

A Review: Role of Silkworm (*Tubifex Tubifex Müller, 1774*) as Bioremediator in Freshwater Ecosystem

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Abstract: *Tubifex tubifex* Müller, 1774 is an oligochaete that is extensively dispersed, in particular in freshwater ecosystems, and plays an important role in the ecosystem. It is one of natural or live feeds for aquatic organisms in nature or cultivation media. Furthermore, *T. tubifex* could be a reasonable living being for the inquiry about the organic impact of different contaminants due to its capacity to decompose matter as well as accumulate metals or chemical pollutants. This review aimed to elaborate on the information about the biology of *T. tubifex* and its role, focusing on its ability as a decomposer in freshwater ecosystems. In the arrangement of this paper, many relevant scientific articles were cited. This paper examined the biology of *T. tubifex* (i.e., morphology, chaetae, segmentation, respiratory, reproduction, and habitat of *Tubifex*) and also the role of *Tubifex* as a bioremediator. This review informed that *Tubifex* has a reddish color due to the presence of erythrocrucorin. As a group of clitellates, *Tubifex* has a clitellum at segments X, XI, and XII. Furthermore, as a group of oligochaetes, it has chaetae that play an important role in burrowing, hooking to the substrate, swimming, crawling, and bioturbating. Tubificidae have the capability of autotomization (autotomy) and regeneration of their missing body parts. *T. tubifex* spends its whole life cycle within the substrate, with its head down within the substrate, whereas part of its posterior may extend over the water column-substrate interface. *T. tubifex* develops a mixed reproductive strategy, self-fertilization or parthenogenesis (pseudogamy), and is hermaphrodite. *Tubifex* undergoes fertilization and produces eggs that are stored in cocoon sacs. The eggs will hatch and then develop into juveniles, adult worms, and individuals that are mature enough to reproduce. This reproductive stage takes about 57-60 days. *Tubifex* is a meiobenthic species of aquatic worm and an important key for organic matter decomposition, heavy metal, or chemical substances reduction and detoxification. It can perform vermicomposting on organic matter and also accumulate chemical substances through its metabolic compounds such as superoxide dismutase enzyme, catalase enzyme, carboxylesterase enzyme, glutathione-S-transferase enzyme, and metallothionein enzyme.

Keywords: Biology, bioremediator, metabolic compound, role, *Tubifex*.

1. Introduction

Tubifex worm (*Tubifex tubifex* Müller, 1774) is included as a group of water worms, generally known as silkworm or hair because it has a soft and very soft body like silk or hair (Yanar et al., 2003; Mahendra et al., 2019). *T. tubifex* is one of the natural feeds commonly used and the most important live feed used in aquaculture or fish cultivation (Mandal et al., 2018; Simangunsong et al., 2023; Kurniawan et al., 2024). This species can play an important role as a supplement because of its high nutritional content (Kautsar et al., 2022). Yanar et al., (2003) explained that this worm contains a percentage of crude protein 11.02 ± 0.58 , lipid 2.14 ± 0.06 , ash 1.83 ± 0.16 , and moisture 18.78 ± 0.83 , and also some fatty acid and amino acid. Oplinger et al., (2011) and Rech et al., (2013) revealed *T. Tubifex* contains protein (50-55%), fat (8-10%), crude fibre (2-5%), ash (4-7%), and water (8-10%). This nutrient content is higher in quantity and of the same quality compared to *Artemia* sp. Mashudi et al., (2023) explained that *T. tubifex* is small in size according to larval mouths,

easier to be digested for larvae of aquatic biota, and according to Conceição et al. (2010) *Tubifex* sp. can supply fundamental nutrients for the great growth performance of larvae.

T. tubifex is grouped in the Oligochaeta class and Naididae (Tubificidae) family, so it is also known as tubificid worms (Erséus & Gustavsson, 2002; Alam et al., 2021). It also has an ability to live in in variety of waters from clean to very contaminated water, especially a habitat with a lot of organic materials (Singh et al., 2007; Yang et al., 2020a) and in the mud or sludge containing rich organic compound particularly in waste water, canals, and drainage basin so that make them known as sewage or sludge worms (Mandal et al., 2016; Haque et al., 2020).

T. tubifex plays a vital role in the ecosystem, in addition to being a natural feed (e.g., for fish, crustacea, etc.), the capability of *T. tubifex* to decompose organic matter has explored through various researches like the pollutant removal of wastewater treatment (Yang et al., 2020a; Yang et al., 2023), include ecotoxicity assessment for metal reduction (Thit et al., 2020). Its bioturbation activity can impact water movement (Mao et al., 2020), which generally influences the materials exchange in the water column-substrate interface (Lagauzère et al., 2009a). *Tubifex*, which lives with its head-down and partially submerged

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in the substrate (e.g., sediment, clay, silt), can function as a conveyor-belt deposit-feeder. Its posterior of the body is exposed to the water above, enabling cutaneous breathing. It consumes substrate particles when foraging in the substrate and excretes them to the surface in mucus-bound fecal pellets, which causes mixing of the substrate particles and influences the distribution of dissolved particles (Lagauzère et al., 2009b). Further, it also improves the bacterial diversity (Yang et al., 2020b), and this association is an effective bioremediation strategy by accelerating organic matter and nitrogen loss in the ecosystem without substantially increasing the nitrogen level in water (Yang et al., 2021). To maintain the quality of the biophysical features of the ecosystem, the remediation can degrade, break down, convert, and/or essentially eliminate toxins from the contaminated environment (Masciandaro et al., 2013).

T. tubifex is a widely distributed invertebrate and can tolerate to the stress factors, so it that, resulting as being a significant bioindicator species (Mermillod-Blondin et al., 2005). Its metabolisms, both of physiological and biological activities, are significantly mediated by many environmental parameters, like hydrodynamics (Mermillod-Blondin et al., 2010), temperature (Przeslawski et al., 2009), low oxygen concentration (Fowler & Goodnight, 1965; Simangunsong et al., 2024), and the contamination of pollutants (Lagauzère et al., 2009a; Mermillod-Blondin et al., 2013; Nie et al., 2017).

T. tubifex is a cosmopolitan worm of freshwater (Beauchamp et al., 2001), it could be a common inhabitant of organic-rich dregs around the world (Hallett et al., 2005), and it has been studied as a bioassay in the aquatic environment (Scopetani et al., 2020). Since as early as 1745, *T. tubifex* has become well-known for its capacity to survive in deoxygenated and often poisonous conditions. Furthermore, Tubifex may survive in extremely polluted waterways with large quantities of complex organic chemicals or metals, and it could be the sole metazoan animal there (Palmer, 1968).

Based on the arguments, the presence and role of *T. tubifex* in the environment, in particular in freshwater ecosystems, were important to be studied. This review was written to elaborate on the information about the biology of *T. tubifex* and its role, focusing on its ability as a decomposer in freshwater ecosystems.

2. Literature Search

The cited articles are the relevant scientific articles. There were 224 cited articles that were published from 1921 to 2024 that were retrieved from Google Scholar based on several keywords, including *Tubifex* sp., *Tubifex tubifex*, role of *Tubifex* sp., Oligochaeta, silkworm, tubificid worms, bioturbation, natural feed for aquatic biota and aquaculture, Tubifex as bioassay and bioaccumulator, and other articles related to it.

The collected information of those articles was systematically analyzed to ensure a comprehensive and detailed review. We have cited articles ranging from Bouché et al., (1999) to Simangunsong et al., (2024), with 20 relevant scientific articles in discussion about the morphology of *Tubifex* sp. We cited Foxon (1936) to Tilic & Bartolomaeus (2016) with total a of 18 relevant

scientific articles in discussion about chaetae of *Tubifex* sp. We also cited Goto et al., (1999) and Bouché et al., (1999) to Walczyńska et al., (2023), with a total of 28 relevant scientific articles in discussion about the segmentation of *Tubifex* sp. We found Zattara & Bely (2015) and Simangunsong et al., (2024) that focus on elaborating about the nervous system of *Tubifex* sp. We cited Palmer (1968) until Méndez-Fernández et al., (2014) with a total of 13 relevant scientific articles in discussion about the respiratory system of *Tubifex* sp. There were 29 relevant scientific articles in discussion about the reproductive system of *Tubifex* sp., ranging from Welch (1921) to Shimizu (2020). Aston (1973) until Simangunsong et al., (2024), with 23 relevant scientific articles, have revealed the habitat of *Tubifex* sp. We cited Aston (1973) until Hertika et al., (2023) and Ratnasari et al., (2023), with a total of 75 the relevant scientific articles in discussion about the role of *Tubifex* sp. as a bioremediator.

