues. For this reason, they are often the only materials that survive in the natural environment (Forbes & Carter, 2015).

Death is a dynamic process, which can occur in a matter of seconds or can be prolonged over a period of time. Death results from the reduction of living matter through a process known as histolysis (i.e., the dissolution of organic tissues), or it can result from the accumulation of foreign substances within the body that are not conducive to the normal living process. The metabolic processes, which are necessary and present in the living, are absent in the deceased. Organisms have the capacity to self-regulate these mechanisms during life, as demonstrated by hibernation. During life, cells and tissues are sustained by highly organized chemical activities that maintain organismal functions. The chemical processes of living cells are defined by biochemical pathways in the body and associated with lipid-bound membranes and organelles (Gill-King, 1997). Following death, chemical disorder and failure of cellular metabolism occur as a result of the lack of cardiac pumping of oxygenated blood throughout the body (Clark *et al.*, 1997; Gill-King, 1997). As the environment within the body becomes more anoxic following circulatory stasis, the disorganization within the cellular chemistry leads to the common macroscopic signs of death and decomposition.

Vertebrate carrion is a nutrient-rich, ephemeral resource that is utilized by many different organisms, ranging from vertebrate and invertebrate scavengers to microbes (Janzen, 1977; Early & Goff, 1986; Braack, 1987). The organisms that consume carrion play an important ecological

role, as decomposition is vital to ecosystem function (Carter *et al.*, 2007). Without these scavengers, vital elements (especially carbon, sulfur, nitrogen, and phosphorus) that are initially trapped in primary producers (plants) and subsequently concentrated in animal tissues would not be released back into the ecosystem in a timely manner (Putman, 1978b; Stevenson & Cole, 1999; Parmenter & MacMahon, 2009).

The removal of soft tissue is not only a chemical process but also a biological process. Bacterial and insect activities are considered two of the most influential factors for carrion decomposition (Anderson & Cervenka, 2002). Arthropods are excellent agents of soft tissue removal particularly, given their prevalence in most natural environments. They hold an important ecological role as they recycle much of the biomass of carrion, making nutrients available for other organisms in the food chain. Animal remains become attractive to insects almost immediately after death; however, a succession of insects will visit the remains as the food resource modifies throughout the decomposition period (Anderson & Cervenka, 2002; Anderson, 2010; Kreitlow, 2010). Ecological succession will vary by region based on climate, intra- and interspecies dynamics, and other random occurrences (Kreitlow, 2010). The carcass, its inhabitants, and the surrounding environment are themselves an ecological community. In most ecosystems, arthropods will be present throughout the early, autolytic, putrefactive, and disintegrative processes, with some carrion beetles sustaining into the skeletonization stage.

Arthropods are attracted to carrion within minutes of death. Without invertebrate or vertebrate scavenging, bacterial decay will be the prominent degradative action and decay will occur at a substantially slower rate (Payne, 1965; Carter *et al.*, 2007; Anderson, 2010). The early-arriving insects will deposit eggs or larvae in the moist regions of the carcass, so that the remains can be used as a food source throughout larval development (Kreitlow, 2010). Diptera larval feeding assists to rupture the skin and liquefy soft tissue during the putrefactive stage (Carter *et al.*, 2007).

Rapid biomass loss is often evidenced during putrefaction as larval masses feed voraciously. Most species do not feed on bone but rather consume the dry, mummified tissue that adheres to the bones. These activities play an important role because they contribute to the cycling of recalcitrant resources.

Temperature is the most influential of the environmental factors as warm conditions encourage the proliferation of microorganisms both within the carcass and in the soil environment. Enhanced enzymatic and microbial degradation will lead to the rapid accumulation and subsequent release of gases from the carcass, which will attract invertebrate and vertebrate scavengers (Carter *et al.*, 2007). Increased larval insect and scavenging activity will assist in the rapid digestion of soft tissue, thus accelerating the process of decomposition (Mann *et al.*, 1990; Bass, 1997; Forbes & Dadour, 2010). In contrast, cold temperatures are less conducive to decomposition due to limited microbial, invertebrate, and vertebrate activity. Temperatures below freezing slow bacterial proliferation and eggs and larvae are unable to survive (Mann *et al.*, 1990). The decomposition process is inhibited, resulting in a lack of putrefactive gases to attract vertebrate scavengers. The result is the preservation of the soft tissue indefinitely or until the environment once more becomes conducive to decomposition (e.g., seasonal changes in temperature) (Rosendahl, 2010).

Moisture is equally important to the survival and proliferation of bacteria and insect larvae (Bass, 1997). Humidity and precipitation are therefore important environmental factors that can mediate these biological activities. Although insects can still be present in arid environments, a lack of moisture will result in the desiccation of fly eggs and inhibition of larval growth. The rapid loss of moisture is associated with desiccation of the soft tissue, leading to preservation by mummification.

The degree of solar radiation to which the remains are exposed will impact the location of larval masses on the carcass. When the carcass is in a shaded environment, larval masses will be visible on the surface of the carcass. However, when the carcass is situated in direct sunlight, larvae will typically source a darker environment within the body cavity (Bass, 1997). Larvae will feed on the internal tissues, leaving the skin as a protective barrier from the sunlight. The resultant effect will be an outer mummified layer of skin resembling the carcass body shape, with few, if any, tissues or organs remaining in the cavity. In addition to climatic conditions, the decomposition environment will also impact the process of soft tissue degradation.

Terrestrial animals that drown or whose remains end up in a water body will be subjected to a different decomposition environment. A similar process will also impact aquatic animals following death. Similar to soil burials, decomposition in aquatic environments is considerably slower than that on the soil surface (Rodriguez, 1997; Madea *et al.*, 2010). This reduced rate of decomposition results from cooler temperatures and differences in dissolved oxygen which impacts microbial degradation. Additionally, invertebrate scavenging is minimal in these types of environments, although scavenging by aquatic animals can still occur. As air escapes from the

lungs, the carcass will sink toward the bottom of the aquatic environment in which early *post mortem* changes will start. Once gases accumulate within the body cavity, the carcass will rise to the surface and float (Allison & Briggs, 1991; Lyman, 1994; Sorg *et al.*, 1997). Decomposition will proceed with the exposure to air, and insects or avian scavengers may be attracted to the remains at this time. Their feeding is predominantly limited to the exposed portions of the body, and their impact on the decomposition rate is noticeably reduced when compared with carcasses on a soil surface. Once the gases are released and the soft tissue is weakened by decay, the carcass will again sink (Haglund & Sorg, 2002). At this time, the soft tissue will continue to undergo degradation and disarticulation or may be preserved by adipocere formation.

The presence of a carcass can have a significant effect on the environment. The magnitude of this effect is related to carcass size (Putman, 1978a,b). Although many details of carrion decomposition have been resolved in recent years, the fundamental effect of a carcass has long been understood: the decomposition of dead animals contributes to the cycling of nutrients within and between habitats (Yang, 2004; Bump *et al.*, 2009). The carrion acts as an island of fertility within an environment and, therefore, contributes to biodiversity by acting as a habitat for species that use carcass material for food and/or shelter. Succession associated with a carcass is driven by changes in the environment (Wells & Greenberg, 1992).

### **1.4 Trophic Roles**

The idea of food chains is quite old and the melding of a set of chains into a web dates back well into the twentieth century. The web starts with a source of energy, frequently the sun, and the raw materials of carbon dioxide, water and the necessary mineral nutrients. Plants and algae are the primary producers, whether in the water or as material washed in from the land and are eaten by grazers (herbivores) or detritus feeders, and those in turn by primary then secondary consumers, and possibly further levels where production is very high (Moss, 2017).

In the carrion system, energy and nutrient transformation processes are initiated by bacterial (and other microbial) species associated with the carrion resource. These groups of organisms have been described as the epinecrotic microbial community of carrion (Pechal *et al.*, 2014b). The species of these groups begin a series of cascading community interactions spanning from intraand interspecific competition and facilitation to complex interdomain (or interkingdom) relationships within a network of organisms associated with carrion that has been defined as the necrobiome (Benbow *et al.*, 2013). This interacting, multidomain network of species associated with carrion does not operate in isolation, but rather influences and is influenced by the surrounding habitat and ecosystem in ways that scientists are only beginning to understand and mechanistically explore (Benbow *et al.*, 2015).

The classification of macroinvertebrates in freshwater is done based on the functional role of organisms rather than their size (Begon *et al.*, 2006). In 1974 Cummins identified the following categories of macroinvertebrates consumers:

- shredders, representing detritivores that feed on coarse organic matter, *i.e.* particles with a diameter greater than 1 mm; their main purpose is to fragment dead organic matter;
- collectors, feeding on fine particulate organic matter, *i.e.* with a diameter of less than 1 mm; they can be divided into collectors-gatherers (feeding on dead organic particles from macroscopic debris or sediments) and collector-filterers (sifting small particles from the water column);
- grazers-scrapers, having mouthparts able to scrape and consume the organic layer adhering to the substrate and / or consisting of algae attached to dead organic substance adsorbed to the substrate surface);
- predators (predators); and
- generalist or omnivorous.

Most orders of aquatic insects (Cummins & Klug, 1979) and other groups of invertebrates contain detritivorous organisms that consume detritus. Any benthic organism that does not exclusively specialize on macrophytes, periphyton, or predation on animals probably consumes detritus as a significant portion of its diet.

Organisms that scrape biofilms can consume a wide variety of microbial species. Scrapers, also referred to as grazers, include snails, some tadpoles, and many types of immature aquatic insects. Scrapers feed on substrata surfaces, consuming attached algae, heterotrophic components of biofilms, and associated deposited organic sediments. Scrapers can also remove heterotrophic biofilms found in groundwaters, so not all scrapers are grazers. In general, scrapers do not discriminate among individual cells because biofilms are generally complex mixtures of many microbial species (Dodds *et al.*, 2014). In general, particulate materials derived from the algal components of biofilms are more easily assimilated than is detrital material derived from the restrial plants because dead leaves and wood tend to be recalcitrant and nutrient-poor. The

nutritional ecology of scrapers can be complex because of differential assimilation of ingested materials (Dodds & Whiles, 2020).

Shredders, including many crustacean and insect species in freshwater habitats, feed on coarse detritus such as wood and leaves. In doing so, they contribute significantly to a key ecosystem function, the degradation of organic materials. Shredders tear leaf and wood material up before ingesting it. This increases rates of microbial degradation of cellulose in their guts and stimulates breakdown of the remaining material by increasing surface area for further microbial colonization. Because they feed on relatively low-quality material, shredders have high ingestion rates, which facilitate breakdown of coarse organic materials and generation of fine particulates (Wallace & Webster, 1996). Feeding activities of shredders generate small particles that feed a variety of collector (Wagner, 1991).

Freshwater organisms that consume relatively small (<1 mm diameter), deposited organic particles from the surface of substrata are termed collector-gatherers. Many of these can eat entire microbial assemblages or pick out particles that are rich in cellular material and are more biologically active. Oligochaetes and chironomids process large quantities of sediments and assimilate the microbial component and any other digestible organic materials in the sediments. As with shredders, this group receives much of its nutrition from microbes associated with the detritus they consume (Eller *et al.*, 2005; Eller *et al.*, 2007). Collector-gatherers can contribute significantly to turnover rates of organic particles (Fisher & Gray, 1983). Collector-gatherers such as oligochaetes and chironomids are often the most abundant macroscopic organisms in many shallow water habitats (Wallace & Webster, 1996), and thus their conversion of detritus and microbial biomass to invertebrate biomass is significant for larger consumers such as fishes and waterfowl, which rely heavily on these groups for food (Dodds & Whiles, 2020).

Organisms can filter particles from water and maintain continuous ingestion. Filter feeders associated with benthic habitats, such as blackfly larvae, freshwater mussels, and net spinning caddisfly larvae can also feed on microbial particles and strongly influence energy flow and nutrient cycling in freshwater habitats. Passive filter feeders, those that let the moving water do the work for them, often dominate filter-feeding assemblages in flowing waters (Dodds & Whiles, 2020).

Omnivores (or generalist) consume materials from multiple trophic levels of the food web or multiple food web compartments (e.g., detritus and algae). Many, if not most, aquatic animals

eat more than one type of food during their life span. Some organisms, such as crayfish, can be predators, herbivores, and detritivores within a life stage. The prevalence of omnivory greatly complicates food web and functional analyses. It can be difficult to determine the trophic position of organisms given omnivory and technical difficulties associated with determination of ingestion and assimilation (Dodds & Whiles, 2020).

### **1.5 Terrestrial vs Aquatic Environment**

Decomposition in terrestrial environments has been studied very well by several researchers on different animal models (Haglund & Sorg, 1997; Amendt et al., 2010; Myburgh et al., 2013; Sutherland et al., 2013). The application of these results has led to successful investigations of many cases on the land (Megyesi et al., 2005; Gelderman et al., 2018). Along with that, the association of specific insect species with various decomposition stages can provide valuable information about the time since death in terrestrial habitats (Payne, 1965; Smith, 1986; Greenberg, 1991; Tomberlin & Adler, 1998). Studying decomposition in an aquatic environment is a very specific field that has minimal research to its credit (Haglund & Sorg, 2002; Heaton et al., 2010; van Daalen et al., 2017). Determining the postmortem index of submerged carcasses is much more difficult than conducting such studies on land. There is a general paucity of researchers who have studied the pattern of decomposition from entomological point of view in the aquatic habitats using different animal models (Haglund & Sorg, 2002; Payne, 1972; Sorg et al., 1997; Hobischak & Anderson, 2002; Anderson & Hobischak, 2004; Alley, 2007; Marhoff et al., 2016). Thus, geographical-region-based studies of the decomposition pattern of the submerged cadavers is the need of the hour. Aquatic insects are extensively used in ecologyrelated research in aquatic systems because of their all-over circulation, respective richness, easy collection, and large size (Merritt et al., 2017). In the aquatic environment, the entomofauna is quite different from the fauna in the terrestrial environment (Haskell et al., 1989; Hobischak, 1997; Keiper et al., 1997; Barrios & Wolff, 2011). No precise predictable succession pattern has been observed in aquatic insect species on submerged carcasses. However, a few studies on the association of aquatic insect species with decomposition of a pig carcass in an aquatic environment exist (Hobischak & Anderson, 2002; Anderson & Hobischak, 2004; Alley, 2007; Marhoff et al., 2016; Ayers, 2010).

An important distinction between terrestrial and aquatic ecosystems in the cycling of carrion resources from both spatial and temporal perspectives is twofold (Stergiou & Browman, 2005). First, the processing of carrion is directly related to the specific properties of water and air, such as narrow temperature ranges, relative density of water, and the inherent three-dimensional nature of aquatic systems that can influence the movement of vertebrate carrion laterally in the water column via wave action, upwelling to the surface, or by sinking to the benthos (Beasley et al., 2012). This relationship may cause the rate of carrion decomposition to be slower in water than on land in some instances, whereas under different circumstances, it may increase the rate. For example, water movement associated with sinking, moving laterally through current and wave action or floating due to bacterial activity resulting in gas accumulation in the gut, facilitates carrion across the aquatic landscape, thus, increasing exposure to scavengers (Britton & Moore, 1994; Merritt & Wallace, 2010; Beasley et al., 2012). Second, carrion indicator species are ecologically characterized in terrestrial systems according to having coevolved to feed on carrion and their trophic relationship with decomposing carcasses (Merritt & Wallace, 2010), whereas in freshwater and marine ecosystems, there is a dichotomy in carrion decomposition with regard to the fauna that utilize it. For example, in freshwater systems, there does not appear to be an evolved "normal" carrion community from a taxonomic standpoint (Fenoglio et al., 2014). Some researchers have also found an association of aquatic insects (orders Ephemeroptera, Coleoptera and Diptera etc.) with the decomposition stages in an aquatic environment (Vance et al., 1995; Keiper et al., 1997). Unfortunately, these insects have been neglected in forensic studies as there did not seem to be a specific aquatic entomofauna that could be associated with decomposition (Haskell et al., 1989). Moreover, a predictable succession pattern of aquatic insects on submerged carcase was not reported until 1997 (Keiper et al., 1997). This has posed a challenge in determining the PMSI of submerged bodies when compared to land. It can also be attributed to a general lack of experimental research in aqueous environments (Sorg et al., 1997; Haglund & Sorg, 2002) due convenience concern, practical restraints, ethical issues regarding the selection of an animal model for research and environmental concerns regarding contamination of water bodies. Other factors that can affect the decomposition of a carcase in an aquatic environment may include vertebrate scavenging (Early & Goff, 1986; Lee & Marzuki, 1993), size of the carcasses (Simmons et al., 2010; Sutherland et al., 2013) and physiological characteristics of the body (Hobischak, 1997; Arnaldos et al., 2004; Chin et al., 2007; Sutherland et al., 2013). Rather, the carrion arthropod fauna in freshwater may be better considered using a functional feeding group (FFG) approach. This approach classifies aquatic invertebrates based on sets of morphological and behavioral adaptations evolved to utilize their basic nutritional resources; in this case, a carcass or algal and fungal matter growing on that carcass (Cummins & Klug, 1979; Minshall *et al.*, 1985; Merritt & Cummins, 2006; Merritt & Wallace, 2010).

In the aquatic environment, the first phase of the degradation of the dead organic substance consists in the leaching of the soluble organic compounds, this phase is mainly abiotic. The soluble organic compounds are also degraded by the first colonizers (bacteria and fungi) who mainly use the simpler substances such as amino acids and sugars, which are liberally diffusible. They act as true pioneers in the degradation of dead organic matter and their activity is mainly determined by the metabolisms of saccharide compounds and is strongly influenced by aeration. When oxygen is freely available, sugars are metabolized into carbon dioxide and water by the growing microorganisms. Under anaerobic conditions, fermentations produce a less efficient degradation of these compounds to by-products such as alcohols and organic acids that modify the nature of the environment for subsequent colonizers. Individual species of decomposing microorganisms are not biochemically very versatile and most of them are capable of using only a limited number of substrates (Begon et al., 2006). It is in fact the diversity of species involved in these processes that allows the decomposition of structurally and chemically complex tissues in the dead body of a plant or animal organism. The second phase of the degradation of the dead organic substance is the fragmentation carried out mainly by detritivorous organisms that process the organic substance and reduce it into smaller and partially digested particles making them more easily accessible to decomposing microorganisms (fungi and bacteria) allowing an increase in speed of the process. The detritivores are made up of numerous organisms including the macroinvertebrates object of this study. The decomposed nutrients are then mineralized, or converted from the organic to the inorganic form to be reused by autotrophic organisms through the process called immobilization which will lead to the formation of new organic substance. Consequently, a given nutrient molecule can subsequently be immobilized and mineralized over a series of cycles. The process of transforming the dead organic substance into inorganic substance is long and complex, the first colonizers tend to use soluble substances, mainly amino acids and sugars, which are freely diffusible, acting as true pioneers of the dead substance, whose activity is dominated by the metabolism of saccharide compounds and is strongly influenced by aeration. When oxygen is freely available, sugars are metabolized into carbon dioxide by growing microorganisms. In anaerobic conditions, fermentations produce a less efficient degradation of these compounds to by-products such as alcohols and organic acids that modify the nature of the environment for subsequent colonizers. Individual species of decomposing microorganisms are not biochemically very versatile, most of them are capable of using only a limited number of substrates. It is in fact the diversity of the species involved that allows the decomposition of structurally and chemically complex tissues in the dead body of an animal. A fundamental role in this process is given by invertebrates, which are mainly responsible for the initial fragmentation of plant or animal residues (Begon *et al.*, 2006).

### **1.6 Decomposition Mechanisms and Stages**

In freshwater environments, detrital biomass has variable dimensions, from coarse fragments (settling on the bottom) to microscopic particles (suspended on the surface). Boling *et al.* (1975) distinguish four fundamental dimensional classes of detrital biomass:

- CPOM (Coarse Particulate Organic Matter) coarse organic matter larger than 16 mm represented by whole leaves, branches and bark;
- LPOM (Large Particulate Organic Matter) medium coarse organic matter between 16 mm and 1 mm represented by fragments of leaves, seeds and shoots;
- FPOM (Fine Particulate Organic Matter) organic matter of fine particulate between 1 mm and 0.45 μm represented by small fragments of leaves and feces of detritivores;
- DOM (Dissolved Particulate Organic Matter) dissolved organic matter less than 0.45 µm, represented by water-soluble organic substance lost during the leaching of detritus.

The metabolism of the first 3 classes takes place mainly in the benthic areas, while the metabolism of the DOM takes place mainly along the water column.

Decomposition is the process through which the energy contained in the dead organic matter is used and represents the first phase of the mineralization process of the organic debris, through which this matter is reduced into smaller dimensional classes and its inorganic constituents. It is achieved through the interaction between microorganisms, decomposing organisms, their predators and abiotic characteristics of the ecosystem (Mancinelli *et al.*, 2005).

A first phase of the decomposition processes of dead organic matter includes cellar lysis, which occurs with the senescence and death of tissues and subsequently, the leaching of soluble materials when the dead organic matter comes into contact with the aquatic medium. The leaching phase can be considered completed within the first 72 hours of immersion in water and the loss of organic matter varies in relationship to the type and chemical composition of the tissues as well as the importance of cell lysis processes. This phase is essentially of a chemical

type and is represented by the loss of water-soluble substances which can also represent 20-40% of the initial weight (Robinson & Jolidon, 2005).

The next phase involves biological decomposition by microorganisms, bacteria, microfungi and detritivorous organisms. During bacterial and fungal colonisation, enzymes are produced within the tissues that facilitate the molecular breakdown of soluble substances, such as amino acids and sugars. In particular physico-chemical conditions or in the presence of significant quantities of debris, both in the aquatic and terrestrial environment, conditions of anaerobiosis generally arise because the organisms present use oxygen much faster than it can spread in the medium. Not only bacteria and fungi colonize the decaying debris, but also invertebrates that feed on the dead substance. The latter are largely general consumers, ie they feed on both the detritus and the populations of microorganisms that colonise the detritus (Robinson & Jolidon, 2005).

In lentic and lotic systems the processes of degradation and utilization of particulate debris were observed to be more intense or actively persistent in postponed seasonal periods with respect to the temporality of debris entries. It has also been assumed that a temporal gradualness in the use of detritus acts as a control over primary lake production by influencing the release rates of nutrients from the debris itself. Following leaching, the debris undergoes the actual degradation of the tissues, which involves a transformation from the largest to the smallest dimensional class, until all the organic matter is oxidised to  $CO_2$ . In each step a part of the organic matter is stored as animal biomass and a part is transformed directly into  $CO_2$ . Decomposition has two main characteristics: the weight loss of the particulate plant material and its fragmentation into lower dimensional classes (Alemanno *et al.*, 2007; Bärlocher, 2005; Sangiorgio *et al.*, 2007; Siligardi *et al.*, 2003).

In order to understand how the biota changes during carrion decomposition, it is critical to understand the stages of decomposition that an animal carcass progresses. Therefore, the stages of decomposition for carrion or corpses in terrestrial settings have been principally characterized in the forensic literature (Payne, 1965; Smith, 1986; Anderson & VanLaerhoven, 1996). Since the Payne and King (1972) treatise of pig decomposition described in six stages in water, more recent work has modified this description by reducing the number of stages as well as the duration of each stage, the carcass condition, and whether aquatic arthropods are present or absent in freshwater, estuarine, or marine habitats (Vance *et al.*, 1995; Minikawa, 1997; Tomberlin & Adler, 1998; Hobischak & Anderson, 2002; Haefner *et al.*, 2004). Due to the influence of terrestrial insects on floating carcasses and the difficulty distinguishing between the two stages of decay as described by Payne and King (1972), the number of stages were reduced

from six to five to include: (1) submerged fresh, (2) early floating, (3) early floating decay, (4) advanced floating decay, and (5) sunken remains (Merritt & Wallace, 2010).

1) First stage (submerged fresh stage): it begins with the deposition of the carcass in the water and ends with the flotation of the same. The carcass remains submerged and there is still no sign of decomposition. (Fig. 1a).

2) Second stage (early floating stage): the carcass is pushed to the surface due to the gases produced by the anaerobic bacterial activity within it. The carcass appears swollen, mostly at the level of the abdomen and an algal patina and a large larval mass may be visible. (Fig. 1b).

3) Third stage (floating decay stage): in this phase the carcass deflates and the deterioration of the body begins to be recognizable through desquamation, loss of muscle mass, deterioration of soft tissues and disarticulation of the hind limbs. The animal is still recognizable in its shape. (Fig. 1c).

4) Fourth stage (advance floating decay stage): it is characterized by greater decomposition as bones such as ribs or limb bones are exposed, the skull disarticles and there is bone loss. The animal is no longer recognizable as such. (Fig. 1d).

5) Fifth stage (sunken remains stage): the carcass is no longer visible on the surface of the water. Inside the cage, only a few bones and flaps of skin remain, which settle on the bottom (Fig. 1e).













(c)



(d)

(e)

**Fig. 1** – Carcass degradation stages: (a) fresh submerged stage, (b) early floating stage, (c) floating decay stage, (d) advanced floating decay stage, (e) sunken remains stage. For panels (b), (c) and (d), photo of the cage containing the carcass soaked in water (left) and photo of the carcass extracted from the water (right).

The succession fauna on carrion in freshwater systems often consists primarily of crayfish (Decapoda: Cambaridae) and other omnivores, whereas shredders and some aquatic predators also engage in scavenging by removing small bits of tissue from the carcass (Wallace *et al.*, 2008; Merritt & Wallace, 2010). Shredders play a more important role in pool habitats than riffles, but for the most part occur on carrion throughout all stages of decomposition. Collector filterers, such as the larvae of net-spinning caddis-flies and black flies (Diptera: Simuliidae) predominate in riffle habitats (Merritt & Wallace, 2010). Interestingly, scraper mayflies (Ephemeroptera: Heptageniidae) were reported to be present during the submerged fresh to floating decay stages presumably grazing on the microbial matrix that included a substantial algal component (Haefner *et al.*, 2004; Zimmerman & Wallace, 2008). The primary issue in associating aquatic invertebrate taxa with a particular stage in carrion decomposition is that it has

been thought that aquatic invertebrates have not evolved as purely sarcophagous feeders (Haskell *et al.*, 1989; Fenoglio *et al.*, 2014).

The degradation of the organic substance inevitably involves a loss of mass of the considered system. The model most used to describe decomposition dynamics considers the mass loss of debris over time and is of a negative exponential type (Olson, 1963):

$$m(t)=m_0\,\mathrm{e}^{-k\,t},$$

where  $m_0$  represents the initial dry mass, m(t) the dry mass of the debris at time t, k the decomposition rate (expressed in days<sup>-1</sup>) and t the time of exposure to decomposition processes. This model is developed for the decomposition of dead organic vegetal matter, under the assumption that the mass loss is constant over time with respect to the remaining amount of matter.

The variables that influence the rate of decomposition processes are divided into internal and external. For the debris coming from the litter of terrestrial environments adjacent to the aquatic systems, a classification of the decay dynamics has been proposed, based on the values of the decomposition rate k; 3 classes are identified:

- slow decomposition,  $k < 0.005 \text{ d}^{-1}$ ;  $t_{90} > 460 \text{ d}$ ;
- intermediate decomposition 0.005  $d^{-1} < k < 0.010 d^{-1}$ ; 230  $d < t_{90} < 461 d$ ;
- fast decomposition,  $k > 0.010 \text{ d}^{-1}$ ;  $t_{90} < 230 \text{ d}$ ;

where  $t_{90}$  indicates the time needed to decompose the 90% of the initial mass (Petersen & Cummins, 1974). The different rates depend on:

- structural factors of the foliar substrates, different according to the taxonomic group,
- ecological factors, such as the presence or absence of detritivores and decomposers, and
- physico-chemical factors, such as dissolved oxygen and temperature.

The analysis of the debris decomposition process as an environmental indicator plays a central role in understanding the functioning of an ecosystem. In fact, the evolution of this ecosystem process is influenced by both abiotic and biotic characteristics of the system, mainly by physico-chemical factors and by the characteristics of the detritivorous communities (Mancinelli *et al.*, 2005, Sangiorgio *et al.*, 2007; Petersen & Cummins, 1974). Natural or anthropogenic modifications, which affect environmental parameters, have repercussions on the ecosystemic

process of decomposition. The study of this process is therefore also a useful tool for assessing the health status of an ecosystem (Pope *et al.*, 1999).

### **1.7 Forensic Applications**

Bridging the intimate relationship between carrion and terrestrial insects with criminal and civil investigations has been facilitated by studies of their life histories and basic ecology (Keiper & Casamata, 2001). Terrestrial insects and their arthropod relatives play a diverse role in such investigations, and the expanding body of knowledge over the past decade has elucidated the effects of temperature on development, behavioral, and dispersal activities, thus providing a firm foundation that is crucial to their application in death scene investigations. To a degree, similar applications of benthic ecology to forensic investigations are becoming more frequent in estimating a PMSI (Post-Mortem Submersion Interval), that is, the time period from corpse submersion in an aquatic ecosystem to the moment of discovery (Keiper & Casamata, 2001). There are several candidates of evidence that can be used to estimate a PMSI: (1) algal growth and succession (Haefner et al., 2004; Zimmerman & Wallace, 2008), (2) adipocere formation (Yan et al., 2001); (3) larval insect activity (Haskell et al., 1989; Hobischak, 1997; Wallace & Merritt, 2008; Myskowiak et al., 2010); and (4) bacterial communities (Benbow et al., 2015). However, there is a paucity of information for using aquatic organisms in PMSI estimates, most probably because there are few evolved necrophagous aquatic insects (Chaloner et al., 2002), and there are few studies that have examined aquatic invertebrate colonization of carrion or corpses (Merritt & Wallace, 2010).

Research efforts addressing the use of aquatic invertebrates to estimate a PMSI have expanded since early studies that focused on developing sampling devices to improve collections of aquatic insect evidence (Vance *et al.*, 1995). Recent statistical modelling research has focused on the modification of unique multivariate approaches (e.g., Wagner's parsimony method typically used in cladistic phylogenetic analyses) to classify different crime scene characteristics and compare them to the collected invertebrates from corpses in order to isolate pertinent bio-indicators and improve accuracy in PMSI estimates (Myskowiak *et al.*, 2010). Recent empirical work has included the identification of indicator taxa (e.g., caddisflies and crayfish) in actual case work that may provide important life history connections to PMSI estimates. The application of Functional Feeding Groups (FFG) classification approaches used in detritus processing may be used to develop the necessary associations between aquatic insect succession and the stage of

decomposition for statistical models (Wallace *et al.*, 2008; Merritt & Wallace, 2010). Forensic entomology in aquatic environments is a rather new concept in Italy, where the estimation of the PMSI using aquatic insects associated with drowned bodies has not been explored yet. On the other hand, the frequency of finding human corpses in an aquatic environment with entomological evidence on them has increased manifold (Heaton *et al.*, 2010; Dalal *et al.*, 2017). In many instances, the dead bodies are found in the aquatic habitats because of suicide or when they are illicitly dumped after a homicide (Lunetta *et al.*, 2004). Ironically, the insect evidence goes unnoticed because of deficiency in studies that could provide strength to scientific investigation.

### **1.8 Purpose of the Thesis**

In this thesis, the entomological successional sequences on carcasses of pigs (*Sus scrofa domesticus*) during their degradation in a freshwater lenthic environment consisting of an artificial pond located in the Modenese Appenines (Emilia-Romagna, Italy) were studied, during four experimental sessions over two years (spring-summer and late summer-autumn, 2018 and 2019).