The references had partially explained the existence of Tubifex biologically and ecologically. Therefore, through this article review, a fairly comprehensive synopsis was made about the biological aspects of Tubifex and the ecological aspects related to its role in waters, especially its bioremediation role.

3. The Biology of *Tubifex tubifex*

Tubifex is the benthic oligochaete worm, one of the important natural foods for water organisms, in particular fish. Some of the Tubificidae were found in marine euryhaline environments, e.g. *Tubifex costatus* (Brinkhurst, 1964), and species of *Ainudrilus* sp. and *Heterodrilus* sp. are found in intertidal and shallow-water subtidal (Wang & Erséus. 2003). However, more of Tubificidae were found in freshwater, e.g. *Tubifex laxus* n. sp., *T. gracilentus* n. sp., *T. conicus* He, Wang & Cui, 2012, *T. tubifex* (Müller, 1774) (Hallett et al., 2005; Peng et al., 2017), *T. montanus* Kowalewski, 1919 (Peng et al., 2014), *T. blanchardi* Vejdovský, 1891 (Marotta et al., 2009), *T. ignotus* (Beauchamp et al., 2001; Negrodo et al., 2003), *T. newaensis* (Yıldız et al., 2007), and *T. nerthus* (Şahin & Yıldız, 2011). Many studies were conducted on *T. tubifex* compared to other species. It has been studied for several reasons, including freshwater indicator, live and natural food for fish, culturable species, host for fish parasites, genetic diversity, and water-polluted resistance.

Tubifex tubifex (Müller, 1774), species of Genus Tubifex, Subfamily Tubificinae, Family Naididae (Tubificidae), Suborder Tubificina, Order Tubificida, Subclass Oligochaeta, Class Clitellata, Phylum Annelida, and Kingdom Animalia. In this taxonomy, many studies have explained that *T. tubifex* is grouped in Family Tubificidae; however, Erséus & Gustavsson (2002) declared Naididae is older than Tubificidae, and Christensen and Theisen (1998) postulated that Naididae arose relatively early in Tubificidae evolution based on relative genetic distances. Benbow (2009) explained different statements that Naididae and Tubificidae are two different families of 10 families in the Subclass Oligochaeta found in freshwater. Referring to various publications, it generally uses the terminology of the Family Tubificidae (e.g., Beauchamp et al., 2001; Erséus & Källersjö, 2004) and up to the latest publication to reveal the family of Tubifex

species. Further, Erséus et al., (2022) have analyzed the Tubificidae'18S rDNA. The result suggests that the Naididae is formed as an equivalent word of the Family Tubificidae, and even Kaster (1980) has written *Tubifex tubifex* Müller, 1774 is a species of Family Tubificidae.

Morphology

At first glance, the *Tubifex* colony looks like a red wave. This is due to the reddish color of its body (Mandal et al., 2016, Haque et al., 2020), particularly the presence of erythrocrucorin (Singh et al., 2010) or dissolved haemoglobin (Vytlačilová et al., 2004) in its blood. Erythrocrucorin (Ec) is a metalloprotein that provides more oxygen-carrying capacity due to the high amount of oxygen binding and high concentration in the blood (Weber & Vinogradov, 2001; Royer et al., 2006; Kruczkowska et al., 2023).

Tubifex sp. has the body length ranged 1-2 cm (Mahendra et al., 2019; Simangunsong et al., 2024), 2-3 cm (Ragi & Jaya, 2014), 4-5 cm (Zhang et al., 2017; Nuraisyah et al., 2023), 2-8.5 cm (Vytlačilová et al., 2004), 5-10 cm (Mewekani & Tampobulon, 2019), and even can reach grow up to 20 cm long (Singh et al., 2010), whereas 0.5 mm in diameter (Kang et al., 2017). *Tubifex* sp. as well as all oligochaetes, has a segmented body ranging from 30-60 segments (Mahendra et al., 2019), 32 to 120 segments (Mariom et al., 2016), or about 130 segments (Vytlačilová et al., 2004).

There are appendages, a prostomium, and a mouth on the segments of its body. There is a peristomium on the second segment of its body (Mariom et al., 2016); however, Bouché et al., (1999) drew that the peristomium is located on the first segment

of the body, together with the mouth and prostomium. There is an anus on the end segment of its body. Each segment of the body has four bundles of chaetae (setae), namely two chaetae in dorsal and two chaetae in ventral position (Mariom et al., 2016). Based on the position of the peristomium at the second segment according to Mariom et al., (2016), they explained that this segment was free of chaetae. Meanwhile, according to other researchers, there are no or free chaetae at the first segment, and the chaetae appear at segment II (Bouché et al., 1999; Vytlačilová et al., 2004; Ragi & Jaya, 2014). Although there is a difference in determining the position of the peristomium, there is a similarity in that there are no chaetae in the segment where the peristomium is located. In addition, there is a clitellum at segments X, XI, and XII, while the ovisac for mature eggs is stored at segments XII to XIV (Bouché et al., 1999; Marotta et al., 2009; Shimizu, 2020).

Clitellates have a clitellum (hence called Clitellata), a glandular structure modification of the epidermis (Schmelz & Collado, 2010) that produces cocoons, namely a sac for encapsulating the eggs. Other characters of Clitellates are the absence of the parapodium and all clitellate species of Clitellates do not have the sensory nuchal organ, asprostomial appendages, and epidermal ciliation (Kuo, 2017). The morphology of *Tubifex* sp. was illustrated in Figure 1 with abbreviations AR: anterior region, consist of prostomium (pr), peristomium (pe), mouth (m), and brain (b); RSR: reproductive system regions (look Figure 7 for detail); CL: clitellum; PR: posterior region; dc: dorsal chaetae; vc: ventral chaetae; and g/i: gut/intestine.

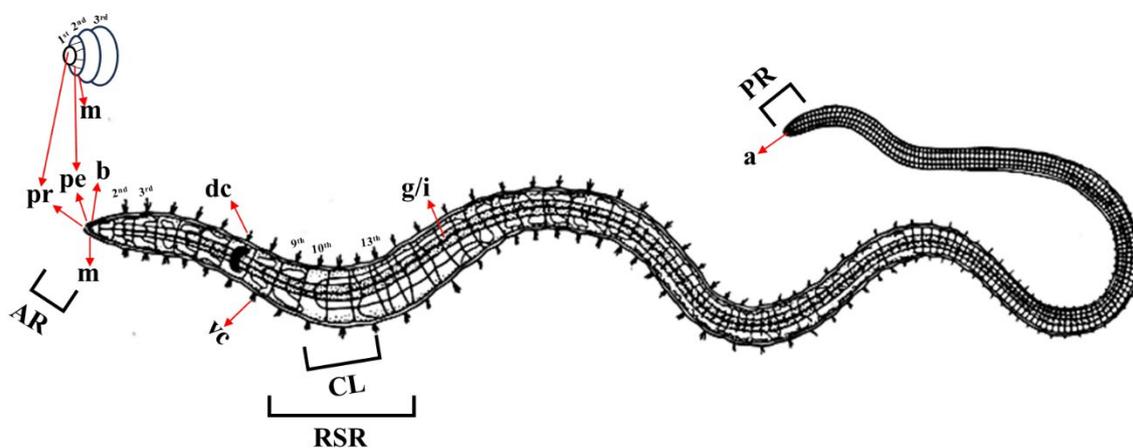


Figure 1. Morphology of *Tubifex* sp.

Chaetae

Oligochaetes have body segments with a small number of chitinous bristles (Bouché et al., 1999) called chaetae or setae. Chaetae are chitinous extracellular structures, and the presence of chitinous chaetae as well as a clitellum is one of the specific features of oligochaete (e.g., *Tubifex*), and the chaetae are an important diagnostic character in Annelida, including oligochaetes. Merz & Edwards (1998) explained that most annelid

species contain dorsal and ventral structures of chaetae connected with the parapodia of the majority of body segments.

The chaetae have important functions for annelids, including *Tubifex*. The chaetae serve a function in creating rubbing between the worm and the substrate when crawling (Gustus & Cloney, 1973; Merz & Edwards, 1998; Hesselberg & Vincent, 2006). The movement of chaetae in crawling is supported by parapodium (parapodia) (Foxon, 1936) Furthermore, Merz & Edwards (1998) revealed that setal sacs hold and bundle the chaetae of

polychaete parapodia. A parapodium may have some setal sacs, each with a connection to muscles, in particular the intrinsic parapodial protractor. These muscles are located in the setal sac to the parapodial wall. An aciculum facilitates connectivity between the setal sacs and tissue, a strong internal chaetae that is connected to protractor muscles and the intrinsic parapodial retractor. The chaetal retractor muscles' contraction reflex can pull the aciculum. The aciculum comes into the parapodium, causing the setal sacs and their associated chaetae to retract. Meanwhile, the protractor muscle's contraction reflex might draw the setal sacs toward the parapodial surface. This condition causes the chaetae to lengthen and the aciculum to shift further away. Thus, the movement of chaetae is indirectly performed by the attachment of muscle to the setal sacs and aciculum, with the worm controlling the movement.

Tubifex uses the chaetae for burrowing (Trevor, 1977) and hooking to the burrow substrate (Woodin & Merz, 1987). The chaetae activity allows the *Tubifex* to rapidly withdraw the body (Clark, 1960), thus it can tribulate a predator from pulling *Tubifex* out of its substrate (Knight-Jones & Fordy, 1979; Woodin & Merz, 1987). The chaeta is also used for bioturbating (Mao et al., 2020), meanwhile the parapodia generate thrust for the worm's movement during swimming (Gray, 1939; Clark & Tritton, 1970). Hesselberg & Vincent (2006) have investigated a correlation of the morphology of chaetae to habitat and locomotory behavior.

The chaetae are epidermal extracellular structures that are in general clearly visible from the exterior of annelids (Annelida). The Polychaeta have highly diverse of chaetae' structure. Each species has a specific pattern of chaetae that can be used as an instrument for species determination (Hausen, 2005). Dumnicka & Poznańska (2006) explained the absence, the changes in shape, number, and dimensions of chaetae depending on environmental conditions.

Furthermore, Hausen (2005) discovered that the microvilli pattern of a single cell—the chaetoblast, the most basal cell of the chaetal follicle—is what dictates the morphology of chaetae. It expands when new chitinous material is deposited at the base. The microvilli produced by the chaetoblast are a highly distinctive category. On these microvilli's exterior, new chitinous material assembles. In spite of the absence of vesicular transport within the chaetoblast' microvilli, the material for apposition to the base of the chaetae may not be directly supplied by the microvilli. On the other hand, the chaetoblast and follicle cells secrete the chitinous material, which then enters into the lumen of the chaetal follicle. After that, it reaches the surface of microvilli by diffusion mechanism, and it releases the substance between the chaetoblast part, i.e., the bases of the microvilli.

Tilic & Bartolomaeus (2016) completed the explanations that chaetae are generated within an ectodermal invagination, namely the chaetal follicle. It is composed of a terminal chaetoblast and follicle cells. Each chaetoblast contains an array of apical microvilli that are changed across time and space as chitin polymerizes along the microvilli. Therefore, the ultimate shape of the chaetae is determined by a controlled alteration of the microvilli pattern.

Many distinct chaetal forms, ranging from extremely complex compound hooked chaetae to simple capillaries, can emerge as a result of changes in spatiotemporal patterns. Chitin release freezes changes in the microvilli pattern, so that the definitive chaetae's structure reflects the changes in the microvilli pattern throughout time. The structure of the chaetae is also influenced by cellular interactions that occur during chaetogenesis.

The chaetae are segmentally arranged bristles. There are many morphologically distinct types of chaetae; however, within a species the chaetae of one type are very similar. The chaetae are classified as either simple (unjointed) chaetae or compound (jointed) chaetae (Gustus & Cloney, 1973). *Tubifex* has pectinate chaetae, hair chaetae, and bifid chaetae on its anterior segments, while bifid chaetae are on the posterior segments (Bouché et al., 1999). The chaetae are illustrated by Timm (2012) and Ragi & Jaya (2014) (Figure 2).

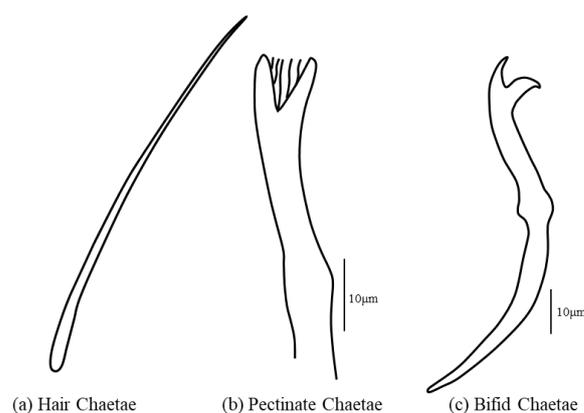


Figure 2. Morphology of *T. tubifex* chaetae. Dorsal chaetae: (a) hair chaetae and (b) pectinate chaetae (modification of short locomotory chaetae); Ventral chaetae: (c) bifid chaetae (Redrawn from Timm, 2012; Ragi & Jaya, 2014).

Segmentation

Oligochaete worms of Phylum Annelida are segmented coelomates that are bilaterally symmetrical, and there are bundles of chaetae on all body segments, however absent on the prostomium at the first segment (Vytlačilová et al., 2004), and an invertebrate whose body plan has a high degree of metamerism (Shimizu & Nakamoto, 2001; Balavoine, 2014). Metamerism or segmentation refers to translational symmetry (Klingenberg, 2015). Zattara & Bely (2015) drew the fundamental annelid body plan that consists of non-segmental regions and segmental regions. There is one non-segmental region at the anterior end composed of the prostomium (pr), peristomium (pe), and mouth (mo), and there is one non-segmental region at the posterior end, called the pygidium (py), composed of the anus. Regions are located between the anterior non-segmental and the posterior non-segmental and consist of segmental units. It also has a growth zone in front of the pygidium at the posterior segment of its body, called the posterior growth zone (pgz) (Figure 3).

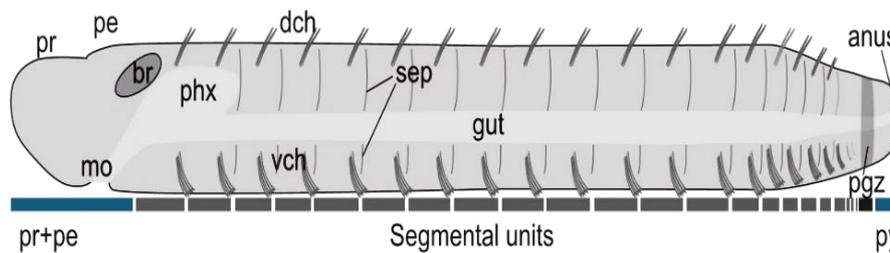


Figure 3. Overview of basic annelid body plan. pr: prostomium; pe: peristomium; mo: mouth; br: brain; phx: pharynx; dch: dorsal chaetae; vch: ventral chaetae; sep: intersegmental septum; gut: ciliated gut; pgz: posterior growth zone; py: pygidium (Zattara & Bely, 2015, cited with permission).