The main objectives of this thesis are summarised as follows:

- Taxonomical identification of the fauna community (in particular, the macroinvertebrates) of an artificial pond; this purpose is part of a broader plan of characterisation, monitoring and enhancement of the fauna communities of the Modena Apennines;
- Design and verification of models for the qualitative and quantitative description of the data relating to the determined taxa and their trophic roles as a function of the degradation stages and some environmental parameters;
- Identification of similarities and differences with respect to the results obtained in similar Literature studies carried out in other regions of the world (e.g., India, Colombia and Canada) in order to understand which aspects may be generalised and which are local in nature;
- Validation of a newly designed sampling protocol in reference to the one developed in the Literature.

To date, in Italy, no experiments of this type have ever been carried out using the pig as a model. In addition to an ecological value, the experiment and the derived models are of applicative interest, in particular in the field of forensic entomology.
# Chapter 2

# Materials and Methods

The experimental activity of the present Thesis consists of two parts, *viz*. field samplings and laboratory activities, which took place over a two-year period, *viz*. 2018 and 2019.

## 2.1 Study Area

The experiment was conducted in a small artificial pond (surface area of 900 m<sup>2</sup> *ca.*, max depth of 5 m; 44°21'06.4"N 10°40'51.1"E) within a private land, located in Piola di Susano (865 m a.s.l.), Municipality of Palagano (MO), in the Modenese Apennines, Emilia-Romagna, Italy. Passing from spring to summer, the water level can retreat by about 1 m in depth. The area is characterised by a mountain environment (Fig. 2.1(a)).

The private land hosts two more ponds; the three water basins are close to each and connected by a stream fed by water coming from the upstream pond, whose water supply very likely derives from rains and snow thaw; this pond presents an inflow for most of the year. The upstream and most easily accessible pond was chosen for the experiment. On the basis of the environmental characteristics, three points of sampling were chosen along the perimeter of the lake, namely P1, P2 and P3 (Fig. 2.1(b)). P1 is located on the east side of the lake, in correspondence of the inflow, in a shaded area, rich in a woody-vegetation that enriches the bottom with plant decomposing allochthonous matter. P2 is located on the south side of the pond, in an area exposed to the sun; it has a silty texture and the arboreal vegetation is less represented. P3 is located on the north side of the perimeter, is characterised by perennial herb (*Mentha aquatica* L., 1753), typically associated with permanently wet habitats adjacent to open water, often partially or wholly submerged and arboreal and shrub vegetation that guarantees shade for most of the morning, the period in which the sampling was carried out.

The shore of the pond is quite varied and offers the conditions for the establishment of rich communities in different points of the pond; in general, the most represented macroinvertebrates' taxonomic groups are Insecta and Clitellata, both in terms of number of individuals and number of species (see Appendix A). Amphibians are well represented within vertebrates community by *Bufo bufo*; moreover, for recreational and angling purposes, specimens belonging to 5 species of bony fish have been introduced into the pond (*viz., Carassius auratus, C. carassius, Cyprinus carpio, Silurus glanis* and *Lepomis gibbosus*).

## 2.2 Pre-Operational Sampling

In 2018, before the sampling activities, the permits required by law were issued by land owner, the Municipality of Palagano, AUSL (Local Health Unit Company) and Arpae (regional prevention agency, environment and energy of Emilia-Romagna).

On the 25<sup>th</sup> of April, 2018, the pre-operational sampling was carried out for each of the points of sampling, in order to study the macroinvertebrate assemblage of the pond before the positioning of the carcasses and therefore the study of their degradation. The sampling was carried out by means of a Suber-type hand net with 21 meshes / cm<sup>2</sup> (aperture of standard size for the sampling of macroinvertebrates), equipped with a blade at the mouth that allows to collect both the substrate and any vegetation anchored to the bottom. The debris withdrawn in a ~3-liter volume is then washed and filtered by means of a sieve having a mesh with a lumen of 0.5 mm<sup>2</sup>. The samples were immediately fixed in ethyl alcohol at 95 ° to be carried to the laboratory later. Through the use of a multiparametric probe (Hanna instruments HI98194, HI98195, HI98196), the physical and chemical-physical parameters were measured in the field: air and water temperature, pH, conductivity and dissolved oxygen (in percentage and in ppm).



(a)



(b)

Fig. 2.1 - Susano area. (a) The pond hosting the study is marked in yellow. (b) Zoom-in of the pond. Points of sampling are depicted in yellow.

Once the samples were carried to the laboratory, the 95° ethyl alcohol was replaced by 75° ethyl alcohol (preservative). Subsequently we proceeded with sorting, an operation which consists of the observation of small quantities of sample through a stereomicroscope (Leica Wild M3B; magnification  $6,4 \times \div 40 \times$ ), in order to separate the animals (object of study) from the sediments. During the sorting, a first rough subdivision of the main taxa found was carried out; the animals thus separated were placed inside test tubes containing 75° ethyl alcohol and labelled with the following data: place, point of sampling and date of sampling and taxonomic group. Once the sorting operation was completed, the taxonomic identification followed by means of dichotomous keys (*vide postea*); the stereomicroscope (Leica Wild M3B; magnification  $6,4 \times \div 40 \times$ ) was used for this purpose. For some taxonomic groups, the morphological characters required by the dichotomous keys were too minute; we therefore proceeded with the preparation of the sampling for optical microscope observation (Zeiss Axioscope 50 upright trinocular Fluorescence Phase and Darkfield Research Routine Microscope w/ Condenser; magnification  $50 \times \div 500 \times$ ). The preparation took place using Faure's liquid, a mounting medium for routine studies.

On the 18<sup>th</sup> of April, 2019, a second pre-operational sampling was carried out for the experimental activity of the second year (2019), following the same operations and collecting samples as for 2018.

## **2.3 Model Species**

Pigs (*Sus scrofa domesticus*) were used as a human proxy for the experiments, because they have been found very similar to humans being omnivorous (Catts & Goff, 1992; Goff, 1993) and has anatomical, genetic and physiological characteristics that make it similar to humans (Meurens, 2012; Matuszewski *et al.*, 2020), so the results obtained could have a forensic application. This animal is therefore an excellent model. Moreover, it is cheap, easily available and relatively manageable; unlike other model species such as primates, it is not subject to "attention" by animal welfare associations (Barone & Guarda, 2017).

Twelve pigs were used (one per point of sampling, for two experimental sessions, for both sampling years) with a mass between 7700 and 26400 g (see Tab. 2.3.1).

		2018	2019				
	S1	S2	S1	S2			
	(13/05 - 01/09)	(08/08 - 17/10)	(02/05 - 13/08)	(16/07 - 03/09)			
– D1	F	М	М	М			
	17500 - 2180 g	24800 - 2194 g	7700 - 729 g	10150 - 756 g			
D1	М	F	Μ	F			
Γ <u>2</u>	19500 - 1375 g	26400 - 3436 g	13350 - 1049 g	17750 - 1747 g			
Р3	М	М	F	М			
	18600 - 1237 g	10200 - 877 g	9150 - 853 g	9100 - 618 g			

**Tab. 2.3.1** - Sex (F: female; M: male) and mass of the different *Sus scrofa domesticus* carcasses used during the experiment over the two years, for the 4 experimental sessions (S) and the different points of sampling (P).

## 2.4 Field Activities

The field activity took place in the years 2018 and 2019 in two partially overlapping time periods: one in spring-summer (from 13/05/2018 to 01/09/2018 and from 02/05/2019 to 13/08/2019) and one in summer-early autumn (from 08/08/2018 to 17/10/2018 and from 16/07/2019 to 03/09/2019). The two periods will be hereafter called "session one" (S1) and "session two" (S2), respectively. All samplings were carried out in a time slot between 9:30 and 13:30.

The designed protocol of sampling is adopted from Barrios and Wolff (2011) and subsequently modified, as described in the following.

(77 cm  $\times$  82 cm  $\times$  122 cm) metal cages were used. A (1.5 cm  $\times$  1.5 cm) mesh was applied to the whole cage's surface. Every twenty meshes *ca.*, two adjacent meshes were joined together in order to obtain a (1.5 cm  $\times$  3.0 cm) wide row. The cages thus arranged allowed the passage of macroinvertebrates and small vertebrates, but prevented as much as possible bone loss and protected the carcass from medium to large vertebrates.

At the beginning of the experiment, the cages containing one pig carcass each were soaked into water and linked by ropes to riparian vegetation of the bank, to prevent them from sinking, being

carried away by the current or moved by the wind. The cages were positioned near the shore in such a way that always about 2/5 of the height of the cage emerged from water.

The sampling was carried out every two days in the first and second stages of carcass degradation, every four days from the third to the fourth stage, and about once a week from the fifth stage until the end of the experiment. The sampled material was transported to the laboratory and subsequently analysed.

At each sampling, each cage was withdrawn from water and transported to the pond's shore, where a tank had been placed. During the transportation, a (80 cm  $\times$  85 cm) wide mouthed net was placed underneath the cage to minimise the loss of any percolating material from the cage and carcass. For each carcass, with the use of tweezers and sometimes a spoon, 3 samplings were carried out from 3 different districts, *viz.* net, carcass and tank. The collected material was fixed in 75 ° ethyl alcohol inside sampling tubes labelled with the date, place, point and district of sampling. When present, adult terrestrial arthropods were captured by means of entomological net and aspirator and placed in labelled test tubes containing 75 ° ethyl alcohol. When present, larvae and eggs of terrestrial diptera were also collected from the carcass and then placed in jars (labelled with the date, place and point of sampling) containing fresh pig liver, so to breed them in the laboratory and identify the species once the imaginal stage was reached.

A first count and identification of taxa were carried out on site. Once the sampling was completed, the cages were repositioned into the pond until the next sampling. At the end of fifth stage, the remains of the carcass were transferred to buckets containing 95  $^{\circ}$  ethyl alcohol, labelled and carried to the laboratory for further analysis.

Each sampling was accompanied by photographs as required by the protocol, respectively of the station, of the various districts and other relevant details. The data collected during the sampling (date, time, weather, chemical-physical and sampled organisms) were recorded on a field sheet (Appendix D).

A multiparametric probe (Hanna instruments HI98194, HI98195, HI98196) was used to measure pH, dissolved oxygen (in percentage and in ppm) and conductivity. The temperature of the air, water and, as far as possible, the temperature of the carcass and the larval mass were measured using a thermometer equipped with a probe. Humidity and the wind speed and direction were also recorded by means of a standard hygrometer, an anemometer and a compass, respectively.

#### 2.5 Laboratory Activities

The collected samples were carried to the laboratory to be sorted and identified. The first phase (sorting) consisted in the observation of the material under the stereoscope to separate animals from sediments. In this initial phase, the collected specimens were also divided at the taxonomic rank of order or class. The samples were stored in 75 ° ethyl alcohol in test tubes labelled with taxon, date, point and district of sampling. In the second phase (taxonomic identification), most of the samples were morphologically identified at species or genus level with the help of dichotomous keys. For some taxonomic groups whose morphological characters were too minute to be appreciated with the aid of a stereomicroscope (Leica Wild M3B; magnification  $6,4 \times \div 40 \times$ ), preparing slides for optical microscopy (of the whole organism or parts of it) using Faure's liquid was necessary.

The used dichotomous keys were: Belfiore, 1983 (Ephemeroptera); Bellmann, 2013 (Odonata); Campaioli *et al.*, 1994; Campaioli *et al.*, 1999 (aquatic macroinvertebrates); Carchini, 1983 (Odonata); Castagnolo *et al.*, 1980 (Bivalvia); Cicolani & Di Sabatino, 2014 (Aquatic mites); Consiglio, 1980 (Plecoptera); Ferrarese, 1983 (Chironomidae, Tanipodine); Ferrarese & Rossaro, 1981 (Diptera: Chironomidae); Fochetti & De Figueroa, 2008 (Plecoptera); Franciscolo, 1979 (Coleoptera: Haliplidae, Gyrinidae and Dytiscidae); Girod, 1980 (Gastropoda); Giusti & Pezzoli, 1980 (Gastropoda); Lencioni *et al.*, 2007 (Chironomidae); Lencioni *et al.*, 2013 (Oligochaeta); Minelli, 1977 (Hirudinea); Moretti, 1983 (Trichoptera); Nocentini, 1985 (Chironomidae, Chironominae); Pirisinu, 1981 (Coleoptera: Palpicorni); Rivosecchi, 1984 (Diptera); Rivosecchi, 1978 (Diptera: Simuliidae); Romi *et al.*, 1997 (Diptera: Culicidae); Rossaro, 1982 (Chironomidae, Ortocladiinae); Tamanini, 1979 (Aquatic Heteroptera); Zullini, 1982 (Nematoda); Karaman Gordan, 1998 (Amphipoda); Zullini, 2010 (Nematoda). Finally, the European fauna checklist was consulted (de Jong *et al.*, 2014).

## 2.6 Statistical Analysis

Three ecological indices are considered, *viz*. the Shannon-Wiener index (or diversity index), the Margalef index (or specific richness index) and the Pielou index (or equipartition index).

The Shannon-Wiener diversity index (H) (Shannon & Wiener, 1949) considers both the number of species present and the way in which individuals are distributed among them; its definition reads

$$H' = -\sum_{j=1}^{S} p_j \ln p_j$$

where  $p_j$  indicates the frequency of the *j*-th species, *i.e.* the ratio between the number of individuals of the *j*-th species and the total number of individuals in the sample ( $\Sigma_j p_j = 1$ ); and *S* the number of species found. The index measures the probability that an individual taken at random from the population belongs to a different species from a species extracted in a previous hypothetical sampling. The greater the value of *H*<sup>'</sup>, the greater the diversity. Its value ranges between 0 and  $+\infty$ .

The Margalef specific richness index (D) (Margalef, 1958) is based on the ratio between the number of species and the total number of individuals. It is calculated as:

$$D = \frac{S-1}{\ln N}$$

where S indicates the number of species found; and N the number of individuals found.

Finally, the Pielou equipartition index (J) (Pielou, 1975) evaluates the degree of uniformity in the distribution of individuals among the different species. It is maximum when all species are present with the same abundance; it has low values instead when there is an abundant species and numerous rare species. The index shows when diversity is due to a balanced relationship between species; it is calculated as:

$$J = \frac{H'}{H_{max}} = \frac{H'}{\ln S}$$

where S indicates the number of species in the sample; H' the Shannon-Wiener index; and  $H_{max}$  the Shannon-Wiener index calculated for a theoretical reference situation.

For the statistical analysis, the Shapiro test (Aldenberg *et al.*, 2002; Schoepf & Schradin, 2012) was first applied to the collected data in order to verify whether their distribution was normal or non-normal.

Subsequently, the data were analyzed using MANOVA (Multivariate Analysis of Variance) and PCA (Principal Component Analysis) to observe the level of similarity between the data by studying the principal components: year, experimental session, point of sampling and carcass degradation stage. The software used for this purpose was R (R Core Team, 2019).

# Chapter 3

# Results

## 3.1 Duration of the Degradation Stages and Sampling Effort

In this work, the degradation of 12 *Sus scrofa domesticus* carcasses in a pond of the Modena Apennines was studied. Tab. 3.1.1 shows the durations in days for the single point of sampling in the two experimental sessions of both years; the average values are  $(99 \pm 3)$  for 2018S1,  $(64 \pm 7)$  for 2018S2,  $(93 \pm 10)$  for 2019S1 and  $(43 \pm 4)$  for 2019S2. The duration of sessions S1 are comparable one to another; likewise the duration of sessions S2. The spring-summer sessions showed a much longer overall duration than the summer- early autumn sessions, for both 2018 (averagely 35 days more) and 2019 (averagely 50 days more) for all carcasses. In general, the experimental sessions of 2018 have a longer duration if compared with the respective ones of 2019 with the exception of only P3 of the two spring-summer periods.

The duration of each decomposition stage and the number of samples performed for each stage are different for each carcass (Tab. 3.1.1). Within the same experimental session, the overall number of sampling is comparable for the three carcasses. The average number of samplings is  $(30 \pm 2)$  for 2018S1,  $(15 \pm 1)$  for 2018S2,  $(30 \pm 3)$  for 2019S1 and  $(10 \pm 1)$  for 2019S2.

The "sampling effort" (s.e.) parameter was also calculated for each stage of carcass degradation, dividing the days of activity in the field for each individual degradation stage by the total number of samplings performed during the entire carcass degradation:

$$s.e.(stage) = \frac{number \ of \ samplings(stage)}{total \ number \ of \ samplings(carcass)}$$

The sampling effort was not calculated in the case of no sampling. From the definition, it follows that  $0 < s.e. \le 1$  (Tab. 3.1.1); the calculated values are within the range 0.0690 and 0.5556.

			duration of	number of		sampling efforts					
			degradation	samplings	1	2	3	4	5		
2018	<b>S</b> 1	P1	100	29	0.0690	0.3103	0.1034	0.3793	0.1379		
		P2	96	28	0.1071	0.2857	0.1071	0.3571	0.1429		
		P3	102	32	0.0938	0.3750	0.0938	0.3125	0.1250		
	<b>S</b> 2	P1	64	16	-	0.1875	0.1250	0.4375	0.2500		
		P2	71	16	-	0.2500	0.1875	0.2500	0.3125		
		P3	57	14	0.0714	0.1429	0.0714	0.4286	0.2857		
2019	<b>S</b> 1	P1	84	27	0.3333	0.3333	0.1481	-	0.1852		
		P2	92	30	0.3000	0.3000	0.1333	0.1333	0.1333		
		P3	104	32	0.2813	0.2813	0.1563	0.1250	0.1563		
	<b>S</b> 2	P1	48	11	0.0909	0.1818	0.1818	0.0909	0.4545		
		P2	41	10	-	0.3000	0.1000	0.1000	0.5000		
		P3	41	9	-	0.1111	0.1111	0.2222	0.5556		

**Tab. 3.1.1** – Duration in days of the degradation and the total number of samplings of the 12 carcasses. The values of sampling efforts per stage are also depicted.

#### **3.2 Physico-Chemical Parameters**

The physico-chemical parameters recorded in the two sampling periods of the two years are shown in Tabs. B.1.1 - B.1.12. The physico-chemical parameters recorded and analysed by Arpae are: pH, conductivity, suspended solids, alkalinity, dissolved oxygen (mg/L), dissolved oxygen (%), BOD 5, COD, ammonia nitrogen, nitric nitrogen, total nitrogen, orthophosphate, total phosphorus, chloride ions, sulphate ions, calcium ions, magnesium ions, sodium ions potassium ions and chlorophyll a (Tab. B.2.1). The points of sampling show different hydrogeomorphological characteristics. P1, especially in S1, shows an intermediate behaviour between lentic and lotic; the inflow stream of the pond pours water and organic debris of terrestrial origin herein (especially on rainy days). P3 is rich in Mentha aquatica which enriches the substrate in this part of the pond. P2 is the point of sampling most exposed to the sun and has a silty texture particularly poor in hydrophytes and dead vegeTab. organic matter. Passing from one experimental session to another, an remarkable difference in height in the coast limit is observed due to the higher temperatures and the lower water supply to the S2 pond compared to S1. From an ecological point of view, the continuous fluctuations in the water level lead to the mixing of nutrients. An environment so rich in nutrients represents a privileged place, both from a trophic and reproductive point of view, for a large number of species (Keddy, 2010). There are missing values (stage 1 of 2018 S2P1, 2018 S2P2, 2019 S2P2, 2019 S2P3 and during stage 4 of 2019 S1P1) and single values for which it was therefore not possible to calculate an average (stages 1

and 3 of 2018 S2R3, stages 3 and 4 of 2019 S2P1, stages 3 and 4 of 2019 S2P2 and stages 2, 3 and 4 of 2019 S2P3) of physico-chemical parameters. This was due to the excessively fast degradation times of the carcass (during these stages) which allowed only one or no measurement during field activity. Noteworthy is also the anomalous snowfall at the beginning of May 2019 which significantly lowered the temperatures of air and water during the first stage of degradation of the carcasses studied in S1 2019.

The average values and standard deviations of the physico-chemical parameters were calculated for each decomposition stage (Tab. 3.2.1). (The plots of the average data along with the leastsquare linear regressions are depicted in Appendix B, Figs. B.1.1-6.) The air and water temperature values calculated for P1, P2 and P3 do not differ significantly from the average of the three values; for 2018S1, 2018S2 and 2019S1 the standard deviations are less than 2 °C, while for 2019S2 wider ranges have been obtained for  $T_{air}$ . The trend observed for S1 of 2018 is very similar to the trend observed in S1 of 2019 (the discrepancies between average temperatures of air and water are in absolute values below 4 °C and 5 °C, respectively); the trend observed during S2 2018 is similar to that of S2 in 2019 although with greater absolute discrepancies (9 °C and 7 °C for  $T_{air}$  and  $T_{water}$ , respectively). During the first stage the differences are more significant (large standard deviation, Tab. 3.2.1), in the intermediate stages of degradation the differences become thinner and increase again in the final stage. It is also observed that the differences are given above all between the values of S2 (Tab. 3.2.1).

Temperature of air									
Stage	<b>2018S1</b>	201882	2019S1	201982					
1	$13.9 \pm 0.9$	26.0 -	$10.4 \pm 0.5$	18.7 -					
2	$18.1 \pm 1.2$	$22.4 \pm 0.9$	$16.8 \pm 0.5$	$22.5 \pm 3.0$					
3	$19.8 \pm 1.4$	$20.6 \pm 2.0$	$21.9 \pm 0.5$	$23.9 \pm 3.6$					
4	$20.2 \ \pm 0.2$	$17.8 \pm 1.1$	$23.4 \pm 0.5$	$22.6 \pm 7.4$					
5	$21.6 \pm 0.5$	$14.5 \pm 2.0$	$21.5 \pm 1.6$	$23.4 \pm 1.1$					
	Temperature of water								
Stage	2018S1	201882	2019S1	201982					
1	$11.1 \pm 0.4$	24.0	$9.0\ \pm 0.4$	20.4					
2	$13.4 \pm 1.4$	$22.8 \pm 0.8$	$12.2 \pm 0.6$	$21.2 \pm 0.5$					
3	$14.6 \pm 0.8$	$21.5 \pm 0.8$	$17.5 \pm 1.2$	$23.1 \pm 2.2$					
4	$18.5 \pm 1.5$	$19.1 \pm 0.8$	$23.5 \ \pm 0.2$	$23.5 \pm 1.8$					
5	$22.4 \pm 0.1$	$15.9 \pm 1.3$	$22.0 \pm 1.0$	$22.9 \pm 0.7$					

**Tab. 3.2.1** – Average air and water temperature values (in Celsius degrees) for the 3 points of sampling in the 4 experimental sessions of 2018 and 2019. The calculated standard deviation values are also reported.

Temperature represents the parameter that most influences the degradation process of the dead organic substance (Wallace, 2015). The general air temperature trend in the two years is congruent with the seasonal one recorded by the Arpae station in Sassostorno (Fig. B.2.1) showing fluctuations from a minimum value of 9 °C to a maximum value of 26 °C in 2018 and from 5.7 °C to 30 °C in 2019. The water temperature in 2018 shows an initial increasing trend and then decreases in the second phase; has a lower degree of variation than air (with values between 10 and 24.1 °C). In 2019 the water temperature shows a decrease in the first days of sampling (up to a minimum value of 7.6 °C) followed by a growth up to a maximum value of 25.6 °C and then decreases again in the final phases of the experiment.

By relating the average temperature values of water and air with the degradation stages of the carcasses, good regression coefficient values are observed (Tab. 3.2.2), especially if we consider the data relating to 2018 and S1 of 2019 (Figs. 3.2.1 and 3.2.2).



2018S1P1



2018S1P3



Fig. 3.2.1 – Average air and water temperatures as a function of the stage 2018.





---- T air (°C) --- T water (°C)

2

3

stage

4

5

5,0

0,0

1

2019S2P2

---- T air (°C) --- T water (°C)

2

3

stage

4

5

5,0

0,0

1

2019S2P3

---- T air (°C) --- T water (°C)

2

3

stage

4

5

5,0

0,0

1



		Temperature of air				Temperature of water					
		Value	Std Err	t-Value	prob >  t	Value	Std Err	t-Value	prob >  t		
2018S1P1	intercept	13.3	0.4	36.98044	0.00004	6.8	1.5	4.46598	0.02091		
	slope	1.7	0.1	15.92833	0.00054	2.8	0.5	6.11725	0.00878		
	$\mathbb{R}^2$	0.98831				0.92578					
2018S1P2	intercept	13.2	1.9	6.88009	0.00629	7.8	1.1	6.77365	0.00658		
	slope	1.8	0.6	3.16466	0.05069	2.7	0.3	7.95966	0.00414		
	$\mathbf{R}^2$	0.76950				0.95479					
2018S1P3	intercept	13.9	2.1	6.46761	0.00750	0.00750 8.7		8.66410	0.00323		
	slope	1.7	0.6	2.57432	0.08218	2.7	0.3	8.96765	0.00293		
	$\mathbb{R}^2$	0.68838				0.96404					
2018S2P1	intercept	27.3	1.5	18.44716	0.00293	27.2	1.7	15.59472	0.00409		
	slope	-2.8	0.4	-6.98143	0.01991	-2.3	0.5	-4.84732	0.04002		
	$\mathbf{R}^2$	0.96058				0.92156					
2018S2P2	intercept	28.7	0.3	97.90154	0.00010	28.3	0.8	36.80604	0.00074		
	slope	-2.9	0.1	-36.13688	0.00076	-2.5	0.2	-12.01878	0.00685		
	$\mathbf{R}^2$	0.99847				0.98634					
2018S2P3	intercept	28.3	0.9	31.02927	0.00007	26.4	0.9	29.57532	0.00008		
	slope	-2.3	0.3	-8.25799	0.00372	-1.7	0.3	-6.18164	0.00852		
	$\mathbf{R}^2$	0.95786				0.92721					
2019S1P1	intercept	10.8	3.4	3.12620	0.08889	5.8	1.2	4.65241	0.04323		
	slope	2.5	1.1	2.30004	0.14814	3.1	0.4	7.83483	0.01590		
	$\mathbf{R}^2$	0.72566				0.96845					
2019S1P2	intercept	9.5	3.3	2.87644	0.06371	5.6	1.9	2.85661	0.06476		
	slope	3.3	1.0	3.29884	0.04577	3.8	0.6	6.49119	0.00742		
	$\mathbb{R}^2$	0.78390				0.93353					
2019S1P3	intercept	10.6	3.6	2.94914	0.06007	6.2	2.4	2.58552	0.08139		
	slope	2.6	1.1	2.35667	0.09970	3.7	0.7	5.08232	0.01472		
	$\mathbf{R}^2$	0.64928				0.89594					
2019S2P1	intercept	20.7	2.7	7.63922	0.00466	20.3	0.5	39.93056	0.00003		
	slope	0.5	0.8	0.57444	0.60592	0.5	0.2	2.92925	0.06104		
	$\mathbb{R}^2$	0.09909				0.74094					
2019S2P2	intercept	25.4	10.6	2.39691	0.13874	22.4	3.5	6.44638	0.02323		
	slope	-0.8	2.9	-0.26995	0.81250	0.3	0.9	0.27020	0.81234		
	R <sup>2</sup>	0.03516				0.03522					
2019S2P3	intercept	16.8	7.7	2.19336	0.15955	18.5	3.2	5.82839	0.02820		
	slope	1.9	2.1	0.92537	0.45247	1.2	0.9	1.40948	0.29408		
	$R^2$	0.29979				0.49832					

**Tab. 3.2.2** – Parameters of linear fit of the values of the average temperature of air and water as a function of the decomposition stage.

The average relative rate of degradation of the carcasses were computed for each carcass of S1 and S2 of both 2018 and 2019 (Fig. 3.2.3(a)). These values were put in correlation with the measured physico-chemical parameters. In particular, upon linear regression, good correlations were shown by considering the average relative rate of degradation as a function of the average water temperature (Fig. 3.2.3, panel (b); intercept  $a_0 = (8.8 \pm 1.6)$  (%/day); slope  $a_1 = (-0.8 \pm 0.1)$  (%/day/°C); correlation coefficient Adj- $R^2 = 0.87557$ ), conductivity (panel (c); intercept  $a_0 = (-36.0 \pm 3.7)$  (%/day); slope  $a_1 = (0.049 \pm 0.006)$  (%/day/( $\mu$ S/cm)); correlation coefficient Adj- $R^2 = 0.87305$ ), pH (panel (d); intercept  $a_0 = (-29.5 \pm 3.6)$  (%/day); slope  $a_1 = (3.5 \pm 0.5)$  (%/day); correlation coefficient Adj- $R^2 = 0.82531$ ) and the dissolved oxygen percentage (panel (e); intercept  $a_0 = (-12.5 \pm 0.6)$  (%/day); slope  $a_1 = (0.21 \pm 0.02)$  (%/day/%); correlation coefficient Adj- $R^2 = 0.93385$ .). The graphs of the average relative rate of degradation versus the other average parameters did not show any evident correlation.

The average temperatures of water and air were related to the overall duration of the degradation stages from 1 to 4 (Fig. 3.2.4). The choice fell on the first 4 stages of degradation as they are the stages in which the carcass still has soft tissues whose degradation is affected by the effects of temperature. Also because the duration of stage 5 was chosen arbitrarily. It can be noted that, with a good regression coefficient value ( $R^2 = 0.9107$  and 0.8365 for water and air, respectively), when the temperatures in these first 4 stages are greater, the duration of the same stages is shorter.



(a)



**Fig. 3.2.3** – Average relative rate of degradation for the different points of sampling (a) and plot as a function of the average water temperature (b), conductivity (c), pH (d) and dissolved oxygen (e). The average rate of degradation is calculated as  $\langle v_{rel} \rangle = (\Delta m / m_{in}) / \Delta t$ . where:  $\Delta m$  is the difference between the initial mass and the final mass of the carcass,  $m_{in}$  is the initial mass and  $\Delta t$  is the duration in days elapsed from the immersion of the carcass to the removal of its remains. In panel (b)-(d), the hyphened line indicates the least-square linear regression.



Fig. 3.2.4 – Total duration of stages 1 to 4 as a function of the average temperature of water (a) and air (b).

### **3.3 Macroinvertebrates**

In the following paragraphs, unless otherwise specified, the term "taxa" refers to the taxonomic level of "genus/species". The sampling of the last date was carried out by collecting the whole carcass; this sampling method is different from those carried out during the rest of the field activity (collection on sight; see Paragraph 2.4 in "Materials and Methods"). The data collected on the last day are therefore not directly comparable with those collected during the other days; they were subsequently excluded from the analysis and will not be shown in this thesis work. In addition, individuals for whom it was not possible to identify the genus or species due to excessive deterioration of the individual were not included in the dataset and were not considered for the subsequent statistical analyses. The classification followed is the one proposed in "European fauna checklist" (de Jong *et al.*, 2014).

A total number of 24383 macroinverbrates belonging to 125 taxa attribuTab. to 7 *phyla* in 2018 and a total number of 6291 macroinverbrates belonging to 98 taxa attribuTab. to 6 *phyla* in 2019 were collected. Among the macroinvertebrates sampled in the 4 experimental sessions of the 2 years, the most represented *phylum* in terms of number of taxa and number of individuals is Arthropoda (Tab. 3.3.1). In fact out of a total of 18049 individuals sampled in S1 in 2018, as many as 15025 were arthropods (83.25%), out of 6334 total individuals in S2 in 2018, 5793 were

arthropods (91.46%). In 2019, respectively, for S1 and S2 out of a total of 5394 and 947 individuals, 4716 and 922 (equal to 87.43% and 97.36% of the total) were arthropods.

Similarly, also for the number of taxa, the majority belongs to Arthropoda for the 4 experimental sessions of the 2 years (Tab. 3.3.1). In fact, out of a total number of 103 taxa sampled in S1 in 2018, 90 were arthropods (87.38%) and out of a total number of 48 taxa in S2 in 2018, 41 were arthropods (85.42%). In 2019, respectively, for S1 and S2 out of a total of 84 and 31 taxa, 67 and 28 (equal to 79.76% and 90.32% of the total) were arthropods. Finally, Tab. 3.3.2 summarises the number of phyla, classes, orders, families, genera and species for each sampling point (P1, P2 and P3), determined in the two experimental sessions (S1 and S2) in the two years (2018 and 2019).

Tab. C.1 shows the list of taxa found in the two years of sampling. The trophic role, the experimental session, the sampling point and the number of individuals sampled are also reported.

2018	S1		S2			
	Nr. of individuals	Nr. of taxa	Nr. of individuals	Nr. of taxa		
Cnidaria	34 (0.19%)	1 (0.97%)	2 (0.03%)	1 (2.08%)		
Platyhelminthes	1 (<0.01%)	1 (0.97%)				
Nematoda			2 (0.03%)	1 (2.08%)		
Mollusca	14 (0.08%)	4 (3.88%)	46 (0.73%)	1 (2.08%)		
Annelida	2716 (15.05%)	6 (5.83%)	208 (3.28%)	3 (6.25%)		
Arthropoda	15025 (83.25%)	90 (87.38%)	5793 (91.46%)	41 (85.42%)		
Bryozoa	259 (1.44%)	1 (0.97%)	283 (4.47%)	1 (2.08%)		
Tot.	18049	103	6334	48		
2019	S1		<b>S2</b>			
	Nr. of individuals	Nr. of taxa	Nr. of individuals	Nr. of taxa		
Cnidaria	20 (0.37%)	1 (1.19%)				
Nematoda	105 (1.97%)	5 (5.95%)				
Mollusca	9 (0.17%)	4 (4.76%)				
Annelida			/	- /		
	429 (8.03%)	6 (7.14%)	25 (2.64%)	3 (9.68%)		
Arthropoda	429 (8.03%) 4714 (88.21%)	6 (7.14%) 67 (79.76%)	25 (2.64%) 922 (97.36%)	3 (9.68%) 28 (90.32%)		
Arthropoda Bryozoa	429 (8.03%) 4714 (88.21%) 65 (1.22%)	6 (7.14%) 67 (79.76%) 1 (1.19%)	25 (2.64%) 922 (97.36%)	3 (9.68%) 28 (90.32%)		

Tab. 3.3.1 – Number of individuals and total aquatic macroinvertebrate taxa for both the 2018 and 2019 experimental sessions.

	2018							2019					
	<b>S1</b>			S2			<b>S1</b>		<b>S2</b>				
	<b>P1</b>	P2	<b>P3</b>	<b>P1</b>	P2	<b>P3</b>	<b>P1</b>	P2	<b>P3</b>	<b>P1</b>	P2	<b>P3</b>	
Phyla	6	6	6	6	6	5	7	7	6	3	3	3	
Classes	10	7	8	6	6	6	10	8	7	3	3	3	
Orders	18	13	13	10	13	11	21	16	15	8	9	7	
Families	41	39	35	17	28	25	46	28	27	14	16	12	
Genera	60*	49*	47*	22*	35*	29*	65*	33*	34*	21*	18*	15*	
Species	69*	56*	53*	24*	37*	29*	73*	40*	40*	24*	20*	15*	

**Tab. 3.3.2** – Number of *phyla*, classes, orders, families, genera and species for each sampling point (P1, P2 and P3), determined in the two experimental sessions (S1 and S2) in the two years (2018 and 2019).

\* Only one genus (or species) was counted when the taxonomical determination ended up to family (or genus).

## 3.3.1 Cnidaria

Only individuals belonging to the *Hydra* genus were found, for which it was not possible to determine the species due to their morphological characters distinguishable only in live animals. In total, 56 *Hydra* sp. were counted corresponding to 0.18% of the total sampled individuals. The presence of *Hydra* sp. was observed almost exclusively in the spring-summer seasons of both years; for S2 only 2 individuals were observed in 2019: one in P1 and one in P2 (Fig. 3.3.1). These Anthoathecata play a role as predators of small animals such as water fleas and copepods (Campaioli *et al.*, 1994).





**Fig. 3.3.1** – Number of individuals of Anthoathecata collected in (a) 2018S1R1, (b) 2018S2R1, (c) 2018S1R2, (d) 2018S2R2, (e) 2018S1R3, (f) 2019S1R1, (g) 2019S1R2, (h) 2019S1R3, as a function of the stage of decomposition.

## **3.3.2** Platyhelminthes

Only one individual of flatworms was found (0.003% of the total) belonging to *Dugesia* sp. in P1 of the S1 of 2018, for which the dichotomous key did not allow a determination to species level. Ascribable to the order of the Tricladida, the representatives of this genus are predators that prefer still or weakly flowing waters (Campaioli *et al.*, 1994).

#### 3.3.3 Nematoda

107 individuals were found (equal to 0.35% of the total) belonging to 2 orders: Enoplida (represented by *Tobrilus* sp.) and Dorylaimida (represented by *Mononchus* sp., *Mylonchulus* sp., *Dorylaimus* sp. and *Laimydorus* sp.). Of these only 2 individuals of *Tobrilus helveticus* were sampled in the late summer session of 2018 in P1 during the fourth stage of carcass degradation, whereas all the remaining nematodes were sampled in 2019 during the spring-summer session (Figs. 3.3.2 and 3.3.3). The order of dorilaimids was mainly distributed in the early stages of carcass degradation (with the exception of a single *Laimydorus* sp. found in stage 5 of P3); the order of enoplids was mainly distributed among the degradation stages depending on the carcass considered. All the sampled nematodes are considered as predators at the expense of small animals (Zullini, 2010).



**Fig. 3.3.2** – Number of individuals of Enoplida collected in (a) 2018S1P1, (b) 2019S1P1, (c) 2019S1P2, (d) 2019S1P3, as a function of the stage of decomposition.



**Fig. 3.3.3** – Number of individuals of Dorylaimida collected in (a) 2019S1P1, (b) 2019S1P2, (c) 2019S1P3, as a function of the stage of decomposition.

### 3.3.4 Mollusca

The 69 individuals (corresponding to 0.23% of the total) sampled belonging to the *phylum* Mollusca are attribuTab. to 4 orders: Neotenioglossa (represented by *Belgrandiella* sp.), Heterostropha (represented by the species *Valvata piscinalis*), Basommatophora (represented by the species: *Ferrissia clessiniana* and *Galba truncatula*) and Veneroida (represented by the species *Sphaerium corneum*).

Among the gastropods, the orders Neothenioglossa and Heterostropha were represented by few individuals, always found in the initial stages of degradation and, for both years, only in P1 during the spring-summer sessions (Figs. 3.3.4 and 3.3.5). Basommatophores were the most represented order of gastropods: *Ferrissia clessiniana* was sampled only in 2018 but in both experimental sessions and always in the final stages of degradation; *Galba truncatula* was sampled only in 2019 with only 3 individuals (respectively 2 in P1 and 1 in P2) in the first two stages of degradation (Fig. 3.3.6). Gastropods are scrapers basing their nutrition on microsediments and organic material encrusting the substrate (Campaioli *et al.*, 1994).



**Fig. 3.3.4** – Number of individuals of Neotenioglossa collected in (a) 2018S1P1, (b) 2019S1P1, as a function of the stage of decomposition.



**Fig. 3.3.5** – Number of individuals of Heterostropha collected in (a) 2018S1P1, (b) 2019S1P1, as a function of the stage of decomposition.





**Fig. 3.3.6** – Number of individuals of Basommatophora collected in (a) 2018S2P1, (b) 2018S1P2, (c) 2018S2P2, (d) 2018S1P3, (e) 2018S2P3, (f) 2019S1P1, (g) 2019S1P2, as a function of the stage of decomposition.

The sampled bivalves were represented by the single species *Sphaerium corneum* present with only 3 individuals in 2018 (respectively 2 and 1 in P1 and P3) during the spring-summer session and by only one individual in 2019 in P1 during the spring-summer session. Like all bivalves, these veneroids are filter feeders that feed mainly on phytoplankton (Campaioli *et al.*, 1994).

### 3.3.5 Annelida

Among the 3378 annelids counted, equal to 11.01% of the total sampled individuals, only one individual belonged to the species *Trocheta bykowskii* (attribuTab. to the order Arhynchobdellida), whereas the remaining part belonged to the order Haplotaxida with representatives of the families Naididae, Enchytraeidae and Lumbricidae.

Among the haplotaxids, the most represented family was Naididae with individuals belonging to Lumbriculus sp., Tubifex sp., Limnodrilus sp., Nais sp. and Haemonais sp. Among these genera the most represented was generally Nais, present in most of the stadiums and in all the points of sampling of all the sessions in both years with the only exception of the S2R3 of 2019 in which was completely absent (Fig. 3.3.7). In some situations (as in S2P1 and S2R2 of 2018) a greater presence of the genus Limnodrilus was observed, but in general as a number of individuals the genus Nais was the most represented. Lumbriculus was found with 4 individuals only in 2018 during the spring summer session in P2 as well as Haemonais, which was only sampled in the spring-summer session of 2018 but in the later stages of the degradation of P1 and P3 carcasses with 6 and 74 individuals respectively. The *Tubifex* genus was found with only one individual in 2019 in S1R2, S2P1 and S2P3. Among the Lumbricidae, Eiseniella tetraedra was sampled for both years with few units and always during S1; the enchitreids were present in both years with few individuals except for the early stages of degradation of P1 during the two spring-summer sessions in which they were abundant (even with more than 100 individuals). In general, haplotaxides are collectors feeding on small particles of organic debris or bacteria present on the substrate (Campaioli et al., 1994; Lencioni et al., 2013).















(i)

















**Fig. 3.3.7** – Number of individuals of Haplotaxida collected in (a) 2018S1P1, (b) 2018S2P1, (c) 2018S1P2, (d) 2018S2P2, (e) 2018S1P3, (f) 2018S2P3, (g) 2019S1P1, (h) 2019S2P1, (i) 2019S1P2, (j) 2019S2P2, (k) 2019S1P3, (l) 2019S2P3, as a function of the stage of decomposition.

*Trocheta bykowskii* was found with only one individual during the first degradation stage of the P1 carcass during the spring-summer session of 2019. The leaches belonging to this genus are macrophages which feed mainly on insect larvae, oligochetes, amphipods and isopods (Campaioli *et al.*, 1994).

### 3.3.6 Arthropoda

26456 individuals of the *phylum* Arthropoda were sampled, corresponding to 86.25% of the total aquatic macroinvertebrates found. Considering the variety of taxa identified, we proceed by analysing the individual orders separately.

#### 3.3.6.1 Acaridida

The collected aquatic arachnids were only 2 mites, both of them found in 2018. Belonging to the genus *Limnesia*, one was found during the second stage of degradation of the P1 carcass during the spring-summer session. The other mite belongs to the *Sperchon* genus and was sampled during the fourth degradation stage of the R3 carcass in the late summer session. The mites belonging to these two genera play roles of parasites at the expense of Diptera (Simuliidae, Tipulidae and Chironomidae) and Trichoptera (Hydroptilidae) (Cicolani & Di Sabatino, 2014).

#### 3.3.6.2 Amphipoda

Over the two years 23 individuals of amphipods were collected, only in the spring-summer sessions, only in the first two stages of carcass degradation and only from the P1 carcasses (Fig.

3.3.8). The two determined species were *Synurella ambulans* and *Niphargus elegans*. In 2018 both species were present with 3 individuals during the second stage of degradation; in 2019 *S. ambulans* was present with 2 individuals in the first stage of degradation and only one individual during the second stage while *N. elegans* was sampled only during the first stage of degradation in a number of 14 individuals. *S. ambulans* plays the trophic role of shredder by feeding and fragmenting the dead organic substance; *N. elegans* is considered as a generalist, since can also prey other living organisms (Campaioli *et al.*, 1994; Karaman, 1993).



**Fig. 3.3.8** – Number of individuals of Amphipoda collected in (a) 2018S1P1, (b) 2019S1P1, as a function of the stage of decomposition.

### 3.3.6.3 Ephemeroptera

The order of Ephemeroptera was present in the samples of both experimental sessions of both years for all carcasses. The 1429 individuals sampled in the two years belong to 3 families: Baetidae, Caenidae and Leptophlebiidae (Fig. 3.3.9). Among baetids 2 genera were found: *Baetis* with 3 species (9 individuals of *Baetis lutheri*, one individual of *B. niger* and 3 individuals of *B. rhodani*) found during stages 3 and 4 of the spring-summer sessions of P1 and P2; *Cloeon* (represented by *C.* gr. *dipterum*) was the most represented genus of ephemerals with a total of 1256 individuals, in 2018 it was the most abundant for all carcasses while in 2019 it had lower numbers and was sometimes not the most represented (stage 4 of S1P2, stage 5 of S1P3, stage 5 of S2P2 and stage 5 of S2P3). The cenids family was represented by 4 species all belonging to the genus *Caenis: Caenis belfiorei* (exclusive of 2018 with only one individual during stage 4 and one during stage 5 of S1P2), *C. lactea* (exclusive of 2019), *C. luctuosa* (present in all sessions and in all carcasses of both years with the exception of S2P1 of 2019 only) and *C. horaria* (present in 2018 in both sessions of P1 and P2 carcasses; in 2019 present in both sessions of the casing P2 and in S1 of P3). The Leptophlebiidae family, represented by the single

species *Habrophlebia fusca*, was found with only one individual during stage 3 and one individual during stage 4 of the 2019S2P3. The nymphs of these families generally feed on organic vegeTab. or animal detritus (Campaioli *et al.*, 1994).





**Fig. 3.3.9** – Number of individuals of Ephemeroptera collected in (a) 2018S1P1, (b) 2018S2P1, (c) 2018S1P2, (d) 2018S2P2, (e) 2018S1P3, (f) 2018S2P3, (g) 2019S1P1, (h) 2019S2P1, (i) 2019S1P2, (j) 2019S2P2, (k) 2019S1P3, (l) 2019S2P3, as a function of the stage of decomposition.

## 3.3.6.4 Odonata

The 720 sampled odonates were attribuTab. to 5 families: Lestidae, Platycnemididae, Coenagrionidae, Aeshnidae and Libellulidae (Fig. 3.3.10). From the lestids family, one individual of *Chalcolestes viridis* and one individual of *Lestes virens* were found respectively in P1 in the fourth stage and R2 during the fifth stage, in the spring-summer session of 2018.

*Platycnemis pennipes* (Platycnemididae) was the most represented odonate, found in all studied carcasses; its frequency was also the highest among odonate taxon. In S1P2 and S2P3 of 2019 these were the only odonates found during the entire degradation of the carcass. The Coenagrionidae family was represented by 6 species: Pvrrhosoma nimphula (with only one individual in the fifth stage of degradation of the P2 carcass of the late summer session of 2018), Erythromma viridulum (in 2018 present only in the fourth stage of S1P2 with one individual, in 2019 mainly represented in P1 for both sessions and during the first session of P3), Enallagma cyathigerum (present only in 2018 in S2P1 during the fourth stage with 4 individuals), Coenagrion puella/pulchellum (found with greater abundance on the P1 carcasses of both sessions), C. hylas (only one individual in 2018 in S1P2) and Ischnura elegans (after P. pennipes it was the most represented species; particularly abundant in 2018, especially during the second experimental session, while in 2019 present only in S1P1 with 2 individuals). Among the escnids 2 genera were found: Anax (present in 2019 as Anax sp. due to the finding of juvaniles for a correct identification to species level, in 2018 as A. imperator found with few units but on all carcasses ) and Aeshna (with the species A. cvanea found with only one individual in 2018 during the fifth stage of S2P2). 3 species belonging to the Libellulidae family were determined, all represented by a single individual and all found in 2018: *Platetrum depressum* (in S1P3), Sympetrum depressiusculum (in S1P2) and S. flaveolum (in S1P3). All odonates are predators both at the adult stage and in the juvenile stages (Campaioli et al., 1994).









stage





(i)

20 Platycnemis pennipes nr. of individuals 15 Pyrrhosoma nymphula 10 Ischnura elegans 5 ■ Aeshna cyanea 0 Anax imperator 1 2 3 4 5 stage





(f)









**Fig. 3.3.10** – Number of individuals of Odonata collected in (a) 2018S1P1, (b) 2018S2P1, (c) 2018S1P2, (d) 2018S2P2, (e) 2018S1P3, (f) 2018S2P3, (g) 2019S1P1, (h) 2019S2P1, (i) 2019S1P2, (j) 2019S2P2, (k) 2019S1P3, (l) 2019S2P3, as a function of the stage of decomposition.

## 3.3.6.5 Plecoptera

The 48 plecoptera sampled belonged to 2 families: Perlodidae and Nemouridae. The perlodids, represented only by the *Isoperla grammatica* species, were only sampled in 2019 with one individual in S1P2 (the only stonefly found on this carcass) and 4 individuals in S1P1 (Fig. 3.3.11). Among the nemurids, *Protonemura ausonia* was found with 3 individuals exclusively in 2018 in S1P1 (the only plecopter species collected this year), the remaining 3 species (*Nemoura cinerea*, *N. flexuosa* and *Protonemura tyrrhena*) were found in S1P1 of 2019 All sampled stoneflies were present in the first two stages of carcass degradation. Nemurids play the role of shredder detritivores, while *Isoperla grammatica* plays the role of predator (Campaioli *et al.*, 1994).





**Fig. 3.3.11** – Number of individuals of Plecoptera collected in (a) 2018S1P1, (b) 2019S1P1, (c) 2019S1P2, as a function of the stage of decomposition.

### 3.3.6.6 Hemiptera

344 Hemiptera belonging to 7 families were sampled: Corixidae, Nepidae, Notonectidae, Pleidae, Gerridae, Veliidae and Hydrometridae. Among the corixids, individuals belonging to two taxa were counted: the sub-family Corixinae (for which a more detailed determination was not possible due to the capture of juvaniles; they were present in both years mainly in the springsummer session with the only exception of a individual found in the fifth stage of S2P3 degradation) and the species Micronecta scholtzi (abundantly present in 2018 in P2 in both sessions while in 2019 it was more represented in S2P1) (Fig. 3.3.12). Nepidae were represented by the Nepa cinerea species (in 2018 in S1P1 and S1P2, in 2019 in S2P1) while in S2R1 of 2018 only one individual of Nepa sp. was found (not determined at a specific level as the individual was excessively immature). The notonectids, represented by the species Notonecta viridis, was exclusive to 2018 with individuals collected on all the carcasses of the spring-summer session and in P3 also during the late summer session (in the latter carcass it was found with the greatest numbers). Plea minutissima (Pleidae) was found with only one individual in S1P2 of 2018 during the first stage of degradation. The gerrids were represented only by the species Gerris lacustris, present in all carcasses of 2018, absent in 2019 for S1P2, S2P2 and S2P1. The velids were collected with a single individual of Microvelia sp. in the fourth stage of S1P1 of 2018; individual examples of Veliidae n.d. were also found in S2P2 of 2018, S1P2 and S2P3 of 2019 (for all the velids sampled it was not possible to determine the species due to the collection of excessively immature individuals). The only species sampled of the hydrometrids was Hydrometra stagnorum found in most of the carcasses of 2018 (except S1P1) and 2019 (except


S1P2); on the S2P2 casing of 2019 these were the only Hemiptera sampled. All sampled Hemiptera play the role of active predators, according to Campaioli *et al.*, 1994.



**Fig. 3.3.12** – Number of individuals of Hemiptera collected in (a) 2018S1P1, (b) 2018S2P1, (c) 2018S1P2, (d) 2018S2P2, (e) 2018S1P3, (f) 2018S2P3, (g) 2019S1P1, (h) 2019S2P1, (i) 2019S1P2, (j) 2019S2P2, (k) 2019S1P3, (l) 2019S2P3, as a function of the stage of decomposition.

# 3.3.6.7 Coleoptera

The 265 individuals sampled of beetles were attribuTab. to 10 families: Haliplidae, Gyrinidae, Dytiscidae, Helophoridae, Hydrochidae, Hydrophilidae, Hydraenidae, Limnebiidae, Scirtidae and Dryopidae. The only carcass in which no beetles were sampled during the entire duration of degradation is S2P3 in 2019. In general the greater abundances of individuals and taxa occured

during the first experimental session than the second and were greater in 2018 compared to 2019 (Fig. 3.3.13). Aliplids (represented by Haliplus obliquus) and scirtids (for which we stopped at the family determination due to the lack of keys for a more detailed determination for the Italian species) were found with only one individual exclusively in S1P2 of 2019. The gyrinids found were larvae of Gyrinus sp. (determining the species was impossible due to the absence of dichotomous keys for the immature stages of this genus) found only in 2018 during stage 4, with 2 individuals for both S1P1 and S1P2. With the 9 sampled genera (Hyphydrus, Hydrovatus, Bidessus, Hydroporus, Laccophilus, Agabus, Ilybius, Acilius and Dytiscus) dytiscids were collected on all carcasses of 2018 except S2P1, while in 2019 they were collected in S1P2 S2P1 and S1R3. The helophorids were represented by 3 species all belonging to the genus *Helophorus*; they were always found in all carcasses during the spring-summer seasons of both years while during the late summer sessions they were found in S2P2 of 2018, S2P1 and S2P2 of 2019. Hydrochus angustatus (Hydrochidae) was present in both years: in 2018 completely absent in P1 both during the first and second experimental session while in 2019 it was missing only in S2P1. The collected hydrophilids were attribuTab. to 2 genera: Laccobius (with 3 species) and Helochares (represented only by H. lividus) both present on all carcasses with the exception of S1 P1 and S2P1 of 2018 and S2P2 of 2019. The limnebids were represented from two species (Limnebius nitidus and L. crinifer) both counted with only one individual respectively in S1P1 of 2018 and S2P3 of 2018. With the species Pomatinus substriatus, dryopids were present during the spring-summer sessions of P2 and P3 of both the years as well as in S2P3 of 2018. The hydrenids were represented by 2 genera: Hydraena (with 5 species) and Ochthebius (with 6 species); these beetles were absent, in 2018, only in S2P3, in 2019 in S1P3. Due to their diversity, beetles play different trophic roles. The aliplids are grazers (with a phytophagous diet based on filamentous algae), gyrinids and dytiscids are predators, hydrochids play the role of shredder detritivores, hydrenids, limnebids and dryopids are collector-gathering detritivores while helophorids, hydrophilids and scirtids play different trophic roles and are therefore included into the generalists, feeding on algae or organic detritus when necessary (Campaioli et al., 1999; Franciscolo, 1979; Pirisinu, 1981).







(c)

(a)









(g)

(e)



















(f)

nr. of individuals



**Fig. 3.3.13** – Number of individuals of Coleoptera collected in (a) 2018S1P1, (b) 2018S2P1, (c) 2018S1P2, (d) 2018S2P2, (e) 2018S1P3, (f) 2018S2P3, (g) 2019S1P1, (h) 2019S2P1, (i) 2019S1P2, (j) 2019S2P2, (k) 2019S1P3, as a function of the stage of decomposition.

## 3.3.6.8 Megaloptera

Only 2 very young larvae belonging to the genus *Sialis* were found: one found during the second stage while the other during the fifth stage of degradation of the S1P1 carcass of 2019. Due to their youth it was not possible to determine the species. The larvae of these insects play the role of predators (Campaioli *et al.*, 1999).

#### 3.3.6.9 Diptera

The order of Diptera was the most represented group of aquatic macroinvertebrates. There are 17 certain families of Diptera (23334 individuals were sampled): Limoniidae, Tipulidae, Psychodidae, Dixidae, Chaoboridae, Culicidae, Simuliidae, Ceratopogonidae, Chironomidae, Stratiomyidae, Tabanidae, Rhagionidae, Empididae, Dolichopodidae, Syrphidae, Sciomyzidae, and Muscidae. Chironomids were the most represented in terms of number of individuals, it was

not possible to determine the individuals at genus and species level but individuals belonging to 3 sub-families were recognised: Chironominae (generally the most represented sub-family, found on all carcasses studied), Orthocladiinae (found on all carcasses studied, during the second and fifth stages of S1P2, during the third stage of S1P1 in 2018 and throughout the degradation of S1P1 in 2019, there were more orthocladins than chironomins) and Tanypodinae (in S2P2 and S2P3 of 2019 it was absent) (Fig. 3.3.14). Tipulids, caoborids, black flies, ragionids, empidids and muscids were found with few units and never on more than one carcass. Dixids, represented by the genus Dixa, were found in all carcasses during the spring-summer session of 2018 and in S1P1 of 2019. The culicides were only sampled in 2018 with the exception of S2P1 and were traceable to the genera Anopheles and Culex. Ceratopogonidae were collected from all carcasses studied with the exception of S2P2 only from 2018 and were represented by 3 genera: Dasyhelea, Culicoides and Stilobezzia. Dolicopodids were found with only one larva in S1P1 and S1P3 of 2018 while straziomids were found with few units only in the spring-summer sessions of P1 in both years. Also the sciomyzids were sampled in the spring-summer sessions of P1 of both years during the first two stages of degradation (with dozens of individuals) while in S2P2 only one individual was found during the fifth stage. Tabanids larvae were only sampled in 2018 in P2 during both the first experimental session and the second during the first and fifth stages respectively. Hoverflies were collected from the three carcasses of the spring-summer session of 2018 while in 2019 from P2 but in both experimental sessions; they were determined to belong to 3 genera (Sericomyia, Helophilus and Parhelophilus) and were all collected during the fourth stage of degradation with the exception of a single very immature larva found during the third stage in S2P2 of 2019. The limonids were represented by 5 genera (Limnophila, Neolimnomyia, Cheilotrichia, Helius and Limonia), all found in the spring-summer sessions of P1 of both years and only one individual of Limonia sp. sampled in S2P2 of 2018. In 2019 the only sampled psychodids were in S1P1, in 2018 they were found on the three carcasses of the spring-summer session and in S2P3; they were determined to belong to 7 genera: Peripsychoda, Trichopsychoda, Berdeniella, Pericoma, Saraiella, Bazarella and Copropsychoda. As beetles, considering their high diversity of taxa, diptera also play different trophic roles within the ecosystem. Among the shredders of dead organic substance we have hoverflies and tipulids, the ceratopogonids play the role of collector-gatherers of fine detritus from the substrate, dixids, culicides and black flies are filterers of suspended debris, psychodids and straziomids scrape encrustations of particles and bacteria from the substrate, limonids, caoborids, dolicopodids, sciomizids, tabanids, empidids and muscids (with the genus found: Lispe) are predators, whereas ragionids show a very diversified diet depending on the species and were therefore counted as

generalists, since it was not possible to determine them at species level (Campaioli *et al.*, 1999; Lencioni *et al.*, 2007; Rivosecchi, 1978; Rivosecchi, 1984). As mentioned above, chironomines, tanipodines and orthocladines were not included.



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**Fig. 3.3.14** – Number of individuals of Diptera collected in (a) 2018S1P1, (b) 2018S2P1, (c) 2018S1P2, (d) 2018S2P2, (e) 2018S1P3, (f) 2018S2P3, (g) 2019S1P1, (h) 2019S2P1, (i) 2019S1P2, (j) 2019S2P2, (k) 2019S1P3, (l) 2019S2P3, as a function of the stage of decomposition.

# 3.3.6.10 Trichoptera

The 288 sampled trichopters were attribuTab. to 3 families: Polycentropodidae, Limnephilidae and Leptoceridae. The Leptoceridae family was exclusive to 2018 and P2 (Fig. 3.3.15); for these trichopters it was not possible to further determine a lower taxonomic level beyond family due to the fact that the 2 larvae found were both excessively immature. With the species *Plectrocnemia* 

*conspersa*, polycentropods were found only in 2019 in P1 and with only one individual for each of the first 3 stages. The limnefilids were the most represented family (both in terms of number of individuals and of species found), which was present both years on all the carcasses of the spring-summer session, and represented with 5 genera: *Limnephilus* (with 5 species), *Halesus*, *Allogamus*, *Stenophylax* and *Chaetopteryx*. The most of them was collected in the first two stages of carcass degradation. Except *Plectrocnemia conspersa* (a predator), all the other sampled trichopters are shredders (Campaioli *et al.*, 1999).





**Fig. 3.3.15** – Number of individuals of Trichoptera collected in (a) 2018S1P1, (b) 2018S1P2, (c) 2018S2P2, (d) 2018S1P3, (e) 2019S1P1, (f) 2019S1P2, (g) 2019S1P3, as a function of the stage of decomposition.

## 3.3.6.11 Lepidoptera

The only larva of *Acentria ephemerella* (Crambidae) was sampled in 2018 in S1P1 during the third stage of degradation. These larval stage moths are phytophages (Campaioli *et al.*, 1999) thus covering the role of grazers.

# 3.3.7 Bryozoa

The found bryozoans all belonged to *Plumatella* and were in a statoblast form. In 2019 they were found on all the carcasses of the spring-summer session, while in 2018 they were found only on P2 and P3, both during the first and second experimental sessions (Fig. 3.3.16). The largest number of individuals met in 2018. These organisms are microphages and capture microorganisms or particles present in the water column (Campaioli *et al.*, 1994).





**Fig. 3.3.16** – Number of individuals of Plumatellida collected in (a) 2018S1P2, (b) 2018S2P2, (c) 2018S1P3, (d) 2018S2P3, (e) 2019S1P1, (f) 2019S1P2, (g) 2019S1P3, as a function of the stage of decomposition.

### 3.4 Chordata

A total number of 1969 individuals belonging to Chordata was collected in the two years. Among these, 87 (equal to 4.42%) were attributable. to the class of Actinopterygii and 1882 (equal to 95.58%) to the class of Amphibia. Actinopterygii was traced back to 3 orders: Cypriniformes (*Carassius auratus, C. carassius* and *Cyprinus carpio*, present in 2018 in S1P2, S2P2 and S2P3, while in 2019 in all carcasses except S1P1; see Fig. 3.4.1), Siluriformes (*Silurus glanis*, in 2018

found on all carcasses always in the last stages, in 2019 present in S1P1, S1R3, S2P2 and S2P3; see Fig. 3.4.2) and Perciformes (*Lepomis gibbosus*, in 2018 found only in S2P2 while in 2019 in S1P2, S1P3, S2P1 and S2P3; Fig. 3.4.3). In addition, individuals at the fry stage were collected for which it was not possible to go beyond determination at the family level.





**Fig. 3.4.1** – Number of individuals of Cypriniformes collected in (a) 2018S1P2, (b) 2018S2P2, (c) 2018S2P3, (d) 2019S2P1, (e) 2019S1P2, (f) 2019S2P2, (g) 2019S1P3, (h) 2019S2P3, as a function of the stage of decomposition.





**Fig. 3.4.2** – Number of individuals of Siluriformes collected in (a) 2018S1P1, (b) 2018S2P1, (c) 2018S1P2, (d) 2018S2P2, (e) 2018S1P3, (f) 2018S2P3, (g) 2019S1P1, (h) 2019S2P2, (i) 2019S1P3, (j) 2019S2P3, as a function of the stage of decomposition.



**Fig. 3.4.3** – Number of individuals of Perciformes collected in (a) 2018S2P2, (b) 2019S2P1, (c) 2019S1P2, (d) 2019S2P2, (e) 2019S1P3, as a function of the stage of decomposition.

The amphibians were all belonging to the *Bufo bufo* species and were present during all stages of all carcasses for both spring-summer sessions (Fig. 3.4.4).



**Fig. 3.4.4** – Number of individuals of Anura collected in (a) 2018S1P1, (b) 2018S1P2, (c) 2018S1P3, (d) 2019S1P1, (e) 2019S1P2, (f) 2019S1P3, as a function of the stage of decomposition.

The sampled actinopterygians play a role of predators of various macroinvertebreats (Tortonese, 1998; Tortonese, 2000). The tadpoles of *Bufo bufo* assume the role of omnivores (Lanza *et al.*, 2016); however, in the present work, we observed that they fragmented and shredded the cadaveric substrate and they therefore played the role of shredders.