Segmentation involves the repetition of units that are arranged along the longitudinal (anterior-posterior) (A-P) axis. Each unit consists of elements from some organ systems and is serially homologous to each other (Fedonkin, 2003; Minelli & Fusco, 2004; Hannibal & Patel, 2013; Isaeva & Kasyanov, 2021). The body part is segmented and limited to the trunk, the head, and the terminal. Its head is represented by the presence of a prostomium, including a brain which is located at no-segment, nor is the pygidium. While the terminal of the body segment is a part of the anus. The prostomium in the anterior part and the pygidium in the posterior part are non-segmental (Starunov et al., 2015). This segment repeats many units of tissues, cells, or organs along the A-P axis, grouping units of various sorts into distinct segments. These units can be excretory organs, sensory organs, skeletal units, nerve cells or ganglia, muscles, and locomotory organs (Chipman, 2010). Externally, the trunk segmentation looks like rings or annuli, and internally, it is separated by intersegmental septa on a serial arrangement of coelomic compartments and also organs and system components in metameric arrangement (Goto et al., 1999; Shimizu & Nakamoto, 2001).

The Oligochaeta, Tubificidae (e.g., *Tubifex tubifex*) have the capability of autotomization (autotomy) and regeneration of their missing body parts. Regeneration is a physiological process that can occur daily or as a direct response to traumatic injury following segmentation or autotomy, regrowth, and restoration of missing body parts. This process occurs in all the different phases of development, from the embryo and larva to the adult phase of an organism undergoing autotomy. Although almost all animals have physiological regeneration (periodic regeneration of cells or tissues, such as epidermal tissue), the ability to regenerate body parts varies greatly, even between closely related species (Kostyuchenko et al., 2016; Kostyuchenko & Kozin, 2021; Walczyńska et al., 2023).

Regeneration, as a result of damaged structures after injury or amputation has been studied in phyla of invertebrates, one of them is annelid that are capable of regeneration at cell until tissue levels, including cell repairing, germ cell rebuilding, structures regrowing, or the regeneration of the all parts of body from the small fragments (Bely, 2006; Bely, 2014; Nikanorova et al., 2020). The regeneration is a complex process, including wound closure

and healing, immune system reaction, aggregation of cells for regeneration, growth of body part generations, patterning, and differentiation. However, generally, there are three processes or steps of this regeneration, namely wound healing phases, dedifferentiation phases, and redevelopment phases. While the duration for each regeneration phase can vary greatly, which can be influenced by the anatomy of the organism, nature of the wound, the location and cause of the amputation, the developmental phase, or the individual age (Acosta et al., 2021; Kostyuchenko & Kozin, 2021; (Walczyńska et al., 2023).

The posterior segment of the *T. tubifex* is the main part for autotomy, such as post-predation, toxicification, etc. This worm can regenerate its functional posterior end as well as a prepygidial segment, which becomes a new regenerated zone. The missing part of body regeneration in *T. tubifex* involves activation of mesoblastocytes and an increasing amount of neoblasts, and there is an increased number of migration of these cells (Bouché et al., 2003). Teloblasts are embryonic stem cells which produce five bilateral pairs of coherent longitudinal columns (bandlets) in the same number of bilateral pairs and then initiate the segmental structures of ectodermal and mesodermal. At the posterior end of the embryo, the teloblasts undergo asymmetrical cell divisions to generate daughter cells. The daughter cells move to the anterior end, so the oldest daughter cells are found at this side of each coherent longitudinal column (Goto et al., 1999; Nakamoto et al., 2000; Takahashi et al., 2008).

Following autotomy, the septum-level circular muscles contracted, and the epidermis then covered them. Large cell migration into the regenerate was observed following the first day of regeneration. Huge nuclei with huge, extremely thick nucleoli and highly basophilic cytoplasm were the characteristics of these cells. The neoblast cell production forms the blastema at the place of section, and these cells have migrated along the nerve cord and are found in the fifth metamere anterior to the site of section (Figure 4A). Neoblasts that were migrating had a pear or spindle shape, whereas those that were immobilized and stuck to the septa (Figure 4B) had a circular shape. The migrating neoblasts started dividing into smaller cells at 24 hours when neoblasts were organized in rows of several cells past the nerve cord's tip (Figure 4C) (Bouché et al., 2003).

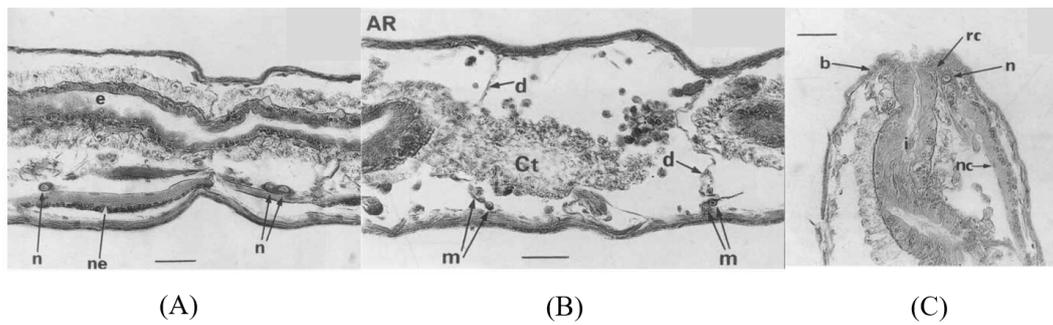


Figure 4. Regeneration in worm (Bouché et al., 2003, cited with permission).

Figure 4 showed (A) worms' regeneration: day 1. Migration of neoblasts (n) to the wound along the nerve cord (ne). Scale bar is 50 μm , and (e) is the intestinal lumen. (B). worms' regeneration: day 1. inosblastocytes position (m) at the postero-ventral face of the dissepiments (d). Scale bar is 50 μm , (ct) is chloragogen tissue, and (AR) is the anterior region. (C). worms' regeneration: day 1. neoblasts accumulation (n) beyond the broken nerve cord (nc). These neoblasts are regulated in rows of some cells and generate into smaller regeneration cells (rc). Scale bar is 50 μm , (i) is intestine and (b) is blastema.

Tubifex sp., like all oligochaetes, is a body-segmented worm with 30-130 segments (Vytlačilová et al., 2004; Mariom et al., 2016; Mahendra et al., 2019). The annelids, in particular, have a body that is segmented into pre-clitellar (pre-clitellum), clitellar (clitellum), and post-clitellar (post-clitellum) segments (Paul et al., 2022).

Tubifex has a mount at the first segment, which has appendages. The prostomium's shape is round or triangular, meanwhile, the terminal of its segment contains the anus. Each segment of the body has four bundles of chaetae (also called setae), namely two chaetae in the dorso-lateral and two chaetae in the ventro-lateral position; however, there are no chaetae in *Tubifex*, which appeared at segment II (Mariom et al., 2016; Bouché et al., 1999; Gline et al., 2011). Marotta et al., (2009) revealed that *T. tubifex* has 2-4 (mostly 3) capillary chaetae for each bundle that is found at the dorsal bundle of the pre-clitellum and also 2-3 (mostly 3) pectinate chaetae, which contain 3 intermediate denticles for each chaeta. Bouché et al., (1999) scanned the distribution of the putative chemoreceptor chaetae (arrowheads) over the anterior region of *T. tubifex*. The many small chaetae are grouped in tufts on the prostomium (P) and peristomium (Pe), and are less dense in other areas. II: segment II (bar = 50 μm) (Figure 5.3).

The brain of *Tubifex* is shifted anteriorly, passing the prostomium or peristomium. There are three pairs of serotonin immunoreactive (SIR), in which the asymmetric trunk pattern was found from the fourth segment, have non-septate ganglia, and the full complement of nerves from the third segment. In the brain of *Tubifex*' juveniles, there are two serotonin-positive cells, meanwhile, the adults have six cells. This fact shows that the number of serotonin-positive cells can change post-embryonically. In addition, the anterior segments of *Tubifex* show a symmetrical pattern of ectodermal segmentation including the autonomous morphogenetic at an early stage, followed by the mesoderm-dependent alignment (Zattara & Bely, 2015).

Furthermore, Mariom et al., (2016) explained there two kinds of chaetae, i.e., pectinate and hair chaetae at the dorsal-lateral. The pectinate chaetae have hooks at the end terminal, formed like combs, and their interior teeth are smaller than the exterior teeth. The hair chaetae have fine elements, called serrations. Meanwhile, all of the ventro-lateral chaetae have the same type, and the chaetae have bifid tips.