#### 3.5 Comparison of the Pre-Operational Sampling between 2018 and 2019

Remarkable differences in abundance values between pre-operational sampling of 2018 and 2019 were identified. This variation is observed as an increase of 472% in the number of sampled macroinvertebrates (4051 individuals in 2019) compared to the 859 organisms found in 2018. The increase mainly involved Clitellata (approx. +323%) and Insecta (approx. +647%). This outstanding increase in abundance of Clitellata was not accompanied by an equivalent increase in taxonomic groups; it essentially depended on variations affecting single species already present such as Nais communis, a detritivorous belonging to oligochetes. If we consider the data of the 3 points of sampling separately, the only group showing a certain decrease was the adenophores. In 2018 in P3 the clitellates had the least abundance. On the contrary, for this group in 2019 in P3 a value almost 4-fold higher than the remaining sampling points was sampled. A significant increase in the number of taxa was also detected. In particular, insects were represented by as many as 26 more taxonomic groups. In the other classes, Clitellata and Gastropoda showed an increase of 3 taxa, while in Adenophorea and Trepaxonemata the increase was 2 and 1 taxa, respectively. In 2019, in P3, there was the appearance of bryozoans, not reported in 2018. The number of taxa did not show remarkable differences in the classes except for an increase of 12 taxa in Insecta at P3.

The comparison of the 3 points of sampling showed that insects was the group most affected by the organic matter. In fact, at all points of sampling, in 2019 there were significantly higher abundances than in 2018, for almost all orders. The only ones to show a slight decrease were the Trichoptera, which in P2 recorded a dozen fewer elements in 2019 than in 2018, and the Coleoptera, totally absent in P1 in 2019. Among all orders, the Diptera showed the greatest increase.

In 2019 an increase in taxa for the different points of sampling was detected. In most cases the change consisted of an increase of a few units, as in Hemiptera (which in the previous year had not been reported) and Odonata. The number of collected taxa, which also included all clitellates, decreased at all 3 sampling points in the pond. For some taxa a decrease in taxonomic groups was observed, such as the decrease of Diptera in P2 and Ephemeroptera in P3. The most conspicuous increase in taxa was observed for Trichoptera at P3. This decreasing trend was mainly due to a decrease in the taxa of Diptera Chironomidae, another large group of collectors, particularly marked in P2 and P3, compared to 2018. Within Insecta, Diptera were by far the dominant order, both in terms of number of individuals and taxa.

Considering the number of taxa for the different trophic roles sampled in the two years, a decrease in collector-gatherers was observed at all points of sampling, in particular at P2. The

greatest growth was recorded at P3 by predators and shredders: the former with doubled the number of taxa, the latter, absent in 2018, counted 7 taxa in 2019. Even generalists showed a consistent increase at P3.

### **3.6 Trophic Roles and Ecological Indices**

The number of sampled individuals and taxa may be influenced by the number of sampling days for each stage. For this reason, the absolute values were accompanied by the values divided by the sampling effort (s.e.) calculated for each stage. The smaller the value of s.e., the larger the weight assigned to that stage. However, in general, considering the absolute values and those divided by s.e., the general picture that emerged was unchanged. Exceptions to the maintenance of proportions were observed for the number of individuals in 2018 S2P3 (stage 1) and 2019 S2P1 (stage 5), and for the number of taxa in 2018 S2P3 and 2019 S2P1 (stage 1), 2019 S1P2 and P3 (stage 4), 2018 S1P1, P2 and P3 2019 S1P1, P2 and P3 (stage 5).

Figs. 3.6.1 - 3.6.5 show the trends, for each stage of degradation, of the number of individuals and taxa as a function of the trophic role played for all 12 carcasses studied in the two years, both considering the absolute values and the values divided by the corresponding sampling effort. The trophic roles considered were: collectors-filterers, collectors-gatherers, shredders, generalists, predators/parasites/parasitoids and grazers/scrapers. For the trophic roles, the data concerning the chironomids were excluded from the sampled fauna since it was not possible to determine them at a taxonomic level lower than the sub-family and, therefore, defining their trophic role for the most part was not possible.

On the basis of the number of individuals, the trophic groups most represented during the first stage of degradation were that of shredders and that of collector-gatherers, followed by predators (Fig. 3.6.1a). The highest numbers of individuals during the first stage were generally recorded during the spring-summer session of 2019.

Considering the number of taxa, the values between the different trophic roles were more uniform. The trophic groups found during this stage on the greatest number of carcasses were the collector-gatherers (found on 8 of the 12 carcasses), the predators (found on 7 of the 12 carcasses) and the shredders (collected from 6 of the 12 carcasses) which were also the three trophic groups with the highest taxa values in this stage (Fig. 3.6.1b).

Also in the second stage, the trophic roles most represented in terms of number of individuals remained the collectors-gatherers and the shredders followed by predators (Fig. 3.5.2a). The different trophic roles were present on a greater number of carcasses. Also in this case the number of taxa with respect to that of individuals was more uniform between the different trophic roles and there was a generic increase in predator taxa with the exception of the S1P1 of 2019 only (Fig. 3.6.2b). The late summer sessions were more represented than the first stage of degradation both in terms of number of individuals and number of taxa.

In the third stage there were fewer individuals of predators but distributed on all 12 carcasses. In general, there was a decrease in the number of individuals of all trophic roles in the different carcasses with the exception of the S2P2 collector-filter units of 2018 which, on the contrary, grow significantly (Fig. 3.6.3a). With the exception of this case, once again the most represented taxonomic groups were generally that of shredders and collector-gatherers. Also in this case, the number of taxa with respect to that of individuals for the different trophic roles was more uniform and the roles that present a greater abundance of taxa were predators and collector-gatherers (Fig. 3.6.3b).

From stage 3 to stage 4, the number of individuals of most of the trophic roles for almost all carcasses increased, in some cases even significantly. The shredders of the 2019 spring-summer session were an obvious exception to what had just been written. The most represented trophic group was that of the collectors-gatherers; collectors-filterers and grazer/scrapers were well represented in the 6 casings of 2018 while they were almost completely missing for this stage in the casings of 2019 (Fig. 3.6.4a). The most common trophic roles among carcasses at this stage (collected on 11 of the 12 carcasses) were collector-gatherers and predators. As for the number of taxa found, the most represented were always collector-gatherers and predators (Fig. 3.6.4b).

In the fifth stage, a large number of individuals belonging to the collector-gatherers were collected especially during the spring-summer session of 2018, and of filterer collectors in S1P2 of 2018. The trophic roles present in all 12 carcasses were the collector-gatherers and predators (Fig. 3.6.5a). Even for the number of taxa determined, the highest values were obtained for predators and collector-gatherers. Shredders, generalists and grazer/scrapers were very little represented (Fig. 3.6.5b).



Fig. 3.6.1 – Stage 1. Absolute number of individuals and taxa (panels (a) and (b), respectively) and values divided by sampling effort (panels (c) and (d), respectively) collected from the 12 carcasses depicted on the basis of their trophic role.





Fig. 3.6.2 – Stage 2. Absolute number of individuals and taxa (panels (a) and (b), respectively) and values divided by sampling effort (panels (c) and (d), respectively) collected from the 12 carcasses depicted on the basis of their trophic role.



Fig. 3.6.3 – Stage 3. Absolute number of individuals and taxa (panels (a) and (b), respectively) and values divided by sampling effort (panels (c) and (d), respectively) collected from the 12 carcasses depicted on the basis of their trophic role.



Fig. 3.6.4 – Stage 4. Absolute number of individuals and taxa (panels (a) and (b), respectively) and values divided by sampling effort (panels (c) and (d), respectively) collected from the 12 carcasses depicted on the basis of their trophic role.



Fig. 3.6.5 – Stage 5. Absolute number of individuals and taxa (panels (a) and (b), respectively) and values divided by sampling effort (panels (c) and (d), respectively) collected from the 12 carcasses depicted on the basis of their trophic role.

In Figs. 3.6.6-9 individuals and taxa were reported for the different trophic roles according to the stage for each carcass. In general, the spring-summer sessions showed communities in the various stages with a greater number of trophic roles than the late-summer sessions. Shredders were mostly represented during the first 3 stages of degradation and, during S1 of 2018, the first 4 stages; generally they were not present and, when present with few individuals and taxa, during stage 5. Predators and collector-gatherers were the most represented trophic groups, they were always present with the only exception of S2 of 2019 in which they were less represented than the previous ones experimental sessions although in S2P3 they were the only two trophic groups found.



**Fig. 3.6.6** – Number of individuals (a, c, e) and number of taxa (b, d, f) of the different trophic roles collected in 2018S1P1 (a, b), 2018S1P2 (c, d) and 2018S1P3 (e, f), as a function of the stage of decomposition.



**Fig. 3.6.7** – Number of individuals (a, c, e) and number of taxa (b, d, f) of the different trophic roles collected in 2018S2P1 (a, b), 2018S2P2 (c, d) and 2018S2P3 (e, f), as a function of the stage of decomposition.



**Fig. 3.6.8** – Number of individuals (a, c, e) and number of taxa (b, d, f) of the different trophic roles collected in 2019S1P1 (a, b), 2019S1P2 (c, d) and 2019S1P3 (e, f), as a function of the stage of decomposition.



**Fig. 3.6.9** – Number of individuals (a, c, e) and number of taxa (b, d, f) of the different trophic roles collected in 2019S2P1 (a, b), 2019S2P2 (c, d) and 2019S2P3 (e, f), as a function of the stage of decomposition.

Fig. 3.6.10 shows, for each trophic role, the number of individuals divided by the s.e. for the different stages of carcass degradation. The collector-filterers were absent in 2019 S2; in 2019 S1 they were found in little amount, while in 2018 they were much more present with the highest values during stages 4 and 5. The collector-gatherers were always present excepted in 8 among the 60 stages; the values in 2018 were averagely higher than those in 2019 for both S1 and S2. For shredders, the values were higher in 2019 than in 2018; in 2019 the values for the first 3 stages were much higher than the remaining ones while in 2018 they were distributed more evenly. Predators were well distributed over most of the stages (49/60).

Fig. 3.6.11 shows the sums (over the three carcasses of the same experimental session) of the number of individuals divided by the sampling effort. The collector-gatherers indicated a trend over the stages and experimental sessions that may be superimposed on the trend shown by the predators; this was reasonable considering that the former were the main preys of the latter.

Fig. 3.6.12 shows the trend in the number of taxa of the different trophic roles divided by the sampling effort for the different stages in the sessions of both years for each carcass. In general, these data were more uniform than those in Fig. 3.5.10. For predators there was always an increase when passing from stage 3 to 4 and from 4 to 5 in 2018 S1. Also in 2019 S2 they were always present in the last 3 stages of degradation.





C.F.

C.G.







G.



P.P.P.



G.S.

**Fig. 3.6.10** – Sum (calculated over the points of sampling) of the number of individuals divided by s.e. as a function of the year, experimental session, point of sampling and stage. Each panel refers to a different trophic role.











S.



G.







G.S.

**Fig. 3.6.11** – Sum (calculated over the points of sampling) of the numbers of individuals divided by s.e. as a function of the year, experimental session and stage. Each panel refers to a different trophic role.











S.











G.S.

**Fig. 3.6.12** – Sum (calculated over the points of sampling) of the number of taxa divided by s.e. as a function of the year, experimental session, point of sampling and stage. Each panel refers to a different trophic role.

Figs. 3.6.13 and 3.6.14 show the graphs relating to the three ecological indices calculated for the communities of the individual stages of degradation. Concerning the Shannon-Wiener index, the higher values of H' were observed in the first two stages for the S1 of both years. With the exception of only S2P1 of 2019, the values of H' during stage 2 were always greater than those calculated for the third stage of degradation. Concerning the Margalef index, a decrease in this value was always observed between stage 2 and stage 3 and an increase between stage 3 and stage 4 for the whole of 2018 and the first experimental session of 2019. Concerning the Pielou index, in general, in 2018 the values for this index were greater during S1 than S2 while in 2019 the differences were smaller between the two experimental sessions and frequently the values were larger for S2 than S1.


Fig. 3.6.13 – Values of ecological indices calculated for the different stages of carcass degradation in the two experimental sessions of 2018. Shannon-Wiener index (H'), Margalef index (D) and Pielou index (J).



Fig. 3.6.14 – Values of ecological indices calculated for the different stages of carcass degradation in the two experimental sessions of 2019. Shannon-Wiener index (H'), Margalef index (D) and Pielou index (J).

#### **3.7 Statistical Analysis**

The data matrix obtained following the taxonomic determination was used to carry out the Shapiro test (W) in order to verify the normal or non-normal trend of the data. The obtained W value (significance level < 0.001) showed that the samples had a non-normal distribution.

We proceeded with MANOVA in order to detect the possible presence of significant differences between the data collected, considering as parameters to be correlated year and experimental session, points of sampling and stage of carcass degradation. PCAs were performed in order to evaluate and obtain a graphical representation of the variance of the two data sets.

Experimental session 1 was characterised by clustered points, with two outliers. Experimental session 2 also presented very clustered points on the diagram and the *p*-value (0.0053) showed a difference in terms of variance between the first and second experimental session (Fig. 3.7a). The axes showed the two main components that had a greater weight on the variance of these two data (the seasons).

In Fig. 3.7b a similar case was presented considering the points of sampling instead of the experimental sessions. Although P1 was the most widely distributed, most of its points were in the same area as the points of P2 and P3 (p-value = 0.0092).

In Fig. 3.7c the degradation stages of the carcasses were taken into consideration; the p-value showed a high value (0.2919), therefore there did not seem to be any significant differences.

Significant differences were observed (p-value = 0.0001) by comparing the data for the year 2018 with those for 2019 (Fig. 3.7d)

However, from the statistical analyses carried out on the 60 observations considered (5 stages for 3 carcasses for two experimental sessions for two years) and related to all the sampled taxa, it emerged that: (1) the 2 sessions, the 3 points of sampling and the 2 years were significantly different from each other; (2) the 5 stages of decomposition, on the other hand, did not present significant differences. This picture confirms what had been previously deduced on the basis of the trends in the number of individuals and number of taxa as a function of the stage of decomposition for the 2 years, for the 2 sessions, for the 3 points of sampling. The apparent inexplicable non-difference between the 5 stages was explained on the basis of the MANOVA results.

Considering the interaction between the sampled taxa and the degradation stages of the carcasses, it was observed that 24 taxa were statistically significantly correlated (see Tab. 3.7).

Of these 10 were taxa that play the trophic role of predators of which 4 (Corixinae, Gerris lacustris, Hydrometra stagnorum and Micronecta scholtzi) had a life cycle closely linked to the surface of the water and it therefore was reasonable that these taxa were more closely related to carcasses in the stages in which this floats, in particular stages 2 and 4 remaining little present during stage 3 to avoid becoming fish prey themselves. Their presence in stage 5 (especially Corixinae and Micronecta scholtzi) was probably attributable to the use by these taxa of the bones (now on the bottom of the cage) as an attachment point and holes for ambushes for prey. Lepomis gibbosus and Silurus glanis, on the other hand, seemed significantly linked to the stages that presented a high source of food coming from the larval masses of terrestrial dipters. There were also practically constant presences during the degradation represented by *Platycnemis* pennipes (a predator odonate) and by the sub-family Chironominae (the taxonomic grouping most represented as number of individuals in most of the samples) for which, however, it was not possible to determine more specific and an accurate reading of this data would be therefore questionable. The shredders limnephilid Limnephilus flavicornis, L. flavospinosus, L. rhombicus and Halesus radiatus also showed a good level of significance when compared with the degradation stages, in fact they had been always found during the first 3 stages when organic matter was still present from fragment.

<i>p</i> -value < 0.0	50	<i>p</i> -value = 0.051 ÷	- 0.099
Taxa	<b>Trophic Role</b>	Таха	<b>Trophic Role</b>
Lymnaea truncatula	G.S.	Nais communis/variabilis	C.G.
Ferrissia clessiniana	G.S.	Enchytraeidae n.d.	C.G.
Cloeon gr. dipterum	C.G.	Erythromma viridulum	P.P.P.
Caenis luctuosa	C.G.	Bidessus minutissimus	P.P.P.
Platycnemis pennipes	P.P.P.	Culex territans	C.F.
Corixinae n.d.	P.P.P.	Chironominae n.d.	-
Micronecta scholtzi	P.P.P.	Sciomyzidae n.d.	P.P.P.
Gerris lacustris	P.P.P.	Limnephilus flavicornis	S.
Hydrometra stagnorum	P.P.P.	Halesus radiatus	S.
Limnephilus flavospinosus	S.	Plumatella sp.	C.F.
Limnephilus rhombicus	S.	Lepomis gibbosus	P.P.P.
Silurus glanis	P.P.P.		
Bufo bufo	S.		

Tab. 3.7 – Taxa with the respective trophic roles which were statistically significant upon MANOVA.



Fig. 3.7 – Graphical representation of the PCA carried out on the data considering: the experimental sessions (a), the sampling points of sampling (b), the degradation stages of the carcasses (c), year (d).

# Chapter 4

# **Discussion and Conclusions**

## 4.1 General Remarks

Despite its artificial origin, the study area has a high ecological value, hosting a large number of taxa and organisms.

Concerning the pre-operational sampling, the comparison between the two years clearly shows an increase of more than 1500 specimens and almost 30 taxa. Most of the diptera belong to Chironomidae which, besides being the most diversified family of freshwater insects, is characterized by species with a great variety of trophic habits (Byrd & Castner, 2001). The increase in taxonomic groups that fall into certain trophic roles, especially generalists and predators, largely depends on the greater number of diptera taxa found in 2019. The study area has a high ecological value hosting a large number of taxa and organisms. Furthermore, although of artificial origin, it can be fully compared to other natural wetlands of the same area; just think that in the Sassomassiccio pond, at the W.W.F. of Sassoguidano (Pavullo nel Frignano), 62 macroinvertebrate taxa have been reported (Benassi *et al.*, 2020), less than half of those detected thanks to this sampling. Taking into account the considerable heterogeneity of the variations found, it is not possible to indicate as a causal factor only the organic material coming from the carcasses.

Initially, six stages of decomposition were observed by Payne and King (1972), and later on, these stages were revised and reduced to five because distinguishing the last two stages was very difficult. In this study, five decomposition stages were observed; and these were submerged fresh, early floating, floating decay, advanced floating decay, and sunken remains. In contrast to this study, Merritt and Wallace (2001), and Hobischack (1998) studied six stages of decomposition. Barrios and Wolff (2011) also studied the colonization of the entomofauna in pig carcasses in two freshwater habitats in Colombia. In contrast to this study, six decomposition

stages were observed in both water systems, whereas five decomposition stages were noted in the present study.

The values of the monitored physico-chemical parameters of the pond (pH, conductivity and dissolved oxygen) show values within the seasonal averages and fluctuations for Apennine ponds for the whole duration of the experiment (fig. B.1.1 - B.1.6). The values of nitrogen, phosphorus and chlorophyll a frame this environment between mesotrophic and eutrophic according to the limits provided by the OECD (Organization for Economic Cooperation and Development). The findings of this work were confirmed by the analyses provided by Arpae (tab. B.2.1). According to Rossaro (1991) and Kagalou *et al.* (2006), dissolved oxygen and temperature are among the main factors that may influence the structure of macrozoobenthic communities. Furthermore, temperature is the physical parameter that most influences the rate of degradation of carcasses (Wallace, 2015) especially when high values are recorded during the first stages.

At the best of our knowledge, one of the used model describing the mass loss as a function of time during a decomposition process is exponential in nature (Olson, 1963). It was derived for the decomposition of dead organic vegetal matter, under the assumption that the mass loss is constant over time with respect to the remaining amount of matter. Despite of its simplicity, this model is hardly applicable to the systems investigated: (i) the type of matter is different; (ii) this implies that in the case of vegetal matter, the mass can tend to zero for long periods of time, whereas in the case of mammalians, a residual mass mainly constituted by bones remains even for long periods of time after the fifth degradation stage; (iii) the assumption that the mass loss is constant over the time is not realistic in the case of decomposition of animals' carcasses until the temperature is constant over the whole decomposition time interval (Wallace, 2015), which was not verified in the present study; (iv) evaluating the trend of the carcasses' mass as a function of time would have dramatically affected the communities, whose study remains the main purpose of this thesis. For these reasons, a different approach was adopted and another model that allows to determine the average decomposition rate developed. It was evidenced that other physicchemical environmental parameters than the water temperature affect the average decomposition rate, such as the conductivity, pH and the % dissolved oxygen (Fig. 3.1.3). The dependence of  $\langle v_{rel} \rangle$  from the temperature and the other parameters may be described by means of first order Taylor expansions, as follows:

$$\langle v_{rel} \rangle = f(u) \cong \langle v_{rel} \rangle_0 + k(u - u_0), \tag{4.1}$$

where u = T,  $\chi$ , pH, or  $[O_2]$ %, and  $k \equiv \left(\frac{\partial \langle v_{rel} \rangle}{\partial u}\right)_0$ . The experimental values confirm that a first order (*i.e.* linear) model suffices for a phenomenological description. The regressions however are meaningful within the range of variability of the measured parameters.

A delay in decomposition was noticed in the aquatic environment compared with the terrestrial environment (Payne, 1965; Tomberlin & Adler, 1998; Tullis & Goff, 1987) because of reduced water temperature and delayed terrestrial succession owing to the submerged carcasses (Dillon, 1997). Decomposition in an aquatic habitat was found to be different from that in a terrestrial habitat as the decomposition stages are different in terms of habitat, decomposition period, and insect association. Also, in an aquatic environment, the decomposition duration greatly varies when it comes to season specificity (de Carvalho & Linhares, 2001). Seasonal water temperature played a great role in the decomposition of the pig carcasses in the aquatic environment (Dalal et al., 2020a). Tomberlin and Adler (1998) also studied the decomposition duration and the succession of insects on a rat carcass in an aquatic environment during the summer and winter seasons and observed differences in decomposition duration during the different seasons. The effect of various parameters on the decomposition process has also been studied by a number of researchers (Hobischak & Anderson, 2002; Tomberlin & Adler, 1998; Rodriguez, 1990). Ayers (2010) studied the differences in the decomposition patterns of terrestrial, freshwater, and saltwater habitats in Texas by using six pig carcasses. Various parameters were studied, such as the weight of the carcass, air temperature, water temperature, humidity, etc.. Various decomposition signs were studied under an aquatic environment in pig carcasses, *i.e.* bloating, wire indentations, skin sloughing, hair shedding, insect activity, disarticulation, putrefying odour, skin blackening etc.. The changes occurred differently in different seasons because the water temperature greatly affected the decomposition process in various seasons (Tomberlin & Adler, 1998; Payne & King, 1972; Anderson & Hobischak, 2004; Barrios & Wolff, 2011). MacDonell and Anderson (1997) studied the decomposition rate using the pig as an animal model. This description of decomposition based on these signs has also been studied by several researchers (Heaton et al., 2010; van Daalen et al., 2017; de Carvalho & Linhares, 2001; Rodriguez, 1990; Anderson & VanLaerhoven, 1996).

Results showed that the degradation is faster in S2 than in S1, because in S2 the stages with soft tissues residuals (whose degradation is influenced by temperature) are at a higher temperature, while in S1 the higher temperatures coincide with the last stages of degradation, when almost only bones (whose degradation is independent of the temperature) remain. In fact, during S2, when temperatures are higher in the first stages of degradation, the degradation times are shorter

than in S1. This is confirmed if the average rate of degradation for the first 4 stages is considered (Fig. 3.1.3), therefore those stages that still have soft tissues whose degradation is influenced by temperature. Moreover invertebrate growth rate is influenced primarily by temperature and food (Chaloner & Wipfli, 2002), which can be difficult to separate because food quality and quantity are often influenced by temperature (Anderson & Cummins, 1979; Sweeney, 1984). Food quality and quantity can affect the growth of most aquatic insect functional-feeding groups (Anderson & Cummins; 1979), including shredders (Sweeney et al., 1986), collectors (Ward & Cummins, 1979), scrapers (Hart & Robinson, 1990), and predators (Martin & Mackay, 1983). A gradient of nutritional quality exists for food items available to stream macroinvertebrates; higher-quality foods contain proportionally more digestible protein and other essential nutrients, and proportionally less recalcitrant structural materials or noxious substances. Food items arranged in order from the most to the least valuable are, respectively, animal material, followed by living algae, decomposing vascular plants, fine particulate detritus, dead leaf material, and wood (Anderson & Cummins, 1979). Salmon meat is, therefore, likely to be a valuable source of food for aquatic macroinvertebrates, influencing the growth and standing stock of taxa having the appropriate feeding morphology and behaviour.

Concerning the number of individuals and the number of taxa as a function of the stage of decomposition, the discovery of some taxonomic groups is to be attributed to an occasional presence of the same on the carcasses or near them. In fact, for these groups the number of individuals collected is extremely small (one or a few units) and their finding is quite occasional and often attributable to a single sampling. Many taxa that can be defined as occasional presences (19 out of 28) are predators or parasites and it is reasonable to think that they have been found on the carcass during their search for prey or hosts in the pond rather than for prey specifically related to the carcasses. A big presence of "not specialized" fauna in carrion decomposition is often acknowledged (Magni et al., 2008). This array of species was ignored for a long time, though their presence is very important as occasional active participant of body degradation. Also these taxa are potential contenders, predators and parasites of carrion fauna, able to partially or totally inhibit the development of some species usually used as foremost indicator of PMI. In some habitats the presence of this fauna is particularly high and when the body is positioned in ecotonal situation, the inference between different sources of degradation agents can complicate the scenario. A part of taxa (Trocheta bykowskii, larvae of the genus Gyrinus, Isoperla grammatica in P2, Dixa present only in S1) were probably found due to the exceptional conditions created during the first periods of the first experimental session of 2019 characterized by low temperatures and frequent rainfall which made the water colder and more

oxygenated (suitable for these taxa) but then, with the advance of the season, these environmental conditions changed and these taxa disappeared. There are also taxa that are well represented during the year but that are punctual presences if a particular experimental session such as for the leptocerid found as the only S2 tricopter in 2018, or if the year as the stoneflies represented in 2018 from only 3 specimens of Protonemura ausonia is considered, while this order is rather represented in the early stages of the S1 of 2019 or Notonecta viridis well represented in 2018 especially in P3 while it is completely absent in 2019 or the culicides well represented in 2018 and completely absent in 2019. Certain species colonize in a predictable sequence, as well, many species colonize seasonally. However, geographical region can have a major impact on terrestrial insect colonization and many other factors may impact aquatic fauna, such as running vs. standing water, season, etc. (Hobischak & Anderson, 1999). Concerning the seasonality of different taxa found, there are taxa that in the aquatic environment complete a part of their life cycle (typically the juvenile one) and end their life exclusively outside the water as adult forms (e.g. tricotterofauna). It is therefore not unusual that for these groups it was possible to find specimens even in large numbers during an experimental session, while in the following session there were no traces. As mentioned, this happens for the order of Trichoptera, which play an important role as shredders during the first experimental session of both years but disappear during the second sessions, concluding their life cycle as non-aquatic adults. Similar is the situation for Bufo bufo present with even thousands of individuals in S1 both years but with the evolution of the tadpole season they pass to the stage of young adults and abandon the strictly aquatic life. During the second experimental sessions, the high temperatures in the first stages of degradation greatly facilitate the carcass degradation, although there are fewer shredders specialized in this role; it is assumed that the activity of generalists may therefore suffice, which can use this resource more during these sessions, having fewer competitors for the fragmentation of dead organic substance. There are groups, even well represented, but closely linked to the conditions of oxygenation, lower temperature and, in R1, of partial current present during S1 such as the order Plecoptera, the genus *Baetis*, the order Amphipoda, the molluscs of the orders Neotenioglossa, Heterostropha and Veneroida which therefore are not found in other periods of the year when these conditions are lacking.

Considering the trend of the different trophic roles during the degradation stages of carcasses, it can be said that during the first two stages of carcass degradation the roles most represented in terms of number of individuals are the collectors gatherer and shredders, especially when considering the S1. This is in fact reasonable because shredders, precisely due to the nature of their trophic source and their role, fragment dead organic substance and therefore are among the

first consumers of the cadaveric substrate. Indeed, shredders are known to use coarse particulate organic material (CPOM) (Chaloner & Wipfli, 2002). By fragmenting the organic substance, they make it accessible and usable for the collectors that use fine particulate organic material (FPOM) (Chaloner & Wipfli, 2002). Overall, Chaloner and Wipfli (2002) and those of other studies in which streams were enriched with sucrose (Warren et al., 1964), cereal grain (Mundie et al., 1983), detritus (Richardson & Neill, 1991), or salmon carcasses (Wipfli et al., 1998, 1999), suggested that collectors and shredders should respond most strongly to the presence of decomposing salmon carcasses in streams. Collectors are the dominant functional-feeding group of macroinvertebrates in southeastern Alaska streams (Oswood et al., 1995). If the number of taxa in these stages is considered, predators are equally represented. During the third stage of degradation there is a downsizing both of the number of individuals and of taxa found which is supposed to be linked to the high competition that is formed during this stage with organisms of terrestrial origin (in particular larvae of calliphorids and sarcophagids) for the cadaveric substrate. This may be also caused by effects of non-consumptive mortality effects like the degree of natural senescence, physiological intolerance (e.g., temperature, physical and chemical thresholds) (Benbow et al., 2020). For instance, non-consumptive mortality is a natural part of the population dynamics of any species, but the overall magnitude is rarely quantified, except for studies related to aquaculture production of commercially important taxa (Rowe et al., 1989; Karunasagar et al., 1994; Chen et al., 1995; Lorenzen, 1996). Furthermore, the mere presence of predators is known to have negative effects on life history traits and fitness of many species (Preisser et al., 2005). Since many organisms are prey for a variety of predators, this collective effect of non-consumptive predation threats can lead to facilitated senescence within many animal populations, although this form of non-consumptive mortality has not been broadly studied. In general, non-consumptive predator effects have been documented to be as strong or stronger than direct predation effects for many species, and the non-consumptive predator presence effect is generally stronger in aquatic than terrestrial ecosystems (Preisser et al., 2005). Non-consumptive effects can come in the form of increased costs of defensive strategies that include lower mating success, increased vulnerability to other predators, energetic investments related to finding resources or defensive structures (e.g., morphology or biochemical), or through reduced survivorship (Kotler et al., 1993; Preisser et al., 2005; Sheriff et al., 2009). During stages 4 and 5, the numbers of individuals and taxa increase again, especially in the case of collector-gatherers and collector-filterers, while the shredders remain little represented. This can be explained by considering that in these final stages the organic substance remaining to be fragmented (CPOM) is now scarce, leaving room for finer particles exploited precisely by

collector-gatherers and filterers rather than shredders. Regarding the degradation of the S2 2019 carcasses, generally little data is available and this is closely linked to the speed with which the degradation took place during this experimental session.

Predators are quite well represented at all stages. In stage 3, like the other trophic roles, they are reduced in number (although together with the collector gatherer they are the most represented as number of taxa). This is probably explained for S2 by the high presence of terrestrial larvae during this stage which attracts not only invertebrate predators, but also fish, which in turn can also prey on the same invertebrate predators. In fact, Silurus glanis and Lepomis gibbosus often begin to appear during the third stage of degradation (especially during S2). The large number of predators in different stages of degradation might be due to the large number of another wellrepresented taxon, i.e. diptera chironomid. Chironomid midges were conspicuous colonists of salmon material in other studies (Minakawa, 1997; Chaloner et al., 2002), and their biomass can be increased by organic and inorganic enrichment (Warren et al., 1964; Mundie et al., 1983; 1991; Richardson & Neill, 1991, Perrin & Richardson, 1997). In environments with a low oxygen concentration, the Chironomidae family may become numerically dominant, due to the presence of hemoglobin in the hemolymph of some larvae (Noncentini, 1985). Organisms of the Chironominae subfamily have this characteristic, a taxon which is generally the most frequent. This fact is in agreement with what reported by Bazzanti et al. (1997), which recorded a dominance of Chironomini, in particular belonging to the genus Chironomus, in environments with average annual dissolved oxygen values between 4 and 5 ppm. In this work, annual average values of dissolved oxygen of  $(2.81 \pm 1.05)$  ppm and  $(4.47 \pm 0.69)$  ppm were recorded, in 2018 and 2019, respectively.

A large number of invertebrate predators (e.g., hemiptera and odonates) were revealed in samples of the different stages of degradation, including the fifth stage. Chaloner and Wipfli (2002) observed that the predator *Rhyacophila* also was expected to respond to the presence of salmon meat, given that predators by definition feed on animal material. Minakawa (1997) found that although the biomass of 2 predators, the stonefly *Sweltsa* and Tanypodinae chironomid midges, increased in the presence of salmon meat, no predators, including *Rhyacophila*, consumed salmon flesh. Chironomid midges, an important prey item, were abundant in rearing containers, but not all *Rhyacophila* species prey upon chironomids (Martin & Mackay, 1983). Other studies that added organic material to streams (e.g., Mundie *et al.*, 1983; Richardson & Neill, 1991) found little or no increase in densities or biomass of predators and scrapers,

although addition of inorganic fertilizer has increased scraper density (Mundie *et al.*, 1991) or biomass (Richardson & Neill, 1991).

Concerning the role played by the 5 species of the hoverfly family, it should be noted that they were found only during the fourth stage of degradation, thus showing a certain degree of specificity linked to this stage. In other studies, Syrphidae was found during the late stages of degradation (which correspond to stages 4 and 5 of the stages recognized in the present work). As noted by Martins *et al.* (2010), although its biology is incompletely known, *Ornidia obesa* frequently breeds on several types of semiliquid synanthropic material, such as animal dung, human feces, sewage, and rotten fruits and vegetables (Sack, 1921; Thompson, 1991; Whittington & Rotheray, 1997; Magni *et al.*, 2008; Carvalho Filho & Esposito, 2009). Moffatt in 2013 identified hoverfly larvae in skeletal human remains, hence a late stage of degradation.

Concerning the communities, those of the spring-summer experimental sessions are richer in taxa and individuals than the late-summer ones. Taxonomic composition in the spring study was greater than in the summer studies (47 versus 33 taxa) (Tabor et al., 2004). The number of taxa reported in similar studies on vertebrate carcasses was between 54 and 522. These included studies that reported 217 species in Tennessee (Reed, 1958), 522 species in South Carolina (Payne, 1965), 101 species in Hawaii (Richards & Goff, 1997), and 54 species in Brazil (Carvalho et al., 2000). The large range of taxa found in these studies can be attributed to differences in climate, sampling frequency, number of animal models that were used, and the reporting of arthropods other than insects. These and other factors are known to affect insect successional patterns on carrion (Anderson, 2001). This is probably caused by several factors: (1) a greater number of samplings carried out during S1 due to their longer degradation duration compared to S2; (2) the intermediate characteristics between the lentic and lotic environment of the points of sampling (especially P1) during S1 following meteorological phenomena of abundant rains and lower temperatures, which lowered the water temperature and created inlet streams originating precisely intermediate conditions, this is probably allowed the establishment of a greater number of taxa otherwise absent in the typically lentic character of the pond environment during S2. Because boundaries often blend elements from the adjacent patches (particularly in the case of ecotones), their structure and composition are often very different from the adjacent patches. Thus, boundaries offer unique habitats with relatively easy access to the adjacent communities. These diverse conditions enable these boundary zones to support species from adjacent patches, as well as those species adapted to the edge environment. As a

result, boundaries are often populated by a rich diversity of life (Smith & Smith, 2015); (3) seasonality, in fact, as already mentioned, several taxa very represented during S1, due to the very nature of their life cycle, are completely missing during S2: (4) since in S1 the degradation stages 1-4 lasted a much longer time than what happened during S2, this has probably allowed the establishment in longer times of more stable and complex communities during the springsummer sessions compared to the late-summer ones. In fact, even considering the trophic roles, during the S1 there are more variegated communities which often, during the various stages of degradation, present all or most of the 6 trophic roles identified. In S2, however, the communities in the various stages of degradation are rarely represented by all 6 trophic roles but are often limited to 2-3 trophic roles per stage. A relationship existing between community stability and diversity was indeed revealed and described by Lehman and Tilman (2000) and by Cardinale and coworkers (2013). Furthermore in R1, especially during S1, there are richer communities probably because it is an environment in which throughout the year there is abundant detritus (even if vegetal) and this may have allowed the establishment of trophic groups (and associated taxa) then they easily adapted to exploit the carcass as an alternative trophic source. In fact the presence of salmon meat used in a stream of Alaska probably also alters resource use by invertebrates, switching diets to salmon-derived FPOM or CPOM from detritus or leaves. For some macroinvertebrates, including caddisfly and chironomid taxa, such diet switching could increase use of other sources of CPOM and FPOM (Chaloner & Wipfli, 2002).

### 4.2 Community Composition and Trophic Role

In the Literature, other Authors have dealt with the decomposition of dead organic matter in an aquatic environment. Parmenter & Lamarra (1991) studied the decomposition of trout and ducks in a lentic system. Through this study it has been shown that the decomposition of vertebrate carcasses represents an important nutritional resource in certain aquatic ecosystems and can regulate the structure and functioning of entire natural communities. Similar experiments have been carried out using salmon as a model species in Northwest America (Chaloner & Wipfli, 2002). A research carried out in Italy took into consideration the degradation of trout carcasses in an Apennine lotic system; the research showed that the carcasses exhibited an exponential loss of mass over time and attracted a rich community of macroinvertebrates (Fenoglio *et al.*, 2005). Some decomposition studies have also been published in the literature, considering mammals as model animals. In particular, rabbits were studied in the terrestrial (Mashaly *et al.*, 2018) and

freshwater environments (Dalal *et al.*, 2020b); pigs in the marine (Anderson & Bell, 2016) and freshwater environments (Dalal *et al.*, 2020a; Hobischak, 1997; Vance *et al.*, 1995; Barrios & Wolff, 2011) were also investigated. In the following a comparison between the findings of the present work and those reported by four of the aforementioned works: Hobischak (1997), Barrios and Wolff (2011) (which inspired the design of the protocol adopted in the present work), and the two works by Dalal *et al.* (2020), will be reported. To facilitate the comparison, only data concerning aquatic insects at the family level collected from a lentic environment for all of the studies were accounted for. Furthermore, with regard to the data collected in this study, the overall data sets at the level of the experimental session were considered, thus merging the data collected in the 3 different sampling points for a single experimental session.

Concerning the characteristics of the biotope, there exist evident differences between the environment investigated in the present study and those employed in the aforementioned Literature. The study areas are substantially different from each other in terms of geographical regions, altitude and climate features, and in particular the average air temperature. The present work was conducted in the Modena Appennins (Italy) at an altitude of 865 m a.s.l.; the average air temperature varies between about 5.7 and 30 °C. In Hobischak (1997) the study was conducted in British Columbia (Canada), at an altitude between 175 and 350 m a.s.l.; the average air temperature varies between about -10.7 °C and 27.8 °C. The degradation of four pig carcasses was investigated during an entire year, by taking into account 5 degradation stages. In both Dalal's works, the study was conducted in Rohtak City, state of Haryana (India), at an altitude of 220 m a.s.l.; the average air temperature varies between about 14.1 and 34 °C. Each study focused on a different animal model, *i.e.* rabbit and pig, whose degradations were sampled over an entire year considering 5 degradation stages. In Barrios and Wolff (2011) the study was conducted in Colombian Andes, at an altitude of 2636 m a.s.l.; the average air temperature varies between about 10 and 17 °C. Therefore the comparison with this study can only be reasonable with the results obtained during the spring-summer sessions of the present study. A pig carcass degradation was investigated from January to April 2007, considering 6 stages of degradation. The two degradation stages acknowledged by Barrios and Wolff as "floating decay" and "bloated deterioration" may be unified since they correspond to stage 4 of the present work; the data corresponding to these two stages were therefore merged and accounted as they came from stage 4.

Despite the evident differences mentioned, it is however useful to carry out a comparison with respect to these studies, because this will allow to highlight the aspects that can be generalised

from those more specific for a single biotope. Tab. 4.2.1 collects the names of insect families found in this work during S1 and S2 of both 2018 and 2019.

2018S1	2018S2	201981	201982
Baetidae	Baetidae	Baetidae	Baetidae
Caenidae	Caenidae	Caenidae	Caenidae
Lestidae	Platycnemididae	Leptophlebiidae	Platycnemididae
Platycnemididae	Coenagrionidae	Platycnemididae	Coenagrionidae
Coenagrionidae	Aeshnidae	Coenagrionidae	Aeshnidae
Aeshnidae	Corixidae	Aeshnidae	Corixidae
Libellulidae	Nepidae	Perlodidae	Nepidae
Nemouridae	Notonectidae	Nemouridae	Gerridae
Corixidae	Gerridae	Corixidae	Veliidae
Nepidae	Veliidae	Gerridae	Hydrometridae
Notonectidae	Hydrometridae	Veliidae	Dytiscidae
Pleidae	Dytiscidae	Hydrometridae	Helophoridae
Gerridae	Helophoridae	Haliplidae	Hydrochidae
Veliidae	Hydrochidae	Dytiscidae	Hydrophilidae
Hydrometridae	Hydrophilidae	Helophoridae	Hydraenidae
Gyrinidae	Hydraenidae	Hydrochidae	Ceratopogonidae
Dytiscidae	Dryopidae	Hydrophilidae	Chironomidae
Helophoridae	Limoniidae	Hydraenidae	Syrphidae
Hydrochidae	Psychodidae	Scirtidae	
Hydrophilidae	Culicidae	Dryopidae	
Hydraenidae	Ceratopogonidae	Sialidae	
Dryopidae	Chironomidae	Limoniidae	
Limoniidae	Tabanidae	Tipulidae	
Tipulidae	Sciomyzidae	Psychodidae	
Psychodidae	Leptoceridae	Dixidae	
Dixidae		Ceratopogonidae	
Chaoboridae		Chironomidae	
Culicidae		Stratiomyidae	
Simuliidae		Rhagionidae	
Ceratopogonidae		Empididae	
Chironomidae		Syrphidae	
Stratiomyidae		Sciomyzidae	
Tabanidae		Muscidae	
Dolichopodidae		Polycentropodidae	
Syrphidae		Limnephilidae	
Sciomyzidae			
Limnephilidae			
Leptoceridae			
Crambidae			

**Tab. 4.2.1** – List of insect families found during the four experimental sessions by adding the three sampling points for each session.

Concerning S1 of 2018, the families with common with (at least) one of the aforementioned studies were: Lestidae, Coenagrionidae, Aeshnidae, Libellulidae, Corixidae, Nepidae, Notonectidae, Gerridae, Gyrinidae, Dytiscidae, Hydrophilidae, Dryopidae, Chironomidae and Limnephilidae. By contrast, the other 25 of the 39 families found during S1 of 2018 were exclusive to this study. Ephemerellidae, Leptophlebiidae, Perlodidae, Elmidae and Brachycentridae were families found exclusively in Canadian work. Gomphidae and Phryganeidae were sampled only in the two works carried out in India and common to both of these works, whereas Noteridae were exclusive of Dalal's work carried out using the rabbit as an animal model. The Colombian work does not show exclusive families of its own. As regards Odonata in the present study, Coenagrionidae and Aeshnidae were always found in all stages of degradation whereas Lestidae and Libellulidae only in the last two stages. It was possible to compare the data concerning the lestids only with Dalal's studies, in fact in both cases this family was absent during the first stage and present in the fourth stage. A similar trend is observed for Libellulidae when comparing with the two Indian studies; on the other hand, if the data of the present study are compared with those of Hobischak, there was congruence for 4 out of 5 stages (the first 3 and the last) while comparing with the Colombian work for this family there was no congruence since Libellulidae were absent for the first 3 stages and present only in the last 2 in the present study, while for Barrios and Wolff they were present for the first 3 stages and absent for the last 2. Coenagrionidae and Aeshnidae were not found by Hobischak. In both of their studies, Dalal et al. find these two families only in stages 3 and 4, whereas Barrios and Wolff found Coenagrionidae during the first 2 stages and Aeshnidae during the first 3 stages. For the order Hemiptera, Corixidae were found common only with the study of Barrios and Wolff whereas Gerridae only with the study of Hobischak. The corixids were found by Barrios and Wolff in all the stages, whereas in the present work they were found only in stages 2, 4 and 5. As in this study, the gerrids were found by Hobischak with a correspondence for the first 4 stages, whereas for the stage 5 were found in the present study, but not in the Canadian one. Notonectidae were found in all stages in the Barrios and Wolff study as well as in the present study, showing a congruence only for the last 3 degradation stages. Nepidae were found only in the two Indian studies in addition to ours showing coincidence of occurrences only in stages 4 and 5; moreover, in all 3 studies it was absent during stage 2. As regards Coleoptera, Gyrinidae was found, as well as in the present study, only by Barios and Wolff (as in this work) in stage 4, but in the Colombian study it was also found during stage 3. Dryopidae were found only by Hobischak and by us; in the present study it was found during stages 2 and 4, whereas in the Canadian study only during the second stage. Dytiscidae and Hydrophilidae were both found in

all the works considered. Dytiscidae were found in the present study in stages 2, 4 and 5; Dalal and Hobischak found them only in stages 4 and 5 whereas Barrios and Wolff in stages 2 and 3. The only family of Diptera found in other studies besides ours was that of chironomids. This family was well represented in all stages of degradation in the present study. This was confirmed by the study of Hobischak and Barrios and Wolff; Dalal found chironomids in the first 4 stages studying the pig carcass and in the first 3 stages studying the rabbit carcass. As for Diptera, also for Trichoptera only one family was found in other studies besides ours: Limnephilidae. Apart from the study of Barrios and Wolff (in which no trichoptera were found), this family was found in all the other 3 studies considered. In this study, limnephilids were sampled during the first 4 stages whereas by Hobischak in all 5 stages; Dalal found these trichoptera only in stages 2, 3 and 4 when used pig as an animal model whereas when used the rabbit only in stages 3 and 4. Among the studies considered there is a correspondence with the present study of 9 families with both Dalal's works, 7 families with Hobischak's work and 9 families with Barrios' and Wolff's work. The families that coincide (in terms of presence or absence) in the different stages with 2018 S1 are: 24/45 in the study of Barrios and Wolff, 29/35 in the study of Hobischak, 23/45 in the study of Dalal with rabbit as an animal model, 25/45 in that with pig as an animal model. Tab. 4.2.2 summarises the comparison between session 2018S1 of this work and the cited Literature, stage by stage (1 to 5), about the different families.

			*					**					***					****		
Family	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Lestidae	0	0	0			0	0	0			0			Х		0			Х	
Coenagrionidae						Х	Х					Х	Х				Х	Х		
Aeshnidae						Х	Х	Х				Х	Х				Х	Х		
Libellulidae	0	0	0		Х						0			Х		0			Х	
Corixidae	0		0				Х		Х	Х	0		0			0		0		
Nepidae		0	0				0	0				0		Х	Х		0		Х	Х
Notonectidae	0	0						Х	Х	Х	0	0				0	0			
Gerridae	Х	Х	0	Х				0					0					0		
Gyrinidae	0	0	0		0	0	0		Х	0	0	0	0		0	0	0	0		0
Dytiscidae	0		0	Х	Х	0	Х				0		0	Х	Х	0		0	Х	Х
Hydrophilidae	0		0	Х	0		X		Χ		0			Х		0			Х	
Dryopidae	0	Х	0		0	0		0		0	0		0		0	0		0		0
Chironomidae	X	X	Х	X	X	X	X	X	X	Χ	Х	Χ	Χ	Х		Χ	Χ	Χ		
Limnephilidae	Χ	Х	Χ	Χ						0		Χ	Х	Х	0			Χ	X	0

**Tab. 4.2.2** – Comparison between session 2018S1 of this work and the cited Literature, stage by stage (1 to 5), about the different families. X = found in both this work and the cited Literature; O = found in neither this work nor the cited Literature; empty cell = no correspondence found.

\* (Hobischak, 1997)

\*\* (Barrios&Wolff, 2011)

\*\*\* (Dalal *et al.*, 2020a)

\*\*\*\* (Dalal et al., 2020b)

Considering S2 of 2018, the common families with at least one of the aforementioned studies were: Coenagrionidae, Aeshnidae, Corixidae, Nepidae, Notonectidae, Gerridae, Dytiscidae, Hydrophilidae, Dryopidae and Chironomidae. By contrast, the other 15 of the 25 families found during S2 of 2018 were exclusive to this study. Ephemerellidae, Leptophlebiidae, Libellulidae, Perlodidae, Elmidae, Brachycentridae, and Limnephilidae were exclusive families of the Canadian study and absent in 2018 S2. Lestidae, Gomphidae, Libellulidae, Phryganeidae and Limnephilidae were exclusive families of the two works carried out in India and common to both of these works whereas Noteridae were exclusive of Dalal's work using rabbit as an animal model. Comparing S2 of 2018 of the present study with the families found by Barrios and Wolff, the Colombian work presents two families instead absent in the present study: Libellulidae and Gyrinidae. Coenagrionidae in the present study were found during all stages of degradation whereas in the two Indian studies only in stages 2 and 3, in the Colombian study only in stages 1 and 2 and were not found in the Canadian study. The other family of odonates found in at least one of the other studies considered (Aeshnidae) shared the absence in the first stage and the presence in the stages 2 and 3 with the two Indian studies, was not found by Hobischak whereas Barrios and Wolff found this family during stages 1, 2 and 3 as opposed to the present study where it was sampled from stage 2 to 5. Corixidae were absent in both the Dalal studies and the Hobischak study; in the study of Barrios and Wolff they were found in all stages of decomposition whereas in the present study in stage 2 to 5. Nepidae were found only in the two Dalal studies, in both cases in stages 3, 4 and 5 whereas in 2018 S2 only in stage 2. Notonectidae were not found in the two Indian works nor in that Canadian; Barrios and Wolff found them during all stages of degradation whereas in the present study they were absent only during stage 3. Gerridae were found only in the Hobischak study and only during stage 4 whereas in the present study they were found in the stages 4 and 5. Dytiscidae were found in both Indian and Canadian works only in the last two stages of degradation; in the present work, only in stages 1 and 2 whereas in the Colombian work only in stages 2 and 3. Hydrophilidae was found in both Dalal's works in stages 2 to 5, from Hobischak only in stage 4, from Barrios and Wolff in all stages while in the present study in stages 2 and 5. Dryopidae were found only by Hobischak in stage 2 whereas in the present work only in stage 4. The trend of the Chironomidae family followed exactly what already described for 2018 S1. Among the studies considered there was a correspondence with the present study of 6 families with both Dalal studies, 5 families with the Hobischak study and 7 families with the Barrios and Wolff study. The families that coincide (for presence or absence) in the different stages with 2018 S2 were: 22/35 (Barrios and Wolff), 13/25 (Hobischak), 12/30 (Dalal; rabbit), and 13/30 (Dalal; pig). Tab. 4.2.3 summarises the comparison between session 2018S2 of this work and the cited Literature, stage by stage (1 to 5), about the different families.

**Tab. 4.2.3** – Comparison between session 2018S2 of this work and the cited Literature, stage by stage (1 to 5), about the different families. X = found in both this work and the cited Literature; O = found in neither this work nor the cited Literature; empty cell = no correspondence found.

			*					**					***					****	:	
Family	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Coenagrionidae						Х	Х					Х	Х				Х	Х		
Aeshnidae	0						X	Х			0	Х	X			0	Х	Х		
Corixidae	0						X	Х	X	Χ	0					0				
Nepidae	0		0	0	0	0		0	0	0	0					0				
Notonectidae			0			X	X		X	Χ			0					0		
Gerridae			0	X		0	0	0			0	0	0			0	0	0		
Dytiscidae			0				X		0	0			0					0		
Hydrophilidae	0		0				X			X	0				Х	0				X
Dryopidae	0		0		0	0	0	0		0	0	0	0		0	0	0	0		0
Chironomidae	Х	X	Х	X	X	X	X	X	X	X	X	X	X	X		X	X	Х		

\* (Hobischak, 1997)

\*\* (Barrios&Wolff, 2011)

\*\*\* (Dalal *et al.*, 2020a)

\*\*\*\* (Dalal *et al.*, 2020b)

Regarding S1 of 2019, the common families with at least one of the studies taken in comparison were: Leptophlebiidae, Coenagrionidae, Aeshnidae, Perlodidae, Corixidae, Gerridae, Dytiscidae, Hydrophilidae, Dryopidae, Chironomidae and Limnophilidae. By contrast, the other 24 of the 35 families found during S1 of 2019 were exclusive to this study. Ephemerellidae, Libellulidae, Elmidae, and Brachycentridae were exclusive families of the Canadian study and absent in 2019 S1. Lestidae, Gomphidae, Libellulidae, Nepidae and Phryganeidae were exclusive families of the two works carried out in India and common to both these works whereas Noteridae is exclusive of Dalal's work using rabbit as an animal model. The Colombian work presented three families instead absent in the present study: Libellulidae, Notonectidae and Gyrinidae. Leptophlebiidae were found only in the Hobischak study only in the fifth stage whereas in Susano they were found during stages 3 and 4. As regards Coenagrionidae and Chironomidae, the trend was similar to that described for 2018 S2. Aeshnidae were found in the present study only during stages 3 and 5, in the two Indian studies during stages 2 and 3, in the Colombian study during the first three stages of degradation while in the Canadian study it was never sampled. Periodidae were found only by Hobischak and only during stage 4 whereas in the present study during the first two stages of degradation. Corixidae were found only in the Colombian study during all stages whereas in the present study during stages 1, 4 and 5. Gerrids were found in the present study during stages 1, 3 and 5; in other studies they were found only by Hobischak during stages 1, 2 and 4. Dytiscidae were found in stages 1, 2 and 5; in both the Indian and Canadian studies only in stages 4 and 5 whereas Barrios and Wolff found this family during stages 2 and 3. Hydrophilidae were found in the present study in stages 1 and 2, in the two Indian studies, on the contrary, only in the last three stages, in the Canadian study only in the fourth stage whereas in the Colombian study they were found during all stages of degradation. Dryopids were found only in the present study during stages 1 and 2 whereas in the Hobischak study only during the second stage. In this study Limnephilidae were found during the first 3 stages of degradation, in the Colombian study were never found whereas in the Canadian one they were found during all stages; Dalal found limnephilids during stages 2, 3 and 4 (the pig study) and during stages 3 and 4 (the rabbit study). Among the studies considered there was a correspondence with the present study of 6 families with both Dalal studies, 8 families with the Hobischak study and 6 families with the Barrios and Wolff study. The families that coincided (presence/absence) in the different stages were: 16/30 (Barrios and Wolff), 16/40 (Hobischak), 12/30 (Dalal; rabbit), 14/30 (Dalal; pig). Tab. 4.2.4 summarises the comparison between session 2019S1 of this work and the cited Literature, stage by stage (1 to 5), about the different families.

**Tab. 4.2.4** – Comparison between session 2019S1 of this work and the cited Literature, stage by stage (1 to 5), about the different families. X = found in both this work and the cited Literature; O = found in neither this work nor the cited Literature; empty cell = no correspondence found.

			*			**							***			****				
Family	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Leptophlebiidae	0	0				0	0			0	0	0			0	0	0			0
Coenagrionidae						Х	Х					Х	Х				Х	Х		
Aeshnidae	0	0		0				Х	0		0		Х	0		0		Х	0	
Perlodidae			0		0			0	0	0			0	0	0			0	0	0
Corixidae		0	0			Х			Χ	Х		0	0				0	0		
Gerridae	X						0		0			0		0			0		0	
Dytiscidae			0		Х		Х		0				0		Х			0		X
Hydrophilidae			0		0	X	Х													
Dryopidae		X	0	0	0			0	0	0			0	0	0			0	0	0
Chironomidae	Х	X	X	Х	X	X	X	Х	Х	Х	Х	Х	Х	Х		X	Х	Х		
Limnephilidae	X	X	X						0	0		Х	Х		0			Х		0

\* (Hobischak, 1997)

\*\* (Barrios&Wolff, 2011)

\*\*\* (Dalal *et al.*, 2020a)

\*\*\*\* (Dalal et al., 2020b)

In S2 of 2019, the common families with at least one of the studies taken in comparison were: Coenagrionidae, Aeshnidae, Corixidae, Nepidae, Gerridae, Dytiscidae, Hydrophilidae and Chironomidae. By contrast, the other 10 of the 18 families found were exclusive to this study. Ephemerellidae, Leptophlebiidae, Libellulidae, Perlodidae, Dryopidae, Elmidae, Brachycentridae, and Limnephilidae were exclusive families of the Canadian study. Lestidae, Gomphidae, Libellulidae, Limnephilidae and Phryganeidae were exclusive families of the two works carried out in India and common to both these works whereas Noteridae were exclusive of Dalal's work using rabbits. The Colombian work presents three families instead absent in the present work: Libellulidae, Notonectidae and Gyrinidae. Coenagrionidae were present only in the last two stages of degradation, they were absent in the work of Hobischak, in the work of Barrios and Wolff were present only in the first two stages of degradation whereas in the two Dalal studies were present only in stages 2 and 3. In this work Aeshnidae were found only during the second stage, in the two Indian studies in the second and third stages, in the Colombian study during the first three stages whereas they were absent in the Canadian study. Croixidae were common only between the present study and that of Barrios and Wolff, in the present study they were sampled only during stages 4 and 5 whereas in the Colombian study they were present in all stages of degradation. Nepidae were only found in this work during stage 5 and in Dalal's two works for both during the last three stages. Gerridae were common to the present study (found

only in stage 5) and to the Hobischak study in which they were found in stages 1,2 and 4. Dytiscidae were found only in stage 5, in the two Indian studies and in the Canadian study in stages 4 and 5 whereas in the Colombian study in stages 2 and 3. Hydrophilidae were found in the present study in stages 2 and 5; in the two Indian studies in the last three stages, by Barrios and Wolff during all stages whereas by Hobischak only in stage 4. Chironomidae followed the same trend observed for the other experimental sessions. There was therefore a correspondence with the present study of 6 families with both Dalal studies, 4 families with the Hobischak study and 6 families with the Barrios and Wolff study. The families that coincided (presence/absence) in the different stages were: 15/30 (Barrios and Wolff), 12/20 (Hobischak), 17/30 (Dalal; rabbit), 18/30 (Dalal, pig). Tab. 4.2.5 summarises the comparison between session 2019S2 of this work and the cited Literature, stage by stage (1 to 5), about the different families.

**Tab. 4.2.5** – Comparison between session 2019S2 of this work and the cited Literature, stage by stage (1 to 5), about the different families. X = found in both this work and the cited Literature; O = found in neither this work nor the cited Literature; empty cell = no correspondence found.

			*					**					***					****	:	
Family	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Coenagrionidae	0	0	0					0			0					0				
Aeshnidae	0		0	0	0		Χ		0	0	0	Х		0	0	0	Х		0	0
Corixidae	0	0	0						Х	Х	0	0	0			0	0	0		
Nepidae	0	0	0	0		0	0	0	0		0	0			Х	0	0			Х
Gerridae			0			0	0	0	0		0	0	0	0		0	0	0	0	
Dytiscidae	0	0	0		Х	0			0		0	0	0		Х	0	0	0		Х
Hydrophilidae	0		0				Х			Х	0				Х	0				Х
Chironomidae	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х		Х	Х	Х		

\* (Hobischak, 1997)

\*\* (Barrios&Wolff, 2011)

\*\*\* (Dalal *et al.*, 2020a)

\*\*\*\* (Dalal *et al.*, 2020b)

In the following, the compare this work and the cited Literature about found/not-found families shall be presented (Tab. 4.2.6). No families were shared by the four aforementioned works, except those shared with ours and Gomphidae and Phryganeidae found in both studies carried out by Dalal *et al.*. (Both of these studies were carried out in the same geographical area.) 7 orders were found in common between the present study and the other studies: Ephemeroptera, Plecoptera, Diptera, Trichoptera (these with one shared family), Odonata, Hemiptera and Coleoptera (these with 4 shared families). Leptophlebiidae (Ephemeroptera) and Perlodidae (Plecoptera) were both found only during S1 2019 (the session of the present experiment that showed lower temperatures) and both were found only in the work by Hobischak. Similarly also

Dryopidae (Coleoptera) were found only in this work and in the Canadian work, but in the present study were sampled in both sessions S1 of 2018 and 2019. 3 families were found in all studies and in the present study in all 4 experimental sessions: Dytiscidae, Hydrophilidae (Coleoptera) and Chironomidae (Diptera). Libellulidae (Odonata) were also found in all the studies considered; in this study, however, they were only sampled during the S1 of 2018. Coenagrionidae and Aeshnidae (Odonata) were always found in the 4 experimental sessions of the present work and sampled both in the two Indian works and in the Colombian work. Corixidae and Gerridae (Hemiptera) were two families found in all the experimental sessions of this work, sometimes even with high numbers of individuals, but they were found respectively only in the works by Barrios and Wolff and Hobischak. Notonectidae (Hemiptera) and Gyrinidae (Coleoptera) were found in the present study in both sessions of 2018 (for notonectids) and only in S1 of 2018 (for gyrinids); both of these families were only reported in the Colombian study. Lestidae (Odonata) and Nepidae (Hemiptera) were sampled only in the present study and the two Indian studies; the first family only during S1 of 2018 whereas the second in both sessions of 2018 and in S2 of 2019. Limnephilidae (Trichoptera) were abundantly found in this study during both S1 2018 and S1 2019, and were found in both studies by Dalal and in that by Hobischak; in the work by Barrios and Wolff they did appear as well as trichoptera do not.

Order	Family		This	Work			Liter	ature	
		2018 S1	2018 S2	2019 S1	2019 S2	*	**	***	****
Ephemeroptera	Leptophlebiidae			×		×			
Odonata	Lestidae	×						×	×
	Coenagrionidae	×	×	×	×		×	×	×
	Aeshnidae	×	×	×	×		×	×	×
	Libellulidae	×				×	×	×	×
Plecoptera	Perlodidae			×		×			
Hemiptera	Corixidae	×	×	×	×		×		
	Nepidae	×	×		×			×	×
	Notonectidae	×	×				×		
	Gerridae	×	×	×	×	×			
Coleoptera	Gyrinidae	×					×		
	Dytiscidae	×	×	×	×	×	×	×	×
	Hydrophilidae	×	×	×	×	×	×	×	×
	Dryopidae	×	×	×		×			
Diptera	Chironomidae	×	×	×	×	×	×	×	×
Trichoptera	Limnephilidae	×		×		×		×	×

**Tab. 4.2.6** – Comparison between this work and the cited Literature about found ( $\times$ ) /not-found families. Those reported are the families which are common with at least one session of this work.

\* (Hobischak, 1997)

\*\* (Barrios&Wolff, 2011)

\*\*\* (Dalal et al., 2020a)

\*\*\*\* (Dalal et al., 2020b)

Although well represented in this study considering the numbers of individuals, several families were not found in the cited Literature. Indeed, among the mayflies, the most represented families were Baetidae and Caenidae, not found in the other works whereas in this work they were found during all the experimental sessions. In general, the order Ephemeroptera was not reported in any of the works considered except for the work of Hobischak who found leptophlebids and only in the last stage of degradation; in this study the presence of these mayflies was much more marginal with respect to the other two families. As for the odonates, with the exception of the Canadian work, wherein only one family is mentioned, in other works, including ours, they are well represented and with several families. What has been said previously about the families found in common with the other works must however take into account the total absence in the other studies of the family was found most (i.e. Platycnemididae), always present in all the sessions and often also the most represented in terms of number of individuals. For the Hemiptera, in the present study the greatest variability of families compared to other studies was found. In fact, among the studies considered, uncommon families were reported among them but they were all present in this study; moreover, Hydrometridae, although not abundantly represented, were still present during all the experimental sessions but not found in any of the other works. Among the Coleoptera at least three families (Hydrochidae, Hydraenidae and Helophoridae) not found in the other works were found in the present work during all the experimental sessions, sometimes even abundantly. The Diptera order was represented in the other studies by the Chironomidae family only; although this was the most represented family also in the present study, the families Psychodidae, Ceratopogonidae Limoniidae and Syrphidae are also all present in all or at least three of the four experimental sessions and in some cases even with high numbers of individuals.