Bouché et al., (1999) revealed that there are differences in the dorsal and ventral chaetae of *Tubifex*. The dorsal chaetae consist of hair and pectinate chaetae. There are fine elements, serrations that adorn the hair chaetae (Figure 5.4), meanwhile pectinate chaetae have an S shape, a distal swelling on the shaft hooks-like combs at the ends with the two exterior larger than the interior teeth (3-4 intermediate teeth). The hair chaetae were interspersed with pectinate chaetae, so that pectinate chaetae were the most external chaetae in the bundles (Figure 5.5 and 5.6). The ventral chaetae have only one type, namely bifid tips (Figure 5.7), which have a longer upper branch than the lower one and also have an S-shaped curve and a swelling, as also owned by the dorsal pectinate chaetae (Figure 5.8).

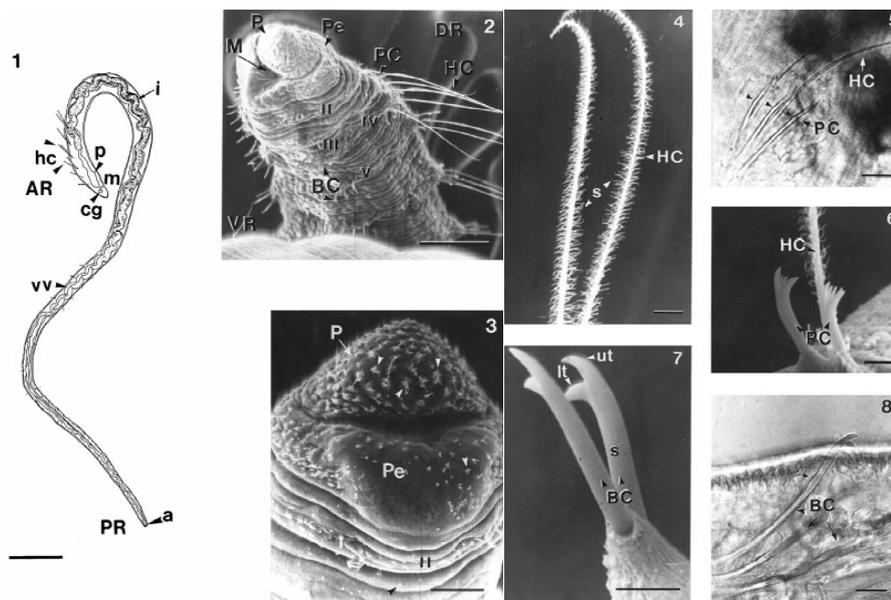


Figure 5. Microscopic electron scanning of the anterior region of *T. tubifex*. Each metamere bears two bundles of ventro-lateral chaetae (bifid chaetae: BC) and two dorso-lateral bundles composed of pectinate chaetae (PC) and hair chaetae (HC) (only one of the two dorso-lateral bundles is visible in the picture). Mouth (M), prostomium (P), peristomium (Pe), dorsal region (DR), ventral region (VR), and metameres II-V (II, III, IV, and V) (scale bar=200 μ m) (Bouché et al., 1999).

Bouché et al., (1999) have revealed that there is a variation in the number of chaetae in the dorsal and ventral bundles in animals and the parts of individuals. There are many chaetae in the front part or anterior of the body and decrease to the rear or posterior of the body. Tubifex has bundles that contain 2-4 pectinate chaetae, 1-3 hair chaetae, and 2-4 bifid chaetae on its anterior segments. However, there is only a single chaeta on the bundles of dorsal and ventral at its third posterior. It's all of the posterior chaetae are bifid type. The size of the chaetae changes along the body, following its diameter function. The chaetae size at the segments II to V becomes larger, then it is the same size up to the clitellum at the segments X-XII, and finally gradually becomes smaller in size until the posterior end. Ventral bifid chaetae have diameter of 300 μ m in the anterior zone and 100

μ m in the posterior zone. The size of hair chaetae was 2-4 times larger than that of the pectinate chaetae.

Nervous System

Tubifex has a segmented body with distinctive anatomical structures. Each segment contains a simple and effective nervous system, while the nervous system consists of ganglia in each segment connected through longitudinal nerve cords, allowing the worms to respond to environmental conditions and stimuli (Simangunsong et al., 2024). Zattara & Bely (2015) revealed that *T. tubifex* has three main parts of the nervous system, namely the anterior brain, and it is joined with the peripheral nervous system, the ventral nerve cord with ganglia, and the segmented peripheral nerves.

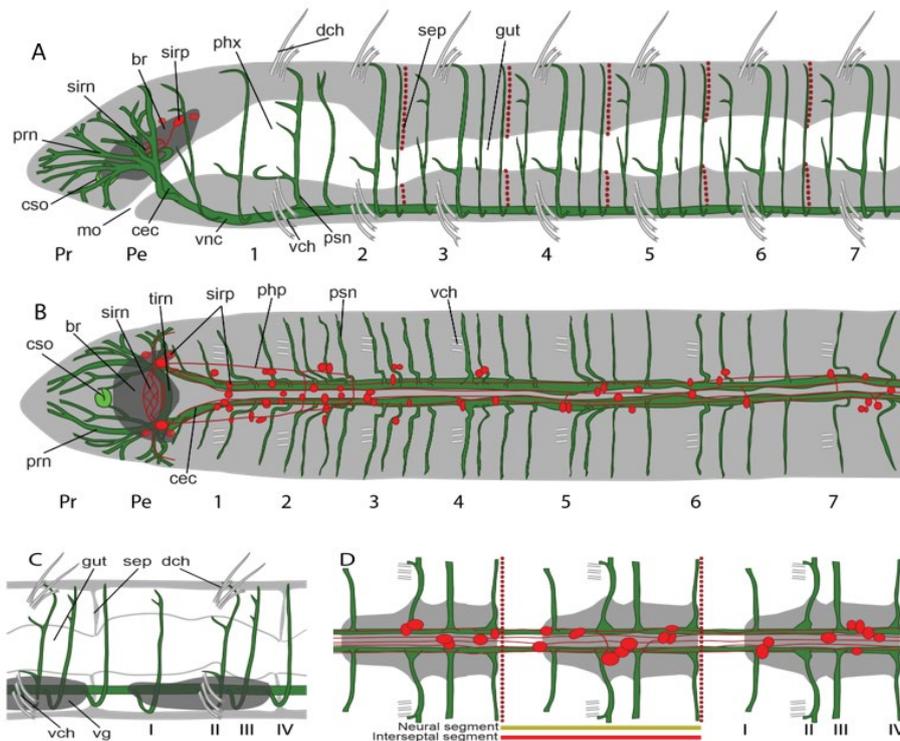


Figure 6. The *T. Tubifex*'s nervous system. A) the anterior terminal' lateral view, B) the anterior terminal' dorsal view, C) lateral view of a typical trunk body segment, and D) schematic of the ventral nerve cord structure (Zattara & Bely, 2015, cited with permission).

Figures 6A and 6B clarify that the green color indicates Acetylated-tubulin immunoreactive (acTIR) neuropil, the red color indicates perikarya and serotonin immunoreactive (SIR) neurites, and the dark gray color indicates the brain. At the anterior end, there is a nervous system that consists of the ventral nerve cord, the segmental peripheral nerve, the prostomial nerve, the circumesophageal connective, the pharyngeal plexus, and the brain. Meanwhile, Figures 6C and 6D reveal the dark gray color showing ventral nerve cord ganglia and the dashed dark red line showing intersegmental septa. These pictures show the position of ganglia, septa, and chaeta relative to peripheral nerve roots (Zattara & Bely, 2015).

Zattara & Bely (2015) discovered that the brain of *T. tubifex* consists of two bilobed structures inside the anterior nervous system. The location of the brain is dorsal to the mouth, with the peristomium encircling the rear edge and the prostomium housing the anterior side. The ventral nerve cord and paired prostomial peripheral nerve cords are connected to the inner neuropil of the brain's external cell cortex via a pair of circumesophageal connectives. The trough-shaped cell cortex is located within the ventral nerve cord, which extends lengthwise along the worm's body. Each piece of its body has a single ganglion and a subesophageal ganglion at the anterior terminal of the cord. The cortical cell is traversed by the neuropil that contains SIR and acTIR neurites. Four peripheral acTIR nerves and recurrent segmental in each fragment's peripheral segmental nerves are present in Tubifex. After emerging from the ganglia and passing ventrally through the body wall's muscular layer, the recurrent segmental nerves run subepidermally to the dorsal

segment of the ventral nerve cord. Each nerve innervates many epidermal sensory hairs. On the front side of the worm, the segmental nerve pattern differs, with segments 1-2 having three nerves rather than four.

Respiratory System

T. tubifex usually lives and feeds with the head inserted into the substrate, while some part of its posterior may appear above the water column-substrate interface (Pelegri & Blackburn, 1995). The posterior part undulates freely in the overlying waters, while partially its part of the body is submerged in the substrate (Bouché et al., 2000; Hare et al., 2001; Lagauzère et al., 2009a). The feed preferences indicate that its feeding behavior is that of an infaunal species. It consumes on the fine-substrate fraction (< 63 µm) (Rodriguez et al., 2001), ingests particles, and digests the attached microflora (e.g., bacteria) mainly on the top 2 cm until 8 cm of substrate e.g., sediment (Pelegri & Blackburn, 1995), and includes organic matter-associated particles in the fractions of the substrate such as sediment, silt, or clay (Méndez-Fernández et al., 2014). Based on this feeding behavior, *Tubifex* is classified as a deposit feeder (Kaster & Wolff, 1982).

The head-down deposit feeder is a species that consumes the organic debris and substrate-associated microorganisms as food by burrowing and swallowing huge volumes of substrate (Lopez & Levinton, 1987). It utilizes the organic matter directly at the substrate surface and interacts with the microbial community inhabiting this area (Amon & Herndl, 1991). The deposit-feeding worm can ingest the substrate, even reaching a depth of 15 cm under the water column-substrate interface (Clough & Lopez,

1993), by burrowing into the substrate and processing it through its digestive tract for substrate alteration. Thus, the deposit feeder can be described as an organism that moves along the surface and burrows into the substrate (e.g., sediment) and digests or consumes organic matter, including living or non-living (dead) organic materials from the substrate.

Therefore, *Tubifex* spends its entire life cycle in the substrate, e.g., sediment, so it has to develop a respiratory system. Its respiratory activity is also carried out in the substrate through the posterior body portion that overlays above the water column-substrate interface to allow cutaneous respiration (Lagauzère et al., 2009b), where its gases exchange with water occurs by diffusion mechanism through the epidermis (Vytláčilová et al., 2004).

T. tubifex uses the permeable integument as the gas exchange organ in its respiratory system. As a deposit feeder, *T. tubifex* places the terminal of the anterior part in the bottom substrate, while its terminal of the posterior part is extended several millimeters into the contiguous water where gas exchange occurs. Its posterior portion's ventilatory movements are used to enhance convective transport of respiratory media, from and to the exchange media surfaces. Ventilatory function can be effective only if an adequate surface area for gas exchange exists and the posterior end has a larger surface area to diffuse the gas. Integumentary convolutions of *Tubifex*'s segments of the posterior play a role to enhance the availability of surface area for gas exchange in the respiratory process. The increase in surface area in this part is more than three times than in the anterior segments. A complicated network of furrows and ridges dominates this convoluted area. The combination of movement of ventilatory and the structure of this area may increase the gas exchange in the respiratory process (Kaster & Wolff, 1982). *T. tubifex* is independent in consuming oxygen in the external environment at an level oxygen of about 1.5 %. This consumption

can change to the dependent type if the oxygen is at 2.5% (Palmer, 1968). In the hypoxia condition, *Tubifex* can survive about 48 hours (Vytláčilová et al., 2004).

Reproduction System

T. tubifex can develop a selfing and outcrossing strategy as a mixed reproductive strategy and a self-fertilization or parthenogenesis (pseudogamy) (Lüscher & Milinski, 2003; Baldo & Ferraguti, 2005; Marotta et al., 2014). Meanwhile, Mahendra et al., (2019) explained *Tubifex* sp. is a hermaphrodite species which has two genital organs in one individual, and it regenerates itself by egg production from mature eggs. Singh et al., 2010 also revealed that *T. tubifex* reproduces through hermaphroditism and sexually matures at about 42 days, and Kaster (1980) explained maturity of *Tubifex*'s sexual was reached in 67 days at low temperature (15° C). Lazim & Learner (1986) revealed *Tubifex* is indicated as a univoltine species (i.e., apparently one sexual generation, one brood of offspring in a year). Although its breeding activity can last for several months, its period is different in each location.

Furthermore, Lazim & Learner (1986) explained *Tubifex* has a reproduction activity over a long period throughout the winter and spring seasons. *Tubifex* is most abundant during April and May, while it is least abundant in October and early November. However, Learner et al., (1978) revealed that *Tubifex* undergoes individual maturity usually during summer and autumn. The adults of *Tubifex* die soon after laying the cocoons.

In our observation, we found *Tubifex* to be most abundant during the dry season and least abundant during the rainy season. It was confirmed by Herawati et al., (2020) that the population growth of *T. tubifex* is influenced by the seasons, so it is easier to find during the dry season than the rainy season. Govedich et al., (2010) drew the reproductive system of Oligochaeta, in particular Tubificidae and Meshcheryakov (1990) for *Tubifex* (Figure 7).

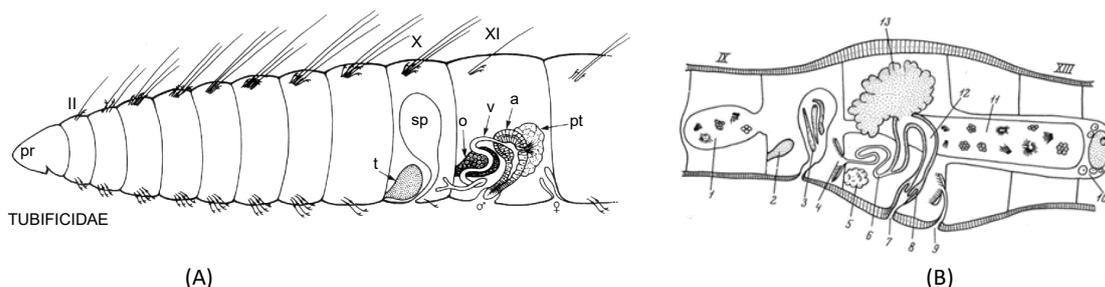


Figure 7. Reproduction organ systems and external characteristics of (A) Tubificidae. Prostomium (pr); spermatheca (sp); testis (t); ovary (o); vas deferens (v); prostate (pt) (Govedich et al., 2010) and (B) 1) anterior seminal sac; 2) testis; 3) spermatheca containing spermatozeugmata; 4) seminal funnel; 5) ovary; 6) seminal duct; 7) male genital pore; 8) penis in the penial sac; 9) female genital pore; 10) egg sac; 11) posterior seminal sac; 12) atrium; 13) prostate (Meshcheryakov, 1990).

In the terminology, parthenogenesis from the Greek, namely parthenos, means virgin, while genesis means origin or creation, interpreted as virgin birth (Kono, 2008; Vijverberg et al., 2019). The parthenogenesis, parthenogenone, parthenogen, or parthenote refers to embryogenesis in unfertilized eggs. It indicates an unfertilized embryo (Parker & McDaniel, 2009) or the

spontaneous embryonic development and naturally occurs in a variety of organisms (Vijverberg et al., 2019), and is a common mechanism of reproduction in most lower species (e.g. rotifers, nematodes, invertebrates) or can also in mammals through inducing process (in-vitro mechanism) (Kharche & Birade, 2013; Ramachandran & McDaniel, 2018).