Barrios and Wolff were the only who accounted for the trophic roles; furthermore, they investigated the degradation over a narrower period of time (four months). For these reasons the comparison between their work and ours deserves some more attention due to these similarities. Concerning the trophic roles, at the best of our knowledge, the only work reporting this classification in the Literature is Barrios and Wolff (2011). The aquatic entomofauna community collected during carcass degradation plays 3 trophic roles: shredders, collectors and predators; the communities of aquatic entomofauna collected in the carcasses studied in the present work during the S1 covers 6 trophic roles: shredders, collectors-gatherers, collectors-filterers, scrapers-grazers, predators and generalists. Concerning collector-gatherers, in the Colombian study the role is played by different taxa of the Chironomidae family (Diptera); in Italy, in addition to the chironomid diptera (which with the sub-families Chironominae and Orthocladinae, probably,

largely cover this role), other important groups of collector-gatherers present both in large numbers and recurrently during degradation are the Ceratopogonidae diptera and the mayflies of the Baetidae family. The shredders in the Colombian study are abundantly represented by hydrophilid beetles, while in this study the entomofauna with the role of shredders is mainly represented by trichoptera, hoverflies and stoneflies. On the other hand, observing the predators, there is a certain similarity between the communities sampled in the two studies; in fact all the orders and families collected by Barrios and Wolff were also found in this study: Odonata (with the families Libellulidae, Aeshnidae and Coenagrionidae), Hemiptera (with the families Corixidae and Notonectidae) and Coleoptera (with the families Dytiscidae and Gyrinidae). In 1995 Vance *et al.* studied 40 small pig carcasses in a 12-ha lake and, they collected aquatic insects belonging to mayflies (of families Baetidae and Caenidae), dragonflies (of the family Coenagrionidae), hemipters (Pleidae and Corixidae) Coleoptera (of the families Dytiscidae and Haliplidae) and dipters chironomids.

Analyzing the trend of trophic roles during the degradation stages in both studies, the hydrophilid beetles in Colombia are well represented during the five stages (they decrease only during stage 3), whereas Payne and King (1972) found hydrophilid only during the first stages, in particular the submerged fresh stage. In this study the shredders (Trichoptera and Plecoptera) are well represented in the first two stages; Trichoptera were not revealed by either Barrios and Wolff (2011) or Vance et al. (1995). In the present work Trichoptera then decrease considerably in the third and fourth while during the fifth stage (as it is reasonable to expect due to CPOM absence) they are absent; on the other hand, the Syrphidae family has another type of trend appearing and acting exclusively during the fourth stage. This suggests that, while in the Colombian environment the presence of shredders (hydrophilid beetles) are not a good indicator of the stage of degradation being a constant presence in the various stages, in the geographical region of this study the discovery of the main groups of shredders (Trichoptera, Plecoptera and Syrphidae) are related to some stages (the first 2 in particular) or to a stage (as in the case of hoverflies), thus possibly providing information on the period of immersion of a carcass in water. Regarding the collectors-gatherers, in the study of Barrios and Wolff they are reported as a constant presence and represented by chironomid diptera once again therefore not good indicators of the degradation stage. As revealed in the literature (Minakawa 1997, Chaloner et al. 2002, Warren et al. 1964, Mundie et al. 1983, 1991, Richardson & Neill 1991, Perrin & Richardson, 1997), also in this study chironomids were found in large quantities and as a constant presence; although it cannot be stated with certainty (since the determination of this family at the level of genus or species has not been possible, yet), it is likely that most of them covers the role of collector-gatherers, as observed in (Chaloner & Wipfli 2002). Furthermore, the role of collectors-gatherers in this study is also covered by two other main groups: the dipterans Ceratopogonidae (also a constant presence more or less abundant during the different stages) and the Ephemeroptera Baetidae which, on the contrary, during the first three stages of degradation are either absent or extremely poorly represented, while they are a well-represented presence during stages 4 and 5. These therefore show a clear preference for the later stages of degradation, when a large part of the soft organic substance has been fragmented.

The predators show a good overlap in the orders and families found between the two studies. As for Odonata, excluding the Platycnemididae (although it is the most represented in the present work) and that of the Lestidae, the trend of the 3 families found in both environments can be compared. These have affinities with the different degradation stages in the two environments: (1) Libellulidae is abundantly represented in Colombia in the first two stages and less in the third, while in Italy was found it with a limited number of individuals and only in stages 4 and 5; (2) Aeshnidae is abundantly represented in the first three stages of degradation in Colombia, while in Italy, although not excessively represented, it appears during all stages; (3) Coenagrionidae in Colombia is present and abundant exclusively in the first two stages, while in Italy it is abundantly represented during all the stages of degradation. As for the Hemiptera, excluding the gerrids and hydrometrids (although well represented in the Italian samplings) and comparing the two families found in both environments, it is observed that: (1) Corixidae is well represented during all stages in Colombia while in Italy it is well represented during stages 4 and 5 and with few sporadic individuals during stages 1 and 2; (2) Notonectidae in Colombia is more or less abundantly represented during all 5 stages while in Italy it is well represented during stages 4 and 5 and only a few individuals during stage 3. The sampled predatory beetles belong to the Dytiscidae and Gyrinidae. The gyrinids found in both studies are unique to stage 4 of degradation. The dytiscids found by Barrios and Wolff are characteristic of stages 2 and 3, while in the present study they were found during most of the degradation (with the exception of stage 3) and with higher numbers during stage 2. Therefore in the two different geographical regions the stage selectivity or the widespread presence during the entire degradation of Odonata and Hemiptera are antithetical. This suggests that in very different environments, insects that play the same trophic role may play the role at different times of degradation having probably developed affinity for different prey. The trend of predatory beetles in the two studies is rather comparable.

The two studies, carried out in very different environments, show differences in terms of fauna found and also of trophic roles found, although 3 of the 6 trophic roles are common. Trophic

roles are often covered by different taxonomic groups, as mentioned for shredders and collectors, although the hydrophilid beetles (the only shredders found in Colombia) probably play a role more like generalists in Italy, being able to exploit different trophic resources. Despite this, similarities are observed in terms of large taxonomic groups (such as orders and families) for predators while frequently showing different affinities at the stages of degradation.

### **4.3 Conclusions**

From this work, the following main conclusions can be drawn:

- Among the chemical-physical parameters measured, temperature most influences the degradation rate, especially during the first degradation stages;
- There are factors that influence the presence of aquatic animals regardless of the presence of the carcass, i.e. the seasonality and conditions that allow the establishment of some taxa over others. For example, heavy rains lead to the establishment of borderline conditions between the lotic and lentic environment; this allows to find taxa that when these conditions disappear, they no longer exist;
- Different seasons and years show very different trends in terms of taxa, while the stages do not seem to show significant differences from each other;
- Trophic roles seem to be correlated with the carcass stage on the basis of how the trophic source is made available during the evolution of carcass degradation. The competition by non-aquatic terrestrial organisms (in particular calliphorids and sarcophagids dipteran larvae) for the trophic source represented by the cadaveric substrate seems to play an important role.
- For now, the importance of this study is purely ecological and entomological in nature. Possible applications in the forensic field might follow further studies that take into account other, for now neglected, parameters, such as for example the different conditions of the carcass, further repetitions of the experiment for a greater number of data, considering the terrestrial fauna collected in the floating stages of the carcass, studying the degradation at different periods of the year, carrying out the study in a lotic or marine environment, evaluating the degradation in adjacent areas between the aquatic and terrestrial environments to observe how much the aquatic environment affects.

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# Appendix A

# Pre-operational Tables

Parameter	25/04/2018	18/04/2019
pН	8.1	7.9
χ / μS/cm	606	676
Susp. solids / mg/L	7	8
Alkalinity / mg/L	508	539
Diss. O <sub>2</sub> / mg/L	4	4
Diss. O <sub>2</sub> / %	43	35
BOD 5 / mg/L	3	2
COD / mg/L	8	8
N amm. / mg/L	0.02	0.02
N nitric / mg/L	0.3	0.3
N tot. / mg/L	< 1	< 1
Orthophosph. / mg/L	< 0.01	< 0.01
P tot. / mg/L	0.02	0,01
Cl <sup>-</sup> / mg/L	7	6
$SO_4^{2-}/mg/L$	93	86
Ca <sup>2+</sup> / mg/L	117	132
$Mg^{2+}/mg/L$	15	15.4
Na <sup>+</sup> / mg/L	22.4	24.1
K <sup>+</sup> / mg/L	1.9	1.8
Chlor. A / $\mu g/L$	< 0.5	< 0.5

**Tab. A.1** – Chemical-physical parameters of water samples analysed by ARPAE laboratories on 25/04/2018 and on 18/04/2019

Tab. A.2 – Physico-chemical parameters recorded in field on 25/04/2018 and on 18/04/2019

		2018			2019	
	P1	<b>P2</b>	<b>P3</b>	P1	P2	<b>P3</b>
T <sub>air</sub> / °C	17.2	14.0	15.0	14.2	12.4	13.6
T <sub>water</sub> / °C	9.5	10.6	10.6	8.4	9.1	9.4
pН	7.70	7.72	7.76	7.31	7.67	7.58
χ / (μS/cm)	722	700	655	770	754	763
Diss. O <sub>2</sub> / %	47.3	54.3	42.7	34.5	40.1	45.4
Diss. O <sub>2</sub> / ppm	4.69	4.94	4.11	3.65	5.14	4.72

ТАХА	T.R.	P1	P2	<b>P3</b>
Hydra sp. Linnaeus, 1758	P.P.P.		2	
Planaria torva (Muller, 1774)	P.P.P.		40	
Theristus sp. Bastian, 1865	P.P.P.			1
Mylonchulus sp. Cobb, 1916	P.P.P.	1		4
Dorylamius sp. Dujardin, 1845	P.P.P.		2	
Laimydorus sp. Siddigi, 1969	P.P.P.	8		2
Stagnicola palustris (O.F. Muller, 1774)	G.S.	4	14	
Sphaerium corneum (Linnaeus, 1758)	C.F.	3		
Nais communis Piguet, 1906 / Nais variabilis	C.G.			
Piguet, 1906		52	90	12
Enchytraeidae n.d.	C.G.	66	20	29
Eiseniella tetraedra (Savigny, 1826)	C.G.	14	12	
Cloeon gr. dipterum (Linnaeus, 1761)	C.G.	2	1	4
Caenis belfiorei Malzacher, 1986	C.G.	1	3	
Caenis horaria (Linnaeus, 1758)	C.G.	1	2	
Caenis sp. Stephens, 1835	C.G.		2	
Platycnemis pennipes (Pallas, 1771)	P.P.P.	11	8	
Coenagrion puella (Linnaeus, 1758) /	P.P.P.			
Coenagrion pulchellum (Vander Linden, 1825)		2	1	3
Platetrum depressum (Linnaeus, 1758)	P.P.P.	4	1	
Nemoura flexuosa Aubert, 1949	S.	6		1
Nepa cinerea Linnaeus, 1758	P.P.P.		1	
Notonectidae n.d.	P.P.P.	2	48	
Gerris lacustris (Linnaeus, 1758)	P.P.P.	1		
Hydrochus sp. Leach, 1817	S.		1	
Hydraena pygmaea Waterhouse, 1833	C.G.	1		1
Normandia nintes (Muller, 1817)	C.G.		1	
Limnophila sp. Macquart, 1834	P.P.P.	1		
Molophilus sp. Curtis, 1833	P.P.P.			1
Culicoides sp. Latreille, 1809	C.G.	13	14	3
Stilobezzia sp. Kieffer, 1911	C.G.	3	2	6
Ablabesmyia longistyla Fittkau, 1962	P.P.P.	3	7	1
Ablabesmyia monilis (Linnaeus, 1758)	P.P.P.	4		
Paramerina divisa (Walker, 1856)	P.P.P.	5		
Diamesa aberrata Lundbeck, 1898	S.G.	1		
Cardiocladius sp. Kieffer, 1912	C.G.	1		
Chaetocladius sp. Kieffer, 1911	C.G.	3		
Cricotopus fuscus (Kieffer, 1909)	C.G.			1
Crictopus sylvestris (Fabricius, 1794)	C.G.			1
Dipocladius cultriger Kieffer, 1908	C.G.	1		1
Hydrobaenus distylus (Potthast, 1914)	C.G.	7	3	14
Paracricotopus niger (Kieffer, 1913)	C.G.	3		

**Tab.** A.3 – List of taxa with the respective trophic rule (T.R.) and numbers of individuals sampled on 25/04/2018 at the 3 points of sampling.

Parametriocnemus sp. Goetghebuer, 1932	C.G.	1		
Paraphaenocladius sp. Thienemann, 1924	C.G.	9		
Kiefferulus tendipediformis (Goetghebuer,	G.S.			
1921)		4	2	
Tvetenia calvescens (Edwards, 1929)	G.S.		2	5
Rheocricotopus effusus (Walker, 1856)	C.G.	14	1	
Dicrotendipes gr. lobiger (Kieffer, 1921)	G.	1		
Dicrotendipes gr. nervosus (Staeger, 1839)	G.		2	
Einfeldia gr. pagana (Meigen, 1838)	G.	1		1
Endochironomus gr. dispar (Meigen, 1830)	G.	1		1
Endochironomus tendes (Fabricius, 1775)	G.		1	1
Glyptotendipes f.l.I Kieffer, 1913	C.F.		1	
Parachironomus f.l. I Lenz, 1921	C.G.		2	10
Micropsectra f.l. II Kieffer, 1908	G.	3		1
Paratanytarus f.l. I Thienemann & Bause,	C.G.			
1913		1		
Polypedilum gr. nubeculosum (Meigen, 1804)	G.	1	2	
Polypedilum (Pentapedilum) sp. Kieffer, 1913	G.		1	
Rheotanytarus sp. Thienemann & Bause, 1913	C.F.		2	
Phaenopsectra sp. Kieffer, 1921	G.	1		4
Chironomini n.d	-		1	
Orthocladiinae n.d.	-		1	1
Solva sp. Walker, 1860	G.S.	2		
Rhagionidae n.d.	G.		1	
Empididae n.d.	P.P.P.	3		
Sciomyzidae n.d.	P.P.P.	1	1	1
Lispe sp. Latreille, 1796	P.P.P.	1		
Limnephilus lunatus Curtis, 1834	S.	2	2	1
Limnephilus rhombicus (Linnaeus, 1758)	S.	39	31	11
Limnephilus vittatus (Fabricius, 1798)	S.		7	
Chaetopteryx gessneri McLachlan, 1876	S.		1	

ТАХА	T.R.	P1	P2	<b>P3</b>
Hydra sp. Linnaeus, 1758	P.P.P.	36	10	14
Planaria torva (Muller, 1774)	P.P.P.		62	
Dendrocoelum lacteum (Muller, 1774)	P.P.P.		6	
Tobrilus sp. Andrassy, 1959	P.P.P.			1
Iotonchus sp. Cobb, 1916	P.P.P.	1		
Laimydorus sp. Siddiqi, 1969	P.P.P.			3
Mesodorylaimus sp. Andrassy, 1959	P.P.P.	1		
Bythinella schmidti (Kuster, 1852)	G.S.	3		
Valvata piscinalis (O.F. Muller, 1774)	G.S.	1		1
Galba truncatula (O.F. Muller, 1774)	G.S.		19	
Limnodrilus claparedeianus Ratzel, 1869	C.G.	7		
Limnodrilus sp. Claparede, 1862	C.G.	6	6	4
Nais communis Piguet, 1906 / Nais variabilis	C.G.			
Piguet, 1906		148	120	509
Enchytraeidae n.d.	C.G.	14	7	170
Eiseniella tetraedra (Savigny, 1826)	C.G.	11	18	5
Pionidae n.d.	P.P.P.			1
Synurella ambulans (Muller, 1846)	S.	8	2	13
Niphargus sp. Schiodte, 1849	G.	10		
Centroptilum luteolum (Muller, 1776)	C.G.		1	
Cleon gr. dipterum (Linnaeus, 1761)	C.G.	1	2	18
Baetidae n.d.	C.G.		1	
Caenis horaria (Linnaeus, 1758)	C.G.	7	4	
Caenis luctuosa (Burmeister, 1839)	C.G.	4	1	
Caenis sp. Stephens, 1835	C.G.	1		
Habrophlebia sp. Eaton, 1881	C.G.			1
Platycnemis pennipes (Pallas, 1771)	P.P.P.	47	37	21
Pyrrhosoma nymphula (Sulzer, 1776)	P.P.P.			1
Ischnura elegans (Vander Linden, 1820)	P.P.P.		1	
Erythromma viridulum (Charpentier, 1840)	P.P.P.	2	3	6
Libellulidae n.d.	P.P.P.	2		5
Nemoura cinerea (Retzius, 1783)	S.	6	1	1
Nemoura flexuosa Aubert, 1949	S.	13	1	3
Protonemoura ausonia (Consiglio, 1955)	S.	3		
Sigara lateralis (Leach, 1817)	P.P.P.	2		
Micronecta sp. Kirkaldy, 1897	P.P.P.	1	177	27
Nepa cinerea Linnaeus, 1758	P.P.P.		1	1
Plea minutissima Leach, 1817	P.P.P.		1	
Gerris lacustris (Linnaeus, 1758)	P.P.P.	3		
Hydrochus angustatus Germar, 1824	S.			1
Laccobius simulatrix Orchymont, 1932	G.		1	4
Hydraena paganetti Ganglbauer, 1901	C.G.		1	

**Tab.** A.4 – List of taxa with the respective trophic rule (T.R.) and numbers of individuals sampled on 18/04/2019 at the 3 points of sampling.

Scirtidae n.d.	G.		14	
Pilaria gr. discicollis (Meigen, 1818)	P.P.P.		2	
Molophilus sp. Curtis, 1833	P.P.P.			3
Bazarella subneglecta (Tonnoir, 1922)	S.G.	2		
Dasyhelea sp. Kieffer, 1911	C.G.		1	2
Heleinae n.d.	C.G.		4	2
Culicoides sp. Latreille, 1809	C.G.	23	27	34
Stilobezzia sp. Kieffer, 1911	C.G.	3	12	14
Ablabesmyia sp. Johannsen, 1905	P.P.P.			2
Monopelopia tenuicalcar (Kieffer, 1918)	P.P.P.		2	
Paramerina sp. Fittkau, 1962	P.P.P.	8		
Prodiamesa olivacea (Meigen, 1818)	C.G.	2		
Chaetocladius sp. Kieffer, 1911	C.G.	3		
Cricotopus bicinctus (Meigen, 1818)	G.	3		
Cricotopus sylvestris (Fabricius, 1794)	C.G.	1		
Cricotopus tibialis (Meigen, 1804)	G.			4
<i>Georthocladius</i> sp. Strenzke, 1941 /	C.G.			
Parasmittia sp. Strenzke, 1950 / Pseudosmittia				
sp. Edwards, 1932		3		
Hydrobaenus sp. Fries, 1830	C.G.			8
Orthocladius (Eudactylocladius) sp.	C.G.			
Thienemann, 1935		1		
Parakiefferiella bathophila (Kieffer, 1912)	C.G.	3		
Rheocricotopus dispar (Goetghebuer, 1913)	C.G.	3		
Rheocricotopus effusus (Walker, 1856)	C.G.	4		
Dicrotendipes gr. nervosus (Staeger, 1839)	G.	197	49	119
Endochironomus tendens (Fabricius, 1775)	G.	1	2	22
Glyptotendipes f.l. I Kieffer, 1913	C.F.			1
Glyptotendipes f.l. II Kieffer, 1913	C.F.	1		
Kiefferulus tendipediformis (Goetghebuer,	S.G.			
1921)		16	3	8
Micropsectra f.l. III Kieffer, 1908	G.	75		
Parachironomus f.l. I Lenz, 1921	C.G.		2	5
Paratanytarsus f.l. II Thienemann & Bause, 1913	C.G.			9
Paratanytarsus f.1. III Thienemann & Bause,	C.G.			
1913		11	12	37
Phaenopsectra sp. Kieffer, 1921	G.	7	2	10
Polypedilum (Pentapedilum) sp. Kieffer, 1913	G.	1		2
Polypedilum gr. nubeculosum (Meigen, 1804)	G.	5	5	5
Stempellinella sp. Brundin, 1947	C.G.	2		
Haematopota sp. Meigen, 1803	P.P.P.	1		
Empididae n.d.	P.P.P.		1	
Chelifera sp. Macquart, 1823	P.P.P.	1		8
Dolichocephala sp. Macquart, 1823	P.P.P.			3
Wiedemannia sp. Zetterstedt, 1838	P.P.P.	1		

Sciomyzidae n.d.	P.P.P.	2	11	1
Scatella sp. Robineau-Desvoidy, 1830	C.G.	1		
Drusus sp. Stephens, 1837	S.			1
Limnephilus lunatus Curtis, 1834	S.	4	18	15
Limnephilus rhombicus (Linnaeus, 1758)	S.	33	19	54
Limnephilus vittatus (Fabricius, 1798)	S.		9	10
Halesus digitatus (von Paula Schrank, 1781)	S.			1
Halesus radiatus (Curtis, 1834)	S.			1
Stenophylax mucronatus McLachlan, 1880	S.	1		
Chaetopteryx gessneri McLachlan, 1876	S.	11	3	6
Limnephilidae n.d.	S.	3		
Plumatella sp. Lamarck, 1816	C.F.			1

Appendix B

**Chemical-Physical Parameters** 

## Legend of labels:

2018S1P1	point of sampling 1, experimental session 1 of 2018
2018S1P2	point of sampling 2, experimental session 1 of 2018
2018S1P3	point of sampling 3, experimental session 1 of 2018
2018S2P1	point of sampling 1, experimental session 2 of 2018
2018S2P2	point of sampling 2, experimental session 2 of 2018
2018S2P3	point of sampling 3, experimental session 2 of 2018
2019S1P1	point of sampling 1, experimental session 1 of 2019
2019S1P2	point of sampling 2, experimental session 1 of 2019
2019S1P3	point of sampling 3, experimental session 1 of 2019
2019S2P1	point of sampling 1, experimental session 2 of 2019
2019S2P2	point of sampling 2, experimental session 2 of 2019
2019S2P3	point of sampling 3, experimental session 2 of 2019

#### ELECTRIC CONDUCTIVITY VS STAGE



Fig. B.1.1 – Average electrical conductivity as a function of the stage 2018



Fig. B.1.2 – Average electrical conductivity as a function of the stage 2019

#### pH VS STAGE



Fig. B.1.3 – Average pH as a function of the stage 2018



Fig. B.1.4 – Average pH as a function of the stage 2019

#### **DISSOLVED OXYGEN (%) VS STAGE**



**Fig. B.1.5** – Average dissolved oxygen as a function of the stage 2018



Fig. B.1.6 – Average dissolved oxygen as a function of the stage 2019



**Fig. B.2.1** – Average daily air temperature at 2 m above the ground recorded by means of the simnpr measurement network of Sassostorno (Lama Mocogno, Modena. Altitude: 971 m a.s.l., longitude: 10.67408; latitude: 44.9524) from 01/01 / 2018 to 01/01/2020. The points corresponding to the 2018 sampling period are shown in red, while those corresponding to the 2019 sampling period are in green.

### Legend of symbols:

- Ta: Temperature air
- Tw: Temperature water
- H: Humidity
- $\chi$ : Electric conductivity
- D.O.: Dissolved oxygen
- Ti: Temperature internal
- Tm: Temperature maggot mass

	2018S1P1																											
	13/05/2018	15/05/2018	17/05/2018	19/05/2018	21/05/2018	23/05/2018	25/05/2018	27/05/2018	29/05/2018	31/05/2018	02/06/2018	04/06/2018	08/06/2018	12/06/2018	16/06/2018	20/06/2018	24/06/2018	28/06/2018	02/07/2018	06/07/2018	10/07/2018	14/07/2018	18/07/2018	22/07/2018	26/07/2018	30/07/2018	06/08/2018	14/08/2018
stage	1	1	2	2	2	2	2	2	2	2	2	3	3	3	4	4	4	4	4	4	4	4	4	4	4	5	5	5
Ta / °C	18,7	11,0	12,0	17,6	12,5	14,6	19,0	20,0	18,7	15,5	22,0	20,1	16,0	21,0	17,7	21,0	20,0	17,0	20,0	21,0	21,6	20,2	22,0	21,0	19,0	22,7	26,0	17,1
Tw / °C	10,6	11,0	10,0	11,1	12,2	12,0	12,5	14,0	12,9	12,9	10,6	13,1	13,4	15,0	12,4	13,7	13,2	14,0	17,6	18,0	19,1	19,1	20,0	20,4	21,2	21,6	24,0	21,2
H / %	47	47	52	59	66	71	73	72	65	73	80	58	76	78	63	53	56	47	61	59	61	74	87	64	60	72	49	64
$\chi$ / $\mu$ S/cm	722	695	690	722	734	715	725	727	680	711	719	718	708	731	722	717	733	665	754	735	692	714	606	706	711	692	664	677
pH	7,70	7,85	7,90	7,70	7,66	7,46	7,57	7,45	7,52	7,50	7,63	7,34	7,36	7,57	7,54	7,43	7,26	7,13	7,11	7,22	7,53	7,29	7,37	7,43	7,48	7,60	7,28	7,38
D.O. / %	96,9	92,8	80,5	85,7	82,5	78,3	74,3	65,6	51,0	38,5	35,5	41,3	26,6	18,7	43,2	32,1	23,4	18,3	19,6	24,0	17,0	23,1	18,5	17,2	32,6	37,5	44,0	27,3
D.O. / ppm	8,61	8,24	7,14	7,60	7,32	6,94	4,69	4,29	3,00	3,40	3,10	3,96	2,56	1,77	4,11	3,20	2,37	1,71	1,78	2,11	1,52	1,92	1,49	1,38	2,63	2,95	3,66	0,84
Ti / °C	12,4	10,7	10,3	11,6	12,4	12,0	13,0	15,0	14,8	14,0	13,0	13,9	13,4	14,1	13,4	13,7	14,4	15,0	18,9	18,4	19,3	19,3						
Tm / °C										19,8	19,0	23,0	23,1	18,0	13,7	15,9												

Tab. B.1.	1 – Chemical	-physical	parameters	recorded	l in th	ne field	l for th	e point of	f sampli	ng 1 d	of the ex	perimental	session	1 of	201	8
			1							<u> </u>		1				

	2018S1P2																										
	13/05/2018	15/05/2018	17/05/2018	19/05/2018	21/05/2018	23/05/2018	25/05/2018	27/05/2018	29/05/2018	31/05/2018	02/06/2018	04/06/2018	08/06/2018	12/06/2018	16/06/2018	20/06/2018	24/06/2018	28/06/2018	02/07/2018	06/07/2018	10/07/2018	14/07/2018	18/07/2018	22/07/2018	26/07/2018	02/08/2018	10/08/2018
stage	1	1	1	2	2	2	2	2	2	2	2	3	3	3	4	4	4	4	4	4	4	4	4	4	5	5	5
Ta / °C	15,0	12,5	12,0	18,0	14,1	16,5	19,8	23,0	21,4	20,1	22,0	19,0	17,7	19,8	17,3	20,0	16,4	19,0	22,6	20,9	23,7	19,4	25,0	20,0	19,0	23,6	22,8
Tw / °C	11.6	11,2	11,0	13,0	12,6	12,4	12,9	15,7	14,3	13,7	13,6	14,0	14,8	15,0	16,0	16,0	15,3	16,1	19,0	19,0	20,1	19,6	21,0	20,5	22,2	22,7	22,5
H / %	74	58	63	54	76	88	79	73	66	38	81	71	58	84	75	76	75	72	72	78	80	87	66	58	67	65	64
$\chi / \mu S/cm$	700	707	706	721	709	708	715	722	664	689	703	707	704	715	711	718	722	724	722	693	719	721	712	672	693	675	666
pH	7,72	7,89	7,80	7,80	7,71	7,64	7,66	7,63	7,64	7,65	7,66	7,72	7,61	7,62	7,61	7,50	7,48	7,38	7,38	7,37	7,39	7,38	7,48	7,68	7,60	7,46	7,58
D.O. / %	92,4	80,5	75,0	62,0	75,5	63,4	52,3	53,5	34,7	34,1	44,2	51,7	33,0	38,9	35,2	27,2	26,9	24,0	33,6	25,8	38,5	26,3	20,9	47,9	42,8	38,2	33,9
D.O. / ppm	8,42	7,31	6,81	5,58	6,88	5,72	4,70	5,00	3,26	3,58	4,16	4,66	3,60	3,46	3,16	2,46	2,39	2,12	2,81	2,14	3,13	2,17	1,69	3,76	3,37	2,99	2,85
Ti / °C	12,0	11,0	10,7	12,1	12,5	12,2	13,7	16,0	15,0	15,0	14,0	14,8	14,6	15,2	14,9	16,3	16,0	16,0	19,6	20,4	20,4	22,0					
Tm / °C										21,0	23,0	23,7	21,0	20,0	16,7	19,0											

Tab. B.1.2 – Chemical-physical parameters recorded in the field for the point of sampling 2 of the experimental session 1 of 2018

	2018S1P3																														
	13/05/2018	15/05/2018	17/05/2018	19/05/2018	21/05/2018	23/05/2018	25/05/2018	27/05/2018	29/05/2018	31/05/2018	02/06/2018	04/06/2018	06/06/2018	08/06/2018	10/06/2018	12/06/2018	16/06/2018	20/06/2018	24/06/2018	28/06/2018	02/07/2018	06/07/2018	10/07/2018	14/07/2018	18/07/2018	22/07/2018	26/07/2018	30/07/2018	03/08/2018	10/08/2018	16/08/2018
stage	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	4	4	4	4	4	4	4	4	4	4	5	5	5
Ta / °C	16,0	12,0	13,0	20,0	14,0	16,0	18,3	22,7	17,0	17,0	19,1	17,0	20,1	15,7	21,0	22,5	19,4	22,4	16,7	14,4	22,0	18,7	18,7	19,3	25,0	22,0	20,5	23,5	23,0	23,1	17,0
Tw / °C	12,0	11.7	11,0	13,0	12,4	18,7	13,6	16,0	14,4	14,0	14,7	14,8	16,0	14,7	14,4	15,6	15,0	15,7	15,7	17,0	19,5	20,0	21,1	21,7	21,1	21,7	22,0	22,2	22,0	23,4	21,6
H / %	21	45	60	42	67	84	54	45	75	54	58	45	65	60	79	61	49	37	46	38	22	67	70	54	41	48	67	63	65	58	55
$\chi / \mu S/cm$	655	688	652	703	709	704	711	720	654	690	703	707	712	701	709	716	709	722	723	727	728	724	725	723	718	711	685	671	681	665	416
pH	7,76	7,80	7,83	7,86	7,75	7,65	7,68	7,71	7,63	7,64	7,76	7,69	7,45	7,63	7,65	7,58	7,56	7,50	7,40	7,45	7,33	7,41	7,36	7,48	7,39	7,48	7,59	7,65	7,49	7,41	7,51
D.O. / %	96,8	82,8	84,8	78,1	66,5	57,1	42,7	42,3	30,1	31,1	37,7	43,1	43,7	42,2	26,6	35,7	32,3	20,1	12,6	17,4	15,0	23,8	22,0	12,3	15,1	19,6	29,8	38,3	26,9	24,9	24,0
D.O. / ppm	9,03	7,68	7,84	7,25	6,15	5,25	4,11	3,94	2,83	2,92	3,58	3,96	3,99	3,97	2,47	3,14	2,98	1,81	1,12	1,52	1,26	1,97	1,81	1,02	1,21	1,56	2,33	2,99	2,12	2,07	1,96
Ti / °C	12,9	11,4	11,1	13,0	12,7	12,7	13,4	16,3	15,5	13,7	13,6	15,0	14,6	14,4	14,7	15,7	17,6	16,0	15,7	16,6	20,1	19,7	21,0	21,3							
Tm / °C										19,2	17,7	19,0	20,8	20,0	28,1	26,0	19,0	19,8	16,2												

Tal	). I	8.1	.3	- (	Che	mic	al-p	ohy	sical	parameters	s recorde	d in	ı th	le f	ielo	1 f	or t	he j	point	of	samp	ling	; 3 (	of	the ex	perimental	sess	ion 1	l of	20	18
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	2018S2P1														
	08/08/2018	10/08/2018	12/08/2018	14/08/2018	18/08/2018	22/08/2018	26/08/2018	30/08/2018	03/09/2018	07/09/2018	11/09/2018	15/09/2018	19/09/2018	26/09/2018	03/10/2018
stage	2	2	2	3	3	4	4	4	4	4	4	4	5	5	5
Ta / °C	21,6	21,0	21,5	17,1	20,5	21,0	12,0	19,7	14,5	17,0	19,0	16,4	17,5	11,2	9,0
Tw / °C	22,0	21,9	21,9	21,2	20,7	21,0	20,0	19,4	17,7	17,6	18,0	18,6	17,9	15,0	11,9
H / %	59	62	63	64	64	63	57	54	81	70	67	64	74	48	53
χ/μS/cm	673	667	664	677	662	657	677	654	655	664	648	646	623	632	621
pН	7,36	7,41	7,39	7,38	7,45	7,48	7,40	7,54	7,55	7,48	7,42	7,36	7,50	7,70	7,76
D.O. / %	33,5	30,3	30,4	27,3	42,0	31,9	26,0	27,9	25,2	28,1	30,8	36,8	33,0	28,7	31,0
D.O. / ppm	1,34	1,07	1,08	0,84	2,01	1,21	0,73	0,88	0,66	0,90	1,12	1,59	1,30	0,94	0,13
Ti / °C	21,0	21,0	21,6	23,0	20,5	21,0									
Tm / °C			26,7	34,0	20,7										

Tab. B.1.4 – Chemical-physical parameters recorded in the field for the point of sampling 1 of the experimental session 2 of 2018
							2018	S2P2								
	08/08/2018	10/08/2018	12/08/2018	14/08/2018	16/08/2018	20/08/2018	24/08/2018	28/08/2018	01/09/2018	05/09/2018	09/09/2018	13/09/2018	19/09/2018	26/09/2018	03/10/2018	10/10/2018
stage	2	2	2	2	3	3	3	4	4	4	4	5	5	5	5	5
Ta / °C	24,2	22,8	22,5	21,6	20,0	22,0	18,7	17,0	15,2	17,3	19,0	20,0	17,7	9,1	11,7	12,6
$Tw \ / \ ^{o}C$	24,1	22,5	23,0	22,0	21,0	21,7	21,0	19,0	18,8	17,6	18,0	19,2	18,0	15,0	12,2	13,0
H / %	80	64	76	74	73	58	53	63	68	58	65	64	78	46	43	77
$\chi$ / $\mu$ S/cm	664	665	664	666	659	657	654	650	646	637	636	632	625	620	614	617
pH	7,55	7,58	7,48	7,55	7,58	7,52	7,54	7,46	7,58	7,56	7,52	7,60	7,43	7,51	7,46	7,61
D.O. / %	42,0	33,9	34,9	37,7	37,0	45,5	29,1	23,0	29,5	29,3	33,0	46,2	27,4	33,2	28,5	36,4
D.O. / ppm	3,38	2,85	2,83	3,05	3,00	3,67	2,37	1,89	2,41	2,39	2,68	2,73	2,24	2,70	2,32	2,95
Ti / °C	23,0	21,4	21,6	22,0	22,1	20,9	20,4	17,9	18,5	17,4	17,7					
Tm / °C			26,0	30,0	26,0	22,0										

Tab. B.1.5 – Chemical-physical parameters recorded in the field for the point of sampling 2 of the experimental session 2 of 2018

						2018S2	2P3						
	08/08/2018	10/08/2018	12/08/2018	14/08/2018	18/08/2018	22/08/2018	26/08/2018	30/08/2018	03/09/2018	07/09/2018	11/09/2018	19/09/2018	26/09/2018
stage	1	2	2	3	4	4	4	4	4	4	5	5	5
Ta / °C	26,0	23,1	22,8	22,7	22,0	24,7	15,0	20,0	15,0	17,7	21,0	19,8	9,0
Tw / °C	24,0	23,4	23,5	22,4	21,4	21,9	20,5	19,9	18,0	18,2	18,7	18,3	15,2
H / %	39	58	69	55	60	56	76	63	64	80	69	84	29
$\chi / \mu S/cm$	665	665	664	665	656	656	651	648	639	635	635	624	621
pH	7,45	7,41	7,49	7,56	7,53	7,48	7,46	7,46	7,46	7,45	7,49	7,05	7,57
D.O. / %	29,2	24,9	30,1	32,5	33,5	28,2	34,7	29,9	28,2	28,4	38,1	29,0	34,9
D.O. / ppm	2,44	2,07	2,51	2,72	2,81	2,36	2,91	2,50	2,35	2,37	3,20	2,42	2,92
Ti / °C	23,5	22,8	22,5	23,3	22,0								
Tm / °C			28,5	30,0									

Tab. B.1.6 – Chemical-physical parameters recorded in the field for the point of sampling 3 of the experimental session 2 of 2018

	2019S1P1																									
	02/05/2019	04/05/2019	08/05/2019	10/05/2019	12/05/2019	14/05/2019	16/05/2019	18/05/2019	20/05/2019	22/05/2019	24/05/2019	26/05/2019	28/05/2019	30/05/2019	01/06/2019	03/06/2019	05/06/2019	07/06/2019	09/06/2019	13/06/2019	17/06/2019	21/06/2019	25/06/2019	03/07/2019	11/07/2019	18/07/2019
stage	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	5	5	5	5
Ta / °C	18,0	10,3	8,9	16,1	6,1	7,6	12,6	8,0	11,0	13,8	17,7	12,6	13,0	11,7	18,2	22,0	22,6	19,0	20,0	22,0	23,0	22,0	25,0	20,5	22,0	18,7
$Tw \ / \ ^{\circ}C$	9,5	10,2	7,6	7,9	8,3	8,0	8,0	8,9	8,9	10,4	10,8	11,0	11,0	10,0	10,7	12,4	13,6	13,5	14,9	15,3	17,4	18,7	20,0	21,8	21,0	20,4
H / %	54	64	43	52	75	59	85	70	67	63	62	82	77	75	68	67	70	52	67	53	58	54	64	67	62	56
$\chi / \mu S/cm$	764	780	729	692	591	643	681	602	633	695	708	669	613	650	668	671	712	714	705	741	719	711	719	719	660	682
pH	7,80	7,89	7,83	8,28	8,10	7,94	7,78	7,95	8,05	7,45	7,45	7,49	8,19	7,98	7,78	7,56	7,41	7,34	7,53	7,44	7,41	7,51	7,40	7,59	7,83	8,50
D.O. / %	41,1	39,4	37,6	56,4	57,9	51,5	41,0	61,0	55,9	35,6	37,1	48,9	69,3	67,7	52,8	49,8	42,6	35,8	60,8	45,4	28,6	55,5	63,2	28,5	38,3	39,7
D.O. / ppm	4,02	3,85	3,67	5,53	5,69	5,07	4,01	6,01	5,49	3,47	3,62	4,80	6,83	6,66	5,18	4,89	4,18	3,48	5,99	4,45	2,70	5,45	6,22	2,77	3,75	3,88
Ti / °C	10,7	10,8	8,4	8,6	8,0	8,0	7,9	8,9	9,0	11,1	13,0	11,6	11,4	10,9	10,8	13,0	13,7	13,9	15,6	15,0	17,6	19,1				
Tm / °C																				19,7	21,7					

Tab. B.1.7 – Chemical-physical parameters recorded in the field for the point of sampling 1 of the experimental session 1 of 2019

													20	)19:	S1P	2														
	02/05/2019	04/05/2019	08/05/2019	10/05/2019	12/05/2019	14/05/2019	16/05/2019	18/05/2019	20/05/2019	22/05/2019	24/05/2019	26/05/2019	28/05/2019	30/05/2019	01/06/2019	03/06/2019	05/06/2019	07/06/2019	09/06/2019	13/06/2019	17/06/2019	21/06/2019	25/06/2019	29/06/2019	03/07/2019	07/07/2019	11/07/2019	18/07/2019	24/07/2019	01/08/2019
stage	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	4	4	4	4	5	5	5	5
Ta / °C	16,8	9,4	6,7	13,4	5,7	9,6	8,2	7,1	11,9	15,5	16,0	11,7	11,7	12,7	19,4	24,0	24,0	20,4	20,6	22,7	24,0	22,7	25,7	26,6	22,2	20,3	22,6	18,4	28,0	24,7
$Tw \ / \ ^{\circ}C$	10,7	10,8	8,0	9,0	8,4	8,5	8,1	9,0	9,3	11,4	12,7	11,8	10,7	11,0	12,0	14,0	14,2	15,0	15,4	16,0	18,0	19,4	21,3	24,0	24,6	23,3	22,0	20,6	25,6	23,0
H / %	36	59	50	53	90	70	68	88	67	30	47	84	89	73	75	59	45	65	80	50	49	36	65	63	56	30	68	64	74	85
$\chi$ / $\mu S/cm$	769	774	726	623	645	598	673	555	609	662	667	668	569	615	654	664	682	687	695	697	707	710	708	719	707	706	700	669	672	638
pН	7,75	8,02	7,88	8,10	7,82	7,98	7,89	7,76	9,30	7,91	7,84	7,78	7,97	7,91	7,86	7,78	7,71	7,94	7,69	7,69	7,69	7,75	7,74	7,84	7,75	7,96	7,76	7,87	7,68	7,81
D.O. / %	45,8	52,0	51,7	51,0	48,7	59,9	49,9	52,8	55,7	58,5	55,8	56,1	55,3	55,2	61,8	64,3	64,5	74,3	68,0	66,1	53,1	65,7	65,9	25,4	35,2	34,3	67,5	38,0	35,8	46,1
D.O. / ppm	4,23	5,14	5,45	5,51	5,05	6,31	4,63	5,38	5,64	5,85	5,32	5,50	5,35	5,51	6,12	6,08	6,04	6,76	6,27	5,95	4,49	5,49	5,35	1,82	2,62	3,12	5,30	3,04	3,27	4,25
Ti / °C	11,0	10,7	8,0	7,7	8,6	8,1	8,0	9,0	9,8	11,4	12,6	12,0	11,6	10,6	12,0	13,6	15,5	14,4	15,4	16,0	18,4	19,7							24,6	
Tm / °C																	29,0	32,7	28,1	25,9	28,5	24,0							38,9	

Tab. B.1.8 – Chemical-physical parameters recorded in the field for the point of sampling 2 of the experimental session 1 of 2019

	2019S1P3																														
	02/05/2019	04/05/2019	08/05/2019	10/05/2019	12/05/2019	14/05/2019	16/05/2019	18/05/2019	20/05/2019	22/05/2019	24/05/2019	26/05/2019	28/05/2019	30/05/2019	01/06/2019	03/06/2019	05/06/2019	07/06/2019	09/06/2019	13/06/2019	17/06/2019	21/06/2019	25/06/2019	29/06/2019	03/07/2019	07/07/2019	11/07/2019	15/07/2019	22/07/2019	28/07/2019	05/08/2019
stage	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3	4	4	4	4	5	5	5	5
Ta / °C	17,4	9,9	7,0	14,6	6,0	9,4	9,7	7,4	13,0	15,0	19,0	11,7	12,6	12,0	15,4	22,6	19,6	18,7	20,0	21,0	21,2	21,0	24,4	26,5	25,5	18,7	21,4	14,2	24,0	18,0	23,3
Tw / °C	11,2	11,0	9,0	8,7	8,4	9,0	8,7	9,0	9,4	11,6	12,0	11,7	11,0	11,0	12,6	14,4	14,0	14,8	17,0	16,3	19,0	19,6	22,4	24,1	24,6	23,3	22,4	20,6	23,4	22,3	23,2
H / %	20	35	62	36	94	51	63	92	54	30	37	78	91	55	40	39	37	40	61	24	46	28	29	32	44	56	29	58	60	93	83
$\chi / \mu S/cm$	765	770	726	604	652	611	663	653	620	663	677	667	572	614	654	670	676	680	688	699	703	705	707	712	708	706	700	686	672	632	629
pH	7,81	7,98	7,89	8,06	7,84	8,11	8,00	7,82	7,98	8,01	7,81	7,72	7,99	7,93	7,90	7,80	7,76	7,80	7,73	7,71	7,70	7,76	7,75	7,72	7,73	7,79	7,78	7,80	7,79	7,76	7,80
D.O. / %	41,2	51,0	51,0	50,4	48,2	56,0	50,6	50,0	54,1	59,6	57,1	59,4	59,9	53,6	60,0	53,3	77,5	62,3	72,9	57,7	61,7	64,2	69,4	32,3	46,3	51,7	66,1	67,2	70,8	66,5	59,4
D.O. / ppm	4,29	5,04	5,37	5,34	4,97	5,75	5,26	5,12	5,47	5,90	5,50	5,80	5,87	5,30	5,92	5,61	7,14	5,79	6,66	5,21	5,31	5,37	5,69	3,65	4,64	4,86	5,13	6,18	6,33	5,99	5,57
Ti / °C	13,0	11,8	9,4	10,9	8,3	8,6	8,0	9,0	9,3	11,6	12,7	11,7	11,6	10,5	12,1	14,4	15,6	15,0	15,7	16,2	19,1	19,6	22,0	24,4							
Tm / °C																	29,4	19,7	27,0	21,0	23,0										

Tab. B.1.9 –	- Chemical-physical	parameters recorded	in the field for the	point of sampling	ng 3 of the ex	sperimental s	ession 1 of 2019
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				2019	S2P1					
	18/07/2019	20/07/2019	22/07/2019	24/07/2019	28/07/2019	01/08/2019	05/08/2019	13/08/2019	20/08/2019	27/08/2019
stage	1	2	2	3	3	4	5	5	5	5
Ta / °C	18,7	25,0	25,6	27,0	16,4	22,6	22,9	23,0	22,0	21,7
Tw / °C	20,4	20,4	22,3	23,5	20,9	22,4	22,4	23,4	21,7	21,0
H / %	56	65	65	67	87	83	70	81	59	68
$\chi / \mu S/cm$	682	675	718	676	483	646	641	625	570	515
рН	8,50	7,87	7,92	7,85	7,93	8,01	7,82	7,67	7,83	7,84
D.O. / %	39,7	31,6	30,8	35,3	48,9	51,1	49,0	40,8	41,8	43,8
D.O. / ppm	3,88	2,96	2,81	3,55	5,89	6,06	5,73	4,42	4,58	4,89
Ti / °C	20,0	19,3	21,2	24,3						
Tm / °C				29,0						

Tab. B.1.10 – Chemical-physical parameters recorded in the field for the point of sampling 1 of the experimental session 2 of 2019

				2019S2P2	2				
	18/07/2019	20/07/2019	22/07/2019	24/07/2019	28/07/2019	01/08/2019	07/08/2019	13/08/2019	20/08/2019
stage	2	2	2	3	4	5	5	5	5
Ta / °C	18,4	25,5	25,0	28,0	15,3	24,7	23,7	24,0	26,0
Tw / °C	20,6	21,0	23,0	25,6	22,4	23,0	23,1	24,4	23,3
H / %	64	73	71	74	90	85	68	83	70
$\chi / \mu S/cm$	669	675	670	672	641	638	634	630	629
рН	7,87	7,78	7,79	7,68	7,70	7,81	7,84	7,64	7,68
D.O. / %	38,0	32,1	36,6	35,8	46,1	46,1	46,3	42,1	44,8
D.O. / ppm	3,48	2,91	3,35	3,27	4,26	4,25	4,28	3,88	4,14
Ti / °C	20,6	23,0	23,2	24,6					
Tm / °C				38,9					

Tab. B.1.11 – Chemical-physical parameters recorded in the field for the point of sampling 2 of the experimental session 2 of 2019

		,	2019S2P3				
	18/07/2019	20/07/2019	24/07/2019	28/07/2019	05/08/2019	13/08/2019	20/08/2019
stage	2	3	4	5	5	5	5
Ta / °C	19,3	22,0	30,0	18,0	23,3	24,0	27,0
Tw / °C	20,6	21,4	25,6	22,3	23,2	24,0	23,5
H / %	61	50	65	93	83	62	31
χ / μS/cm	673	669	670	632	629	627	631
pH	7,86	7,82	7,75	7,76	7,80	7,68	7,83
D.O. / %	72,5	70,8	66,5	66,5	59,4	57,3	48,9
D.O. / ppm	6,34	6,23	5,98	5,99	5,57	5,45	4,95
Ti / °C	20,8	24,0					
Tm / °C		31,2					

Tab. B.1.12 – Chemical-physical parameters recorded in the field for the point of sampling 3 of the experimental session 2 of 2019

	2018																			
date	pН	χ / μS/cm	Susp.solid s / mg/L	Alkalinity / mg/L	Diss. O <sub>2</sub> / mg/L	Diss. O <sub>2</sub> / %	BOD 5 / mg/L	COD / mg/L	N amm. / mg/L	N nitric / mg/L	N tot. / mg/L	Orthophosp h. / mg/L	P tot. / mg/L	Cl <sup>-</sup> / mg/L	SO4 / mg/L	Ca <sup>2+</sup> / mg/L	Mg <sup>2+</sup> / mg/L	Na <sup>+</sup> / mg/L	K <sup>+</sup> / mg/L	Chlor. A / µg/L
11/05	8,2	654	5	507	10	93	4	7	0,02	0,3	<1	<0,01	0,02	6	92	119	14	22,4	1,8	<0,5
24/06	8,1	638	11	532	2,4	25	3	7	0,07	0,2	<1	<0,01	0,02	6	79	124	14,2	22,5	2	<0,5
02/08	7,8	624	268	515	4,6	54	3	27	<0,02	<0,2	<1	<0,01	0,05	7	61	113	13,8	23,1	1,9	21
16/08	7,8	606	24	510	3	34	2	13	0,04	<0,2	<1	<0,01	0,11	8	54	110	13,4	22,5	2	10
27/08	7,5	605	166	478	2,6	29	2	14	0,06	<0,2	1	<0,01	0,05	8	49	101	13,6	24,9	2,3	1,8
03/10	7,9	567	10	490	3,2	30	<2	10	0,27	<0,2	1	<0,01	0,04	10	46	100	14,3	24,7	2,5	2,4
17/10	8	568	13	480	2,9	27	2	11	0,19	<0,2	1	<0,01	0,04	8	48	95,8	13,8	23,5	2,7	1,5
										2019										
date	рН	χ / μS/cm	Susp. solids / mg/L	Alkalinity / mg/L	Diss. O <sub>2</sub> / mg/L	Diss. O <sub>2</sub> / %	BOD 5 / mg/L	COD / mg/L	N amm. / mg/L	N nitric / mg/L	N tot. / mg/L	Orthophosp h. / mg/L	P tot. / mg/L	Cl <sup>-</sup> / mg/L	SO4 / mg/L	Ca <sup>2+</sup> / mg/L	Mg <sup>2+</sup> / mg/L	Na <sup>+</sup> / mg/L	K <sup>+</sup> / mg/L	Chlor. A / µg/L
15/04	7,9	676	8	539	4	35	2	8	0,02	0,3	<1	<0,01	0,01	6	86	132	15,4	24,1	1,8	<0,5
03/07	7,9	615	22	516	3,6	43	<2	7	<0,02	<0,2	<1	0,01	0,04	6	58	101	13,2	21,9	1,6	<0,5
03/09	8,1	535	25	438	4,1	45	<2	15	0,18	0,3	<1	<0,01	0,05	7	49	91,2	13,3	23,7	1,8	11,5

Tab. B.2.1 – Chemical-physical parameters monitored by Arpae during the field activities in 2018 and 2019.

## Appendix C

Fauna

## Legend of labels:

2018S1P1	point of sampling 1, experimental session 1 of 2018
2018S1P2	point of sampling 2, experimental session 1 of 2018
2018S1P3	point of sampling 3, experimental session 1 of 2018
2018S2P1	point of sampling 1, experimental session 2 of 2018
2018S2P2	point of sampling 2, experimental session 2 of 2018
2018S2P3	point of sampling 3, experimental session 2 of 2018
2019S1P1	point of sampling 1, experimental session 1 of 2019
2019S1P2	point of sampling 2, experimental session 1 of 2019
2019S1P3	point of sampling 3, experimental session 1 of 2019
2019S2P1	point of sampling 1, experimental session 2 of 2019
2019S2P2	point of sampling 2, experimental session 2 of 2019
2019S2P3	point of sampling 3, experimental session 2 of 2019

				Nr. o	f Indiv	iduals		P	rese	nce/.	Abse	nce
Таха	T.R.	Carcass	1	2	3	4	5	1	2	3	4	5
Hydra sp. Linnaeus, 1758	P.P.P.	2018S1P1	0	10	0	0	0	0	1	0	0	0
		2018S1P2	1	1	0	0	13	1	1	0	0	1
		2018S1P3	0	0	0	0	9	0	0	0	0	1
		2018S2P1		0	0	0	1		0	0	0	1
		2018S2P2		1	0	0	0		1	0	0	0
		2019S1P1	1	2	0		0	1	1	0		0
		2019S1P2	9	1	0	0	1	1	1	0	0	1
		2019S1P3	3	2	0	0	1	1	1	0	0	1
Dugesia sp. Girard, 1850	P.P.P.	2018S1P1	0	1	0	0	0	0	1	0	0	0
Tobrilus helveticus Hofmanner, 1914	P.P.P.	2018S2P1		0	0	2	0		0	0	1	0
Tobrilus sp. Andrassy, 1959	P.P.P.	2019S1P1	0	6	2		0	0	1	1		0
		2019S1P2	1	2	0	0	0	1	1	0	0	0
		2019S1P3	18	1	0	1	8	1	1	0	1	1
Mononchus sp. Bastian, 1865	P.P.P.	2019S1P1	4	0	0		0	1	0	0		0
		2019S1P2	2	0	0	0	0	1	0	0	0	0
		2019S1P3	33	0	0	0	0	1	0	0	0	0
Mylonchulus sp. Cobb, 1916	P.P.P.	2019S1P1	1	0	0		0	1	0	0		0
Dorylaimus sp. Dujardin, 1845	P.P.P.	2019S1P1	8	0	0		0	1	0	0		0
Laimydorus sp. Siddiqi, 1969	P.P.P.	2019S1P1	12	4	0		0	1	1	0		0
		2019S1P2	0	1	0	0	0	0	1	0	0	0
		2019S1P3	0	0	0	0	1	0	0	0	0	1
Belgrandiella sp. A.J. Wagner, 1928	G.S.	2018S1P1	0	1	0	0	0	0	1	0	0	0
Belgrandia sp. Bourguignat, 1870	G.S.	2019S1P1	4	0	0		0	1	0	0		0
Valvata piscinalis (O.F. Muller, 1774)	G.S.	2018S1P1	0	1	0	0	0	0	1	0	0	0
		2019S1P1	1	0	0		0	1	0	0		0
Galba truncatula (O.F. Muller, 1774)	G.S.	2019S1P1	1	1	0		0	1	1	0		0
		2019S1P2	1	0	0	0	0	1	0	0	0	0
Ferrissia clessiniana (Jickeli, 1882)	G.S.	2018S1P2	0	0	0	0	6	0	0	0	0	1
		2018S1P3	0	0	0	0	3	0	0	0	0	1
		2018S2P1		0	0	2	2		0	0	1	1
		2018S2P2		0	0	11	14		0	0	1	1
		2018S2P3	1	0	0	11	5	1	0	0	1	1
Sphaerium corneum (Linnaeus, 1758)	C.F.	2018S1P1	0	2	0	0	0	0	1	0	0	0
		2018S1P3	0	0	0	0	1	0	0	0	0	1
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**Tab. C.1** – List of taxa with the respective trophic role (T.R.). The total number of individuals and presence(1)/absence(0) descriptor for each taxon in the different carcasses are also depicted for each stage of decomposition.

		2019S1P1	1	0	0		0	1	0	0		0
Lumbriculus variegatus (Muller, 1774)	C.G.	2018S1P2	0	0	0	4	0	0	0	0	1	0
Tubifex sp. Lamarck, 1816	C.G.	2019S1P2	0	0	1	0	0	0	0	1	0	0
		2019S2P1	0	0	1	0	0	0	0	1	0	0
		2019S2P3		0	0	0	1		0	0	0	1
Limnodrilus hoffmeisteri Claparede, 1862	C.G.	2019S1P2	0	0	1	1	0	0	0	1	1	0
Limnodrilus sp. Claparede, 1862	C.G.	2018S1P2	0	10	0	62	12	0	1	0	1	1
		2018S1P3	0	1	0	0	1	0	1	0	0	1
		2018S2P1		4	9	3	4		1	1	1	1
		2018S2P2		34	28	2	51		1	1	1	1
		2019S2P1	0	0	2	0	7	0	0	1	0	1
		2019S2P3		0	0	0	1		0	0	0	1
Nais communis Piguet, 1906 / Nais variabilis Piguet, 1906	C.G.	2018S1P1	4	138	5	430	6	1	1	1	1	1
		2018S1P2	40	73	5	70	509	1	1	1	1	1
		2018S1P3	1	11	1	18	1067	1	1	1	1	1
		2018S2P1		6	0	2	0		1	0	1	0
		2018S2P2		21	6	1	18		1	1	1	1
		2018S2P3	1	1	0	4	9	1	1	0	1	1
		2019S1P1	47	33	16		27	1	1	1		1
		2019S1P2	31	5	0	1	2	1	1	0	1	1
		2019S1P3	29	18	2	4	7	1	1	1	1	1
		2019S2P1	0	4	0	0	6	0	1	0	0	1
		2019S2P2		0	1	0	2		0	1	0	1
Haemonais waldvogeli Bretscher, 1900	C.G.	2018S1P1	0	0	0	6	0	0	0	0	1	0
		2018S1P3	0	0	0	0	74	0	0	0	0	1
Enchytraeidae n.d.	C.G.	2018S1P1	0	140	2	3	0	0	1	1	1	0
		2018S1P2	0	7	0	0	0	0	1	0	0	0
		2018S1P3	0	2	0	0	0	0	1	0	0	0
		2018S2P2		2	0	0	1		1	0	0	1
		2018S2P3	0	1	0	0	0	0	1	0	0	0
		2019S1P1	160	32	0		0	1	1	0		0
		2019S1P2	1	0	0	0	0	1	0	0	0	0
Eiseniella tetraedra (Savigny, 1826)	C.G.	2018S1P1	0	10	0	1	0	0	1	0	1	0
		2018S1P2	0	0	0	0	1	0	0	0	0	1
		2018S1P3	0	0	0	0	2	0	0	0	0	1
		2019S1P1	4	3	0		0	1	1	0		0
		2019S1P2	1	0	0	0	0	1	0	0	0	0
		2019S1P3	2	0	0	0	0	1	0	0	0	0
Trocheta bykowskii Gedroyc, 1913	P.P.P.	2019S1P1	1	0	0		0	1	0	0		0

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Sperchon sp. Kramer, 1877	P.P.P.	2018S2P3	0	0	0	1	0	0	0	0	1	0
Limnesia sp. Koch, 1836	P.P.P.	2018S1P1	0	1	0	0	0	0	1	0	0	0
Synurella ambulans (Muller, 1846)	S.	2018S1P1	0	3	0	0	0	0	1	0	0	0
		2019S1P1	2	1	0		0	1	1	0		0
Niphargus elegans Garbini, 1894	G.	2018S1P1	0	3	0	0	0	0	1	0	0	0
		2019S1P1	14	0	0		0	1	0	0		0
Baetis lutheri Muller-Liebenau, 1967	C.G.	2018S1P1	0	0	0	3	0	0	0	0	1	0
		2018S1P2	0	0	1	5	0	0	0	1	1	0
Baetis niger (Linnaeus, 1761)	C.G.	2018S1P1	0	0	0	1	0	0	0	0	1	0
Baetis rhodani (Pictet, 1843)	C.G.	2019S1P1	0	1	2		0	0	1	1		0
Cloeon gr. dipterum (Linnaeus, 1761)	C.G.	2018S1P1	0	1	0	110	60	0	1	0	1	1
		2018S1P2	0	0	0	101	145	0	0	0	1	1
		2018S1P3	0	0	1	293	64	0	0	1	1	1
		2018S2P1		45	11	40	5		1	1	1	1
		2018S2P2		92	19	7	32		1	1	1	1
		2018S2P3	16	39	3	55	24	1	1	1	1	1
		2019S1P1	1	0	0		15	1	0	0		1
		2019S1P2	2	1	0	4	10	1	1	0	1	1
		2019S1P3	1	0	0	13	22	1	0	0	1	1
		2019S2P1	0	1	0	1	15	0	1	0	1	1
		2019S2P2		2	0	0	1		1	0	0	1
		2019S2P3		0	0	1	3		0	0	1	1
Caenis belfiorei Malzacher, 1986	C.G.	2018S1P2	0	0	0	1	1	0	0	0	1	1
Caenis horaria (Linnaeus, 1758)	C.G.	2018S1P1	0	1	0	11	0	0	1	0	1	0
		2018S1P2	0	1	0	14	2	0	1	0	1	1
		2018S2P1		2	0	0	1		1	0	0	1
		2018S2P2		0	3	1	0		0	1	1	0
		2019S1P2	0	2	0	3	1	0	1	0	1	1
		2019S1P3	0	1	2	0	0	0	1	1	0	0
		2019S2P2		0	0	0	3		0	0	0	1
Caenis lactea (Burmeister, 1839)	C.G.	2019S1P1	0	1	0		0	0	1	0		0
		2019S1P2	0	0	0	0	1	0	0	0	0	1
		2019S2P2		0	0	0	2		0	0	0	1
Caenis luctuosa (Burmeister, 1839)	C.G.	2018S1P1	0	0	0	6	0	0	0	0	1	0
		2018S1P2	0	0	2	17	4	0	0	1	1	1
		2018S1P3	0	0	0	4	0	0	0	0	1	0
		2018S2P1		0	1	1	0		0	1	1	0
		2018S2P2		2	7	6	1		1	1	1	1
		2018S2P3	0	0	0	6	1	0	0	0	1	1