The terminology of parthenogenesis is used to explain pseudogamy. Pseudogamy is a reproductive mechanism where sperm of males activate females' oocytes, but there is no contribution of the genome from the sperm to the offspring's genome (Launay et al., 2020). Pseudogamy was also known as gynogenesis, namely, the natural parthenogenetic development. In this mechanism, sperm only stimulate or trigger the embryogenesis phases without the contribution of the sperm's genome in this process (Schlupp, 2005). This condition is also called pseudogamous parthenogenesis (sperm-independent reproduction). Sperms penetrate ovum and initiate embryogenesis development. However, sperms do not contribute their genetic material to the zygote, including the syngamy process i.e., the fusion process between the egg and sperm pronuclei which results in no parental genetic expression in the fertilization (Beukeboom & Vrijenhoek, 1998).

Hermaphrodite is the expression of reproductive function in a single individual, both male and female (Nakadera & Koene, 2013). It is capable of self-fertilization (parthenogenesis); however, it is also capable of cross-fertilization by copulation (Hill et al., 2018). Its entire life cycle takes place within the substrate (Méndez-Fernández et al., 2014). The *T. tubifex*, oligochaetes,

Clitellata, a monophyletic group of Annelida, reproduce through hermaphroditism (i.e., an individual, male or female, contains a single reproductive system). Clitellata produce gonopores that protrude from the clitellum of the worm, both male and female. Then, there is a slime secretion and a protective cocoon to protect embryo development. The sexual way matures at about 42 days (Singh et al., 2010; Hill et al., 2018; Iyer et al., 2019).

This worm has two genital organs in one individual and regenerates itself by egg production. Tubifex can produce cocoons, and this production can occur for 40-45 days since first copulation. The ovoid-shaped cocoons with a diameter of 0.7 mm and 1 mm in length deposit the eggs. Furthermore, the eggs in the cocoons undergo division and develop into segments. There are 4-5 grains in each cocoon, which are bred until they hatch into embryos for 10-12 days. Tubifex needs 50-57 days through all phases of its life cycle from the copulation, laying eggs and cocooning phase, hatching to adulthood phase, until producing and removing its cocoons (Mahendra et al., 2019). Kaster (1980) revealed that cocoons of Tubifex commonly contain 4-9 embryos, and they can contain 14-17 embryos in each cocoon. Referring to those arguments, we proposed an illustration of the life cycle of Tubifex as oviparous in cross-fertilization by copulation (Figure 8).

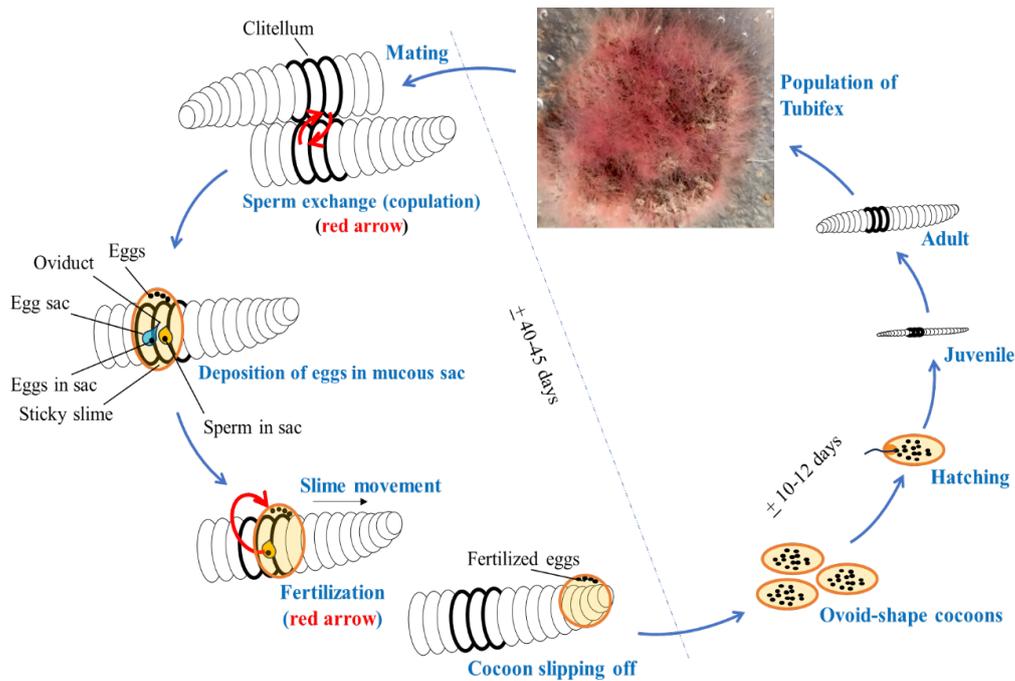


Figure 8. Schematic of life cycle of Tubifex as oviparous in cross fertilization by copulation.

Shimizu (2020) explained that Tubifex has oviposition behaviour. This behavior consists of four phases, i.e., cocoons move around the area of the clitellum, ovisac eggs budge to the ovarian coelom at around segment 11, coelomic eggs are deposited to outside of the cocoons, and then the cocoons are released to the environment. Meanwhile, Hirao (1965) explained Tubifex has two main successive phases of the oviposition behaviour, i.e., the forming of cocoons around the clitellum and the releasing or depositing of the cocoons.

The embryonic development of Tubifex starts from stages 1-cell, 2-cell, 4-cell, quadrant micromeres, bilateral cell division, mesoteloblasts, endodermal precursors, and ectoteloblast precursors. Tubifex produces eggs in metaphase I, and then the zygote forms two polar bodies. After that, the process gets into the first mitosis phase. The cytoplasm of the egg cell lacking yolk is concentrated at both poles of the egg cell's polar plasma before the first cell division. In the early development, there is a stereotyped sequence of cell divisions. In the second division, cells undergo division and separation. In the third division, there

is an unequal repetition of the quadrants to produce D quadrant micromeres on the animal side in three and four production and macromeres on the vegetal side. During cell division, the polar plasma is segregated, and these cells undergo bilateral cell divisions in the next divisions. Meanwhile, there is quadrantal cells dividing evenly in the sixth division (Nakamoto et al., 2011; Nakamura et al., 2017). Urbisz et al., (2015) found that *Tubifex*'s ovary consists of only one large ovary with more than 2,600 cells.

Clitellate have a special reproductive organ, which is distinguished from other worms, namely the clitellum. The egg capsule (i.e., cocoon) is produced in this organ. The clitellum looks like a thickened sleeve or saddle (Hill et al., 2018) at segments X, XI, and XII of the anterior regions (Bouché et al., 1999; Marotta et al., 2009; Shimizu, 2020). The clitellum produces proteinaceous material for the protection of eggs, building the cocoons in the mature sexual periods within thousands of glands, or provides a microenvironment for embryonic development (Rossi et al., 2013; Mc Loughlin et al., 2016). The reproduction mechanism, hermaphrodite involve gonopores of male and female that are discharged from the clitellum (Parish, 1981).

The fertilization process of clitellate can also involve physical contact. The copulation occurs between two individuals, and both of them mutually transfer their sperm from a spermatophore (i.e., sperm-filled sac) that involves an intromittent organ (Hill et al., 2018). Kelly & Moore (2016) explained that males have intromittent organs. These organs enter the genital tract of the female, and in this tract, the female deposits sperm in internal fertilization. Rodriguez & Fend (2018) explained that the spermatophores are attached to the prostate bulb. The sperms are transferred to the clitellar area of its partner at the ventral body surface.

Hill et al., (2018) explained that sperm come out of the spermatophore and move to the ovaries via the coelomic sinuses for egg fertilization internally. The mechanism of spermatogenesis occurs in paired testes. It produces a mature spermatozoa, and then the spermatozoa are collected in the epididymis for a longer time. On the other hand, a paired ovisacs produce oogonia and oocytes. They move for congregating and toward the female pore through the oviducts. The sperm is stored in spermathecae, and it will be released during internal fertilization process or into the cocoon. Within egg development, the vitellogenesis process provides yolk for the eggs. Therefore, the yolk can be quite large, so it may be stored in the cocoon fluid. It will become albumenotrophy, a nutritional source during the long embryonic development period. Further, the proliferation of clitellum-specific granular cells occurs as the first process in the secretion of proteinaceous cocoons together with egg laying. It is proliferated to make fibrous protein as material for the cocoon's wall; meanwhile, another cell will produce a sticky matter like glue to seal the terminal regions of the cocoon. The granules of each component, including cocoon walls, are needed to construct a single cocoon. Each cocoon can deposit between 1 and 100 eggs, depending on the species. Welch (1921) explained that the cocoons' colour is whitish or greyish. The cocoons appear semi-

transparent, although the eggs cause the cocoons to appear opaque.