		2019S1P1	1	0	0		0	1	0	0		0
		2019S1P2	0	0	0	5	1	0	0	0	1	1
		2019S1P3	0	1	1	1	30	0	1	1	1	1
		2019S2P2		0	0	0	2		0	0	0	1
		2019S2P3		0	0	0	4		0	0	0	1
Habrophlebia fusca (Curtis, 1834)	C.G.	2019S1P3	0	0	1	1	0	0	0	1	1	0
Chalcolestes viridis (Vander Linden, 1825)	P.P.P.	2018S1P1	0	0	0	0	1	0	0	0	0	1
Lestes virens (Charpentier, 1825)	P.P.P.	2018S1P2	0	0	0	1	0	0	0	0	1	0
Platycnemis pennipes (Pallas, 1771)	P.P.P.	2018S1P1	3	19	9	61	19	1	1	1	1	1
		2018S1P2	3	7	2	19	3	1	1	1	1	1
		2018S1P3	1	3	1	4	4	1	1	1	1	1
		2018S2P1		12	10	75	23		1	1	1	1
		2018S2P2		11	15	15	11		1	1	1	1
		2018S2P3	2	0	2	30	3	1	0	1	1	1
		2019S1P1	13	16	10		17	1	1	1		1
		2019S1P2	7	11	2	10	16	1	1	1	1	1
		2019S1P3	0	2	2	12	15	0	1	1	1	1
		2019S2P1	0	0	5	4	13	0	0	1	1	1
		2019S2P2		2	3	0	6		1	1	0	1
		2019S2P3		0	0	0	5		0	0	0	1
Pyrrhosoma nymphula (Sulzer, 1776)	P.P.P.	2018S2P2		0	0	0	1		0	0	0	1
Ischnura elegans (Vander Linden, 1820)	P.P.P.	2018S1P3	1	7	1	5	6	1	1	1	1	1
		2018S2P1		0	1	17	2		0	1	1	1
		2018S2P2		1	1	1	9		1	1	1	1
		2018S2P3	1	5	1	34	9	1	1	1	1	1
		2019S1P1	2	0	0		0	1	0	0		0
Enallagma cyathigerum (Charpentier, 1840)	P.P.P.	2018S2P1		0	0	4	0		0	0	1	0
Coenagrion hylas (Trybom, 1889)	P.P.P.	2018S1P2	0	0	0	1	0	0	0	0	1	0
Coenagrion puella (Linnaeus, 1758) / Coenagrion pulchellum (Vander Linden, 1825)	P.P.P.	2018S1P1	1	5	1	2	0	1	1	1	1	0
		2018S1P2	0	1	0	1	0	0	1	0	1	0
		2018S2P1		1	0	0	0		1	0	0	0
		2019S1P1	1	4	2		0	1	1	1		0
		2019S1P3	1	1	1	0	1	1	1	1	0	1
		2019S2P1	0	0	0	0	2	0	0	0	0	1
		2019S2P2		0	0	0	1		0	0	0	1
Erythromma viridulum (Charpentier, 1840)	P.P.P.	2018S1P2	0	0	0	1	0	0	0	0	1	0
		2019S1P1	0	4	1		1	0	1	1		1
		2019S1P3	1	0	0	2	2	1	0	0	1	1
		2019S2P1	0	0	0	1	8	0	0	0	1	1
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Aeshna cyanea (Muller, 1764)	P.P.P.	2018S2P2		0	0	0	1		0	0	0	1
Anax imperator Leach, 1815	P.P.P.	2018S1P1	0	0	0	2	1	0	0	0	1	1
		2018S1P2	1	1	1	0	1	1	1	1	0	1
		2018S1P3	1	0	0	0	0	1	0	0	0	0
		2018S2P1		0	0	0	1		0	0	0	1
		2018S2P2		0	1	1	1		0	1	1	1
		2018S2P3	0	1	0	1	2	0	1	0	1	1
Anax sp. Rambur, 1842	P.P.P.	2019S1P3	0	0	1	0	1	0	0	1	0	1
		2019S2P2		1	0	0	0		1	0	0	0
Platetrum depressum (Linnaeus, 1758)	P.P.P.	2018S1P3	0	0	0	1	0	0	0	0	1	0
Sympetrum depressiusculum (Selys, 1841)	P.P.P.	2018S1P2	0	0	0	0	1	0	0	0	0	1
Sympetrum flaveolum (Linnaeus, 1758)	P.P.P.	2018S1P3	0	0	0	1	0	0	0	0	1	0
Isoperla grammatica (Poda, 1761)	P.P.P.	2019S1P1	1	3	0		0	1	1	0		0
		2019S1P2	1	0	0	0	0	1	0	0	0	0
Nemoura cinerea (Retzius, 1783)	S.	2019S1P1	11	0	0		0	1	0	0		0
Nemoura flexuosa Aubert, 1949	S.	2019S1P1	14	4	0		0	1	1	0		0
Protonemura ausonia (Consiglio, 1955)	S.	2018S1P1	0	3	0	0	0	0	1	0	0	0
Protonemoura tyrrhena (Festa, 1938)	S.	2019S1P1	11	0	0		0	1	0	0		0
Corixinae n.d.	P.P.P.	2018S1P2	0	1	0	14	5	0	1	0	1	1
		2018S1P3	0	1	0	1	0	0	1	0	1	0
		2019S1P1	0	0	0		2	0	0	0		1
		2019S1P2	1	0	0	1	4	1	0	0	1	1
		2019S1P3	0	0	0	1	2	0	0	0	1	1
		2019S2P3		0	0	0	1		0	0	0	1
Micronecta scholtzi (Fieber, 1860)	P.P.P.	2018S1P2	0	0	0	36	24	0	0	0	1	1
		2018S1P3	0	0	0	1	4	0	0	0	1	1
		2018S2P1		0	0	1	0		0	0	1	0
		2018S2P2		9	9	23	2		1	1	1	1
		2018S2P3	0	0	0	3	1	0	0	0	1	1
		2019S1P3	0	0	0	3	0	0	0	0	1	0
		2019S2P1	0	0	0	5	15	0	0	0	1	1
Nepa cinerea Linnaeus, 1758	P.P.P.	2018S1P1	0	0	0	3	1	0	0	0	1	1
		2018S1P2	1	0	0	1	1	1	0	0	1	1
		2019S2P1	0	0	0	0	3	0	0	0	0	1
Nepa sp. Linnaeus, 1758	P.P.P.	2018S2P1		1	0	0	0		1	0	0	0
Notonecta viridis Delcourt, 1909	P.P.P.	2018S1P1	0	0	0	1	0	0	0	0	1	0
		2018S1P2	0	0	0	4	2	0	0	0	1	1
		2018S1P3	0	0	1	12	2	0	0	1	1	1
		2018S2P3	3	3	0	16	7	1	1	0	1	1
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Plea minutissima Leach, 1817	P.P.P.	2018S1P2	1	0	0	0	0	1	0	0	0	0
Gerris lacustris (Linnaeus, 1758)	P.P.P.	2018S1P1	0	0	0	14	3	0	0	0	1	1
		2018S1P2	0	1	0	2	1	0	1	0	1	1
		2018S1P3	1	0	0	1	0	1	0	0	1	0
		2018S2P1		0	0	17	3		0	0	1	1
		2018S2P2		0	0	0	3		0	0	0	1
		2018S2P3	0	0	0	1	1	0	0	0	1	1
		2019S1P1	2	0	2		0	1	0	1		0
		2019S1P3	0	0	0	0	2	0	0	0	0	1
		2019S2P3		0	0	0	2		0	0	0	1
Microvelia sp. Westwood, 1834	P.P.P.	2018S1P1	0	0	0	1	0	0	0	0	1	0
Veliidae n.d.	P.P.P.	2018S2P2		0	0	1	0		0	0	1	0
		2019S1P2	0	1	0	0	0	0	1	0	0	0
		2019S2P3		0	0	0	1		0	0	0	1
Hydrometra stagnorum (Linnaeus, 1758)	P.P.P.	2018S1P2	0	0	0	10	5	0	0	0	1	1
		2018S1P3	0	1	0	4	4	0	1	0	1	1
		2018S2P1		1	0	0	0		1	0	0	0
		2018S2P2		0	0	1	5		0	0	1	1
		2018S2P3	0	0	0	0	3	0	0	0	0	1
		2019S1P1	0	0	0		3	0	0	0		1
		2019S1P3	0	1	0	0	1	0	1	0	0	1
		2019S2P1	0	0	0	0	3	0	0	0	0	1
		2019S2P2		0	0	0	2		0	0	0	1
		2019S2P3		0	0	2	5		0	0	1	1
Haliplus obliquus (Fabricius, 1787)	G.S.	2019S1P2	0	0	0	1	0	0	0	0	1	0
Gyrinus sp. O. F. Muller, 1764	P.P.P.	2018S1P1	0	0	0	2	0	0	0	0	1	0
		2018S1P2	0	0	0	2	0	0	0	0	1	0
Hyphydrus ovatus (Linnaeus, 1761)	P.P.P.	2018S1P1	0	1	0	0	0	0	1	0	0	0
Hydrovatus cuspidatus Kunze, 1818	P.P.P.	2018S1P1	0	14	0	0	0	0	1	0	0	0
Bidessus delicatulus (Schaum, 1844)	P.P.P.	2018S2P3	1	0	0	0	0	1	0	0	0	0
Bidessus minutissimus (Germar, 1824)	P.P.P.	2019S1P3	0	0	0	0	1	0	0	0	0	1
		2019S2P1	0	0	0	0	1	0	0	0	0	1
Hydroporus pubescens (Gyllenhal, 1808)	P.P.P.	2018S1P2	0	5	0	0	0	0	1	0	0	0
		2018S1P3	0	1	0	2	0	0	1	0	1	0
		2019S1P1	0	5	0		0	0	1	0		0
Laccophilus hyalinus (De Geer, 1774)	P.P.P.	2018S1P3	0	1	0	0	0	0	1	0	0	0
Agabus brunneus (Fabricius, 1798)	P.P.P.	2018S1P2	0	0	0	1	0	0	0	0	1	0
Agabus dilatatus (Brullé, 1832)	P.P.P.	2019S1P1	3	2	0		0	1	1	0		0
Agabus solieri Aubé, 1836	P.P.P.	2018S1P1	0	0	0	1	0	0	0	0	1	0
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Ilybius sp. Erichson, 1832	P.P.P.	2018S1P3	0	2	0	0	0	0	1	0	0	0
Acilius sulcatus (Linnaeus, 1758)	P.P.P.	2018S1P1	0	0	0	0	1	0	0	0	0	1
Dytiscus marginalis Linnaeus, 1758	P.P.P.	2018S1P3	0	0	0	0	1	0	0	0	0	1
		2018S2P2		1	0	0	0		1	0	0	0
Helophorus flavipes Fabricius, 1792	G.	2018S1P1	0	4	4	4	0	0	1	1	1	0
		2018S1P2	0	9	4	5	0	0	1	1	1	0
		2018S1P3	0	28	2	9	0	0	1	1	1	0
		2018S2P2		0	1	0	0		0	1	0	0
		2019S1P1	1	1	2		1	1	1	1		1
		2019S1P2	0	1	0	4	0	0	1	0	1	0
		2019S1P3	0	0	1	3	0	0	0	1	1	0
		2019S2P1	1	1	0	0	0	1	1	0	0	0
		2019S2P2		3	0	0	2		1	0	0	1
Helophorus griseus Herbst, 1793	G.	2018S1P3	0	0	0	1	0	0	0	0	1	0
Helophorus pallidipennis Mulsant & Wachanru, 1852	G.	2018S1P2	0	0	1	0	0	0	0	1	0	0
		2018S1P3	0	7	0	1	0	0	1	0	1	0
		2019S1P2	0	0	1	1	0	0	0	1	1	0
		2019S1P3	0	0	0	5	0	0	0	0	1	0
Hydrochus angustatus Germar, 1824	S.	2018S1P2	3	3	0	0	1	1	1	0	0	1
		2018S1P3	0	3	0	0	0	0	1	0	0	0
		2018S2P2		1	0	0	1		1	0	0	1
		2018S2P3	0	0	0	0	1	0	0	0	0	1
		2019S1P1	1	1	0		0	1	1	0		0
		2019S1P2	0	0	0	0	3	0	0	0	0	1
		2019S1P3	2	0	0	0	0	1	0	0	0	0
		2019S2P2		2	0	0	0		1	0	0	0
Helochares lividus (Forster, 1771)	G.	2018S1P2	0	0	0	2	0	0	0	0	1	0
		2018S2P3	0	0	0	0	1	0	0	0	0	1
Laccobius minutus (Linnaeus, 1758)	G.	2018S2P2		1	0	0	0		1	0	0	0
		2019S1P1	0	1	0		0	0	1	0		0
		2019S1P3	0	1	0	0	0	0	1	0	0	0
Laccobius neapolitanus Rottenberg, 1874	G.	2019S1P1	2	0	0		0	1	0	0		0
		2019S1P3	0	2	0	0	0	0	1	0	0	0
Laccobius striatulus (Fabricius, 1801)	G.	2018S1P2	0	0	0	1	0	0	0	0	1	0
		2018S1P3	0	1	0	2	0	0	1	0	1	0
		2019S1P1	0	1	0		0	0	1	0		0
		2019S1P2	0	1	0	0	0	0	1	0	0	0
		2019S2P1	0	1	0	0	1	0	1	0	0	1
Hydraena andreinii d'Orchymont, 1934	C.G.	2019S2P1	0	0	0	0	2	0	0	0	0	1
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Hydraena bohemica Hrbacek, 1981	C.G.	2019S2P1	1	0	0	0	0	1	0	0	0	0
Hydraena paganettii Ganglbauer, 1901	C.G.	2018S1P1	0	1	0	0	0	0	1	0	0	0
		2018S1P2	0	7	0	1	0	0	1	0	1	0
		2019S2P1	0	0	0	0	1	0	0	0	0	1
Hydraena pygmaea Waterhouse, 1833	C.G.	2018S2P1		0	0	1	0		0	0	1	0
Hydraena similis d'Orchymont, 1930	C.G.	2018S2P1		1	0	2	1		1	0	1	1
Hydraena subimpressa Rey, 1885	C.G.	2018S1P1	0	10	1	2	0	0	1	1	1	0
		2018S1P3	0	0	0	3	1	0	0	0	1	1
		2018S2P2		0	1	0	0		0	1	0	0
		2019S1P1	0	1	0		0	0	1	0		0
		2019S1P2	1	0	0	0	0	1	0	0	0	0
		2019S2P1	0	0	0	0	2	0	0	0	0	1
Hydraena testacea Curtis, 1830	C.G.	2018S1P3	0	2	0	0	0	0	1	0	0	0
Ochthebius aeneus Stephens, 1835	C.G.	2018S1P1	0	2	0	0	0	0	1	0	0	0
		2019S2P2		1	0	0	0		1	0	0	0
Ochthebius gibbosus Germar, 1824	C.G.	2018S1P1	0	1	0	0	0	0	1	0	0	0
Ochthebius meridionalis Rey, 1885	C.G.	2018S1P1	0	2	0	0	0	0	1	0	0	0
Ochthebius minimus (Fabricius, 1792)	C.G.	2018S2P2		1	0	0	1		1	0	0	1
Ochthebius viridis Peyron, 1858	C.G.	2018S1P3	0	2	0	0	0	0	1	0	0	0
		2018S2P2		1	0	0	0		1	0	0	0
Ochthebius (Henicocerus) sp. Agassiz, 1846	C.G.	2018S1P1	0	3	0	2	0	0	1	0	1	0
Limnebius crinifer Rey, 1885	C.G.	2018S2P3	1	0	0	0	0	1	0	0	0	0
Limnebius nitidus (Marsham, 1802)	C.G.	2018S1P1	0	0	0	1	0	0	0	0	1	0
Scirtidae n.d.	G.	2019S1P2	1	0	0	0	0	1	0	0	0	0
Pomatinus substriatus (Muller, 1806)	C.G.	2018S1P2	0	0	0	2	0	0	0	0	1	0
		2018S1P3	0	1	0	0	0	0	1	0	0	0
		2018S2P3	0	0	0	1	0	0	0	0	1	0
		2019S1P2	0	1	0	0	0	0	1	0	0	0
		2019S1P3	1	0	0	0	1	1	0	0	0	1
Sialis sp. Latreille, 1802	C.G.	2019S1P1	0	1	0		1	0	1	0		1
Limnophila sp. Macquart, 1834	P.P.P.	2018S1P1	0	1	0	0	0	0	1	0	0	0
Neolimnomyia gr. nemoralis (Meigen, 1818)	P.P.P.	2018S1P1	0	2	0	0	0	0	1	0	0	0
Cheilotrichia sp. Rossi, 1848	P.P.P.	2019S1P1	1	0	0		0	1	0	0		0
Helius sp. Lepeletier & Serville, 1828	P.P.P.	2018S1P1	0	1	0	0	0	0	1	0	0	0
Limonia sp. Meigen, 1803	P.P.P.	2018S2P2		0	0	1	0		0	0	1	0
		2019S1P1	0	1	0		0	0	1	0		0
Tipula sp. Linnaeus, 1758	S.	2018S1P3	0	1	0	0	0	0	1	0	0	0
		2019S1P1	3	0	0		0	1	0	0		0
Peripsychoda sp. Enderlein, 1935	G.S.	2018S1P1	0	10	2	100	0	0	1	1	1	0
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		2018S1P2	0	0	0	39	0	0	0	0	1	0
		2018S1P3	0	0	4	97	0	0	0	1	1	0
Trichopsychoda hirtella (Tonnoir, 1919)	G.S.	2018S1P1	0	1	0	0	0	0	1	0	0	0
		2019S1P1	1	0	0		0	1	0	0		0
Berdeniella sp. Vaillant, 1976	G.S.	2018S1P1	0	25	0	3	0	0	1	0	1	0
		2019S1P1	2	0	0		0	1	0	0		0
Pericoma sp. Walker, 1856	G.S.	2019S1P1	6	0	0		0	1	0	0		0
Saraiella sp. Vaillant, 1973	G.S.	2019S1P1	1	0	0		0	1	0	0		0
Bazarella subneglecta (Tonnoir, 1922)	G.S.	2018S1P1	0	27	0	1	0	0	1	0	1	0
		2019S1P1	13	0	0		0	1	0	0		0
Copropsychoda sp. Tonnoir, 1940	G.S.	2018S2P3	0	0	0	2	0	0	0	0	1	0
Dixa sp. Meigen, 1818	C.F.	2018S1P1	0	4	1	7	0	0	1	1	1	0
		2018S1P2	0	0	0	1	0	0	0	0	1	0
		2018S1P3	0	1	0	0	0	0	1	0	0	0
		2019S1P1	1	2	0		0	1	1	0		0
Chaoborus pallidus (Fabricius, 1794)	P.P.P.	2018S1P1	0	0	0	0	1	0	0	0	0	1
Anopheles maculipennis Meigen, 1818	C.F.	2018S1P1	0	0	0	2	0	0	0	0	1	0
		2018S1P2	0	0	0	59	18	0	0	0	1	1
		2018S1P3	0	0	0	26	5	0	0	0	1	1
		2018S2P2		4	2	0	0		1	1	0	0
		2018S2P3	5	1	0	1	1	1	1	0	1	1
Culex pipiens Linnaeus, 1758	C.F.	2018S2P2		1	0	0	0		1	0	0	0
Culex territans Walker, 1856	C.F.	2018S1P1	0	0	0	1	0	0	0	0	1	0
		2018S1P2	0	0	0	3	1	0	0	0	1	1
		2018S1P3	0	0	0	1	2	0	0	0	1	1
		2018S2P3	0	1	0	0	0	0	1	0	0	0
Simulium ornatum Meigen, 1818	C.F.	2018S1P3	0	3	0	0	0	0	1	0	0	0
Dasyhelea sp. Kieffer, 1911	C.G.	2018S1P1	0	2	0	0	0	0	1	0	0	0
		2019S1P1	5	6	0		0	1	1	0		0
Dasyhelea sp. B Kieffer, 1911	C.G.	2019S1P1	1	0	0		0	1	0	0		0
Culicoides sp. Latreille, 1809	C.G.	2018S1P1	0	1	0	1	1	0	1	0	1	1
		2019S1P1	40	5	0		0	1	1	0		0
		2019S1P3	0	0	0	0	1	0	0	0	0	1
Stilobezzia sp. Kieffer, 1911	C.G.	2018S1P2	4	9	3	14	81	1	1	1	1	1
		2018S1P3	5	7	1	19	14	1	1	1	1	1
		2018S2P1		0	1	1	0		0	1	1	0
		2018S2P3	16	16	0	1	7	1	1	0	1	1
		2019S1P1	24	7	0		5	1	1	0		1
		2019S1P2	4	6	0	0	3	1	1	0	0	1

		2019S1P3	6	0	0	2	1	1	0	0	1	1
		2019S2P1	1	1	0	0	11	1	1	0	0	1
		2019S2P2		0	0	0	1		0	0	0	1
		2019S2P3		1	0	0	2		1	0	0	1
Chironominae n.d.	-	2018S1P1	5	353	91	3090	353	1	1	1	1	1
		2018S1P2	25	65	158	1842	605	1	1	1	1	1
		2018S1P3	14	299	331	2431	973	1	1	1	1	1
		2018S2P1		164	198	402	41		1	1	1	1
		2018S2P2		341	444	403	437		1	1	1	1
		2018S2P3	112	328	95	1558	242	1	1	1	1	1
		2019S1P1	112	59	11		0	1	1	1		0
		2019S1P2	308	138	90	496	295	1	1	1	1	1
		2019S1P3	315	96	155	647	280	1	1	1	1	1
		2019S2P1	27	21	14	48	108	1	1	1	1	1
		2019S2P2		62	16	8	173		1	1	1	1
		2019S2P3		11	7	26	173		1	1	1	1
Tanypodinae n.d.	P.P.P.	2018S1P1	1	17	4	25	5	1	1	1	1	1
		2018S1P2	3	2	1	8	7	1	1	1	1	1
		2018S1P3	0	1	0	11	15	0	1	0	1	1
		2018S2P1		2	0	0	0		1	0	0	0
		2018S2P2		11	7	6	2		1	1	1	1
		2018S2P3	2	3	0	15	0	1	1	0	1	0
		2019S1P1	3	1	1		0	1	1	1		0
		2019S1P2	3	0	0	1	0	1	0	0	1	0
		2019S1P3	0	0	0	0	2	0	0	0	0	1
		2019S2P1	0	0	0	0	1	0	0	0	0	1
Orthocladiinae n.d.	-	2018S1P1	1	1103	205	213	0	1	1	1	1	0
		2018S1P2	12	81	32	246	62	1	1	1	1	1
		2018S1P3	1	75	9	53	22	1	1	1	1	1
		2018S2P1		0	1	0	1		0	1	0	1
		2018S2P2		5	1	2	22		1	1	1	1
		2018S2P3	5	3	0	13	3	1	1	0	1	1
		2019S1P1	117	284	89		0	1	1	1		0
		2019S1P2	14	15	30	62	29	1	1	1	1	1
		2019S1P3	11	6	116	62	17	1	1	1	1	1
		2019S2P1	0	1	1	0	5	0	1	1	0	1
		2019S2P2		13	4	2	20		1	1	1	1
		2019S2P3		7	1	5	6		1	1	1	1
Beris sp. Latreille, 1802	G.S.	2018S1P1	0	3	0	0	0	0	1	0	0	0
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		2019S1P1	1	1	0		0	1	1	0		0
Oplodontha viridula (Fabricius, 1775)	G.S.	2019S1P1	1	0	0		0	1	0	0		0
Oxycera sp. Meigen, 1803	G.S.	2018S1P1	0	1	0	0	0	0	1	0	0	0
Rhagionidae n.d.	G.	2019S1P1	5	0	0		0	1	0	0		0
Atylotus fulvus (Meigen, 1804)	P.P.P.	2018S2P2		1	0	0	0		1	0	0	0
Atylotus sp. Osten-Sacken, 1876	P.P.P.	2018S1P2	0	0	0	0	5	0	0	0	0	1
Empididae n.d.	P.P.P.	2019S1P1	1	0	0		0	1	0	0		0
Dolichopodidae n.d.	P.P.P.	2018S1P1	0	1	0	0	0	0	1	0	0	0
		2018S1P3	0	0	0	1	0	0	0	0	1	0
Sericomyia lappona (Linnaeus, 1758)	S.	2018S1P1	0	0	0	2	0	0	0	0	1	0
		2018S1P2	0	0	0	5	0	0	0	0	1	0
		2018S1P3	0	0	0	8	0	0	0	0	1	0
Helophilus hybridus Loew, 1846	S.	2018S1P1	0	0	0	2	0	0	0	0	1	0
		2018S1P2	0	0	0	8	0	0	0	0	1	0
		2018S1P3	0	0	0	17	0	0	0	0	1	0
Helophilus pendulus (Linnaeus, 1758)	S.	2018S1P3	0	0	0	8	0	0	0	0	1	0
Helophilus trivittatus (Fabricius, 1805)	S.	2019S1P2	0	0	0	1	0	0	0	0	1	0
Helophilus sp. Meigen, 1822	S.	2019S2P2		0	1	0	0		0	1	0	0
Parhelophilus frutetorum (Fabricius, 1775)	S.	2018S1P3	0	0	0	5	0	0	0	0	1	0
Sciomyzidae n.d.	P.P.P.	2018S1P1	0	20	0	0	0	0	1	0	0	0
		2018S2P2		0	0	0	1		0	0	0	1
		2019S1P1	40	7	0		0	1	1	0		0
		2019S2P1	0	0	0	0	0	0	0	0	0	0
Lispe sp. Latreille, 1796	P.P.P.	2019S1P1	7	0	0		0	1	0	0		0
Plectrocnemia conspersa (Curtis, 1834)	P.P.P.	2019S1P1	1	1	1		0	1	1	1		0
Limnephilus flavicornis (Fabricius, 1787)	S.	2019S1P1	1	7	0		0	1	1	0		0
		2019S1P2	0	1	0	0	0	0	1	0	0	0
		2019S1P3	3	5	0	0	0	1	1	0	0	0
Limnephilus flavospinosus (Stein, 1874)	S.	2018S1P1	0	3	0	0	0	0	1	0	0	0
		2018S1P2	1	0	0	0	0	1	0	0	0	0
		2019S1P1	6	9	1		0	1	1	1		0
		2019S1P2	10	3	0	0	0	1	1	0	0	0
		2019S1P3	15	3	0	0	0	1	1	0	0	0
Limnephilus lunatus Curtis, 1834	S.	2018S1P1	1	1	0	0	0	1	1	0	0	0
		2018S1P2	1	1	1	2	0	1	1	1	1	0
		2018S1P3	0	1	0	0	0	0	1	0	0	0
		2019S1P1	1	4	0		0	1	1	0		0
		2019S1P2	2	2	0	0	0	1	1	0	0	0
		2019S1P3	12	10	1	0	0	1	1	1	0	0

Limnephilus rhombicus (Linnaeus, 1758)	S.	2018S1P1	1	19	1	0	0	1	1	1	0	0
		2018S1P2	15	16	2	0	0	1	1	1	0	0
		2018S1P3	1	4	0	1	0	1	1	0	1	0
		2019S1P1	23	11	1		0	1	1	1		0
		2019S1P2	13	5	1	0	0	1	1	1	0	0
		2019S1P3	12	7	1	0	0	1	1	1	0	0
Limnephilus vittatus (Fabricius, 1798)	S.	2018S1P2	0	1	0	0	0	0	1	0	0	0
Halesus radiatus (Curtis, 1834)	S.	2019S1P1	16	9	0		0	1	1	0		0
		2019S1P3	2	1	0	0	0	1	1	0	0	0
Stenophylax permistus McLachlan, 1895	S.	2018S1P1	0	1	0	0	0	0	1	0	0	0
Allogamus antennatus (McLachlan, 1876)	S.	2019S1P1	1	1	0		0	1	1	0		0
Chaetopteryx gessneri McLachlan, 1876	S.	2019S1P1	8	1	0		0	1	1	0		0
Leptoceridae n.d.	S.	2018S1P2	0	0	0	1	0	0	0	0	1	0
		2018S2P2		1	0	0	0		1	0	0	0
Acentria ephemerella (Denis & Schiffermüller, 1775)	G.S.	2018S1P1	0	0	1	0	0	0	0	1	0	0
Plumatella sp. Lamarck, 1816	C.F.	2018S1P2	0	0	0	11	193	0	0	0	1	1
		2018S1P3	0	0	0	1	54	0	0	0	1	1
		2018S2P2		7	109	17	14		1	1	1	1
		2018S2P3	0	0	0	136	0	0	0	0	1	0
		2019S1P1	0	3	0		0	0	1	0		0
		2019S1P2	10	5	2	0	0	1	1	1	0	0
		2019S1P3	21	0	4	1	19	1	0	1	1	1
Carassius auratus (Linnaeus, 1758)	P.P.P.	2019S1P2	0	1	0	0	0	0	1	0	0	0
Carassius carassius (Linnaeus, 1758)	P.P.P.	2018S1P2	0	0	2	2	0	0	0	1	1	0
		2018S2P2		1	0	0	0		1	0	0	0
		2018S2P3	0	0	0	1	0	0	0	0	1	0
		2019S1P2	0	1	0	6	0	0	1	0	1	0
		2019S1P3	0	0	0	1	0	0	0	0	1	0
		2019S2P1	0	0	0	0	1	0	0	0	0	1
		2019S2P2		6	0	1	0		1	0	1	0
		2019S2P3		0	2	2	0		0	1	1	0
Cyprinus carpio Linnaeus, 1758	P.P.P.	2019S2P1	0	0	1	0	0	0	0	1	0	0
		2019S2P2		1	0	0	0		1	0	0	0
		2019S2P3		0	0	3	0		0	0	1	0
Cyprinidae n.d.	P.P.P.	2018S2P3	0	1	0	1	0	0	1	0	1	0
Silurus glanis Linnaeus, 1758	P.P.P.	2018S1P1	0	0	0	0	4	0	0	0	0	1
		2018S1P2	0	0	0	0	3	0	0	0	0	1
		2018S1P3	0	0	0	0	3	0	0	0	0	1
		2018S2P1		0	1	4	1		0	1	1	1
			I									

		2018S2P2		0	2	8	8		0	1	1	1
		2018S2P3	0	0	0	1	2	0	0	0	1	1
		2019S1P1	0	0	1		0	0	0	1		0
		2019S1P3	0	0	1	2	1	0	0	1	1	1
		2019S2P2		2	0	0	0		1	0	0	0
		2019S2P3		0	0	2	0		0	0	1	0
Lepomis gibbosus (Linnaeus, 1758)	P.P.P.	2018S2P2		0	1	0	0		0	1	0	0
		2019S1P2	0	0	0	0	1	0	0	0	0	1
		2019S1P3	0	0	0	1	0	0	0	0	1	0
		2019S2P1	0	0	1	0	2	0	0	1	0	1
		2019S2P2		0	0	0	1		0	0	0	1
		2019S2P3		0	0	0	0		0	0	0	0
Bufo bufo (Linnaeus, 1758)	S.	2018S1P1	0	80	4	81	0	0	1	1	1	0
		2018S1P2	11	73	15	42	0	1	1	1	1	0
		2018S1P3	9	56	17	37	0	1	1	1	1	0
		2019S1P1	305	170	133		44	1	1	1		1
		2019S1P2	206	219	119	15	0	1	1	1	1	0
		2019S1P3	95	125	24	0	0	1	1	1	0	0
			I									

Appendix D

Field Sheet

// SCHED	DI CAMPO	luoro	A	Â
sereno poco nuvoloso foschia velature lievi	elature scarse nubi sparse stazion	ne: OA OB OC OD		(V)
nuvoloso stratificazioni nubi basse nebbioso	tratificazioni coperto Tacq	°C σμS/cm		
banchi di nebbia pioviggine debole pioggia rovesci isolati	piggia/rovescio pioggia/rovescio protection forte			
roverci piogia Alterna de la construcción de la co	temporali pioggia e neve re osservazioni: w 270	direzione del verto		
variabile neve nevicitio neve debole forte	Aussta m/s		ET. D	A TATATA
stadio carcassa: ① subm. fresh ② early floating   ③ floating dec. ④ adv. fl. dec. ⑤ sunken remains			En the	
macroinvertebrati da fissare     Nº   taxa   distre		distretto		
			note:	
macroinvertebrati da allevare				
N <sup>v</sup> taxa		distretto		
N° tot camp. fissati: N° tot camp. vivi:				

## Acknowledgment

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