Dumnicka & Poznańska (2006) revealed *T. tubifex* developed spermathecae containing spermatozeugma, and its spermatozeugma has a different shape in each species (Figure 9). As a group of Annelida, some organisms show diversity of reproductive behaviors, including fertilization or asexual fragmentation. Although almost all of Oligochaeta reproduce asexually as the main form of their life (Timm, 2012), there is no publication or evidence that indicates *Tubifex* can do asexual fragmentation except the segmentation-autotomization for regeneration of its missing body part.

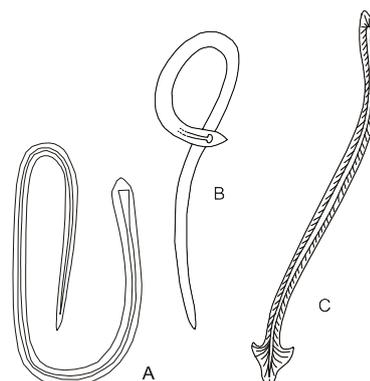


Figure 9. Comparison of the spermatozeugma shapes. A- *Tubifex blanchardi* from Włocławek reservoir; B- *Tubifex tubifex* (after Hrabě 1981); C- *T. tubifex* (after Stephenson 1930) (Dumnicka & Poznańska, 2006).

Habitat of *Tubifex*

Tubifex is a meiobenthic (meiofauna) species of aquatic worm, dwelling in the substrate or other stationary surfaces and a burrowing organism. *Tubifex tubifex* thrives in silt-clay and fine sand substrate, in mud rich in organic waste, including in waste canals, open drainage, and associated in slow water flow.

It forms reddish colonies in the substrate and consumes particles in the silt-clay, bacteria, and organic debris via its body wall (Rodriguez et al., 2001; Kaeser & Sharpe, 2006; Anlauf & Moffitt, 2008; Mandal et al., 2016; Haque et al., 2020). *Tubifex* is often found at the lower layer of water bodies, where its optimal habitat characteristics are nutrient-rich muddy substrates that provide organic matter, such as dead leaves, plant debris, and remnants of other organisms as a feed source. It also needs relatively low oxygen levels, that can reduce competition with other organisms less tolerant to anaerobic conditions. According to Singh et al., (2010), *Tubifex* can live and survive in the water with physico-chemical properties as shown in Table 1.

Tubifex sp. can play a role as a bioindicator of ecosystem status, water quality, and polluted water, although its presence is low in density in oligotrophic ecosystems (Milbrink et al., 2002). *Tubifex* is often an important part of the aquatic food chain and can adapt to the various environmental conditions, including the ability to survive in anaerobic (anoxia) or aerobic conditions, in non- or slightly flowing water, in cool and stable temperature, in acidic to base pH value, in highly polluted or toxic environment

(Reynoldson, 1987; Kaeser & Sharpe, 2006; Lagauzère et al., 2009a; Singh et al., 2010; Łuszczek-Trojnar et al., 2014; Gusakov et al., 2023; Simangunsong et al., 2024). Tubifex’s respiratory physiology can adapt to very low dissolved oxygen, so it can survive for a long time in an anaerobic environment (Aston, 1973; Simangunsong et al., 2024). Oxygen-poor conditions can also

provide advantages for Tubifex because they can increase significant bioturbation compared to aerobic environments (Nie et al., 2011). Its ability to bioturbate places it as one of the important meiobenthic bioturbators or burrowing meiofauna in a habitat (de Lucas Pardo et al., 2013).

Table 1. The physico-chemical properties of water for Tubifex.

Parameters	Sampling Locations				
	intake and residential wastewater	during culture period using pig dung	during culture period using dairy sludge	during culture period using poultry excreta	
pH	6.9-7.2	7.01 ± 0.15 to 7.08 ± 0.26	6.87 ± 2.64 to 7.01 ± 2.34	6.5 ± 0.51 to 7.8 ± 0.54	
Alkalinity (mg/l)	144-296	205.35 ± 6.92 to 211.42 ± 8.49	250.42 ± 2.00 to 259.14 ± 2.10	58.14 ± 1.14 to 128.75 ± 1.16	
Salinity (ppt)	Nil				
Dissolved oxygen (ppm)	1.8-4.6	3.06 ± 1.15 to 1.23	1.22 ± 0.007 to 0.009	0.45 ± 0.006 to 0.002	1.4 ±
Free carbon dioxide (ppm)	6-16	3.00 ± 1.13 to 1.23	2.25 ± 0.009 to 0.010	0.7 ± 0.11 to 5.3 ± 0.19	
Total dissolved solids (ppm)	298-431				
Total suspended solids (ppm)	30-400				
Biological oxygen demand (ppm)	25-310				
Chemical oxygen demand (ppm)	90-640				
Ammonia-nitrogen (NH ₄ ⁺ -N) (ppm)	1.5-35	0.009 ± 0.001 to ± 0.002	0.58 ± 0.008 to 0.69 ± 0.009	0.1 ± 0.001 to 0.3 ± 0.001	
Nitrite-nitrogen (NO ₂ -N) (ppm)	0.30-2.73				
Nitrate-nitrogen (NO ₃ -N) (ppm)	1.12-5.35	0.30 ± 0.05 to 0.09	0.58 ± 0.004 to 0.67 ± 0.005	0.32 ± 0.04 to 1.23 ± 0.10	
Phosphate (PO ₄ -P) (ppm)	0.50-2.53	0.32 ± 0.04 to 0.10	1.05 ± 0.10 to 1.7 ± 0.008	1.02 ± 0.10 to 2.48 ± 0.04	
Chloride (ppm)	51-70				
Hardness (ppm)	230-260				

Source: Singh et al., (2010)

4. The Role of *Tubifex* sp. as Bioremediator

Tubifex sp. is a capable invertebrate for the study of the biological effects caused by a variety type of contaminants (Vytlačilová et al., 2004) due to its tolerance and resistance to pollution (Lucan-Bouché et al., 1997; 1999a, b), thus, its presence can be an indicator of polluted water (Bustami et al., 2019). It can be marked as an essential aquatic riverine indicator species and can be used as a bioindicator organism for pollution monitoring and riverine health (Roy et al., 2022). Figure 10 showed harvesting activity in *Tubifex*'s habitat for a study.



Figure 10. Harvesting *T. tubifex* in its habitat, i.e., drain (personal documentation).

In ecological engineering, many aquatic organisms, one of them is *T. Tubifex*, are considered for bioremediation (Gifford et al., 2007). *T. tubifex* is able to reduce nutrients such as nitrogen and phosphorus (Kang et al., 2016) and organic matter (Yang et al., 2021); meanwhile, the presence of *T. tubifex* can also enhance the microbial decomposition rates (Kristensen, 2000). Furthermore, *T. tubifex* can improve water quality (Lou et al., 2009; Kang et al., 2018) and substrate (Arrate et al., 2004; Yang et al., 2021). The

ability to reduce dependence on the concentration of pollution (Aston, 1973).

In the implementation, as a deposit feeder (Kaster & Wolff, 1982), *T. tubifex* has been used as a bioremediator in wastewater treatment to remediate the domestic and slaughterhouse wastewater which have a high content of organic material (Taufik & Warganegara, 2013), so it can also as decomposers and serve as nutrient-rich natural feed sources for various fish species and other aquatic organisms (Singh et al., 2010). *T. tubifex* is important for inorganic pollutants treatment, so its role in the context of ecological health is crucial (Sharma et al., 2024). The role of *T. tubifex* in the environment based on its capability to live in organic matter, or non-organic matter, and Table 2 shows some materials that are used for cultivation medium for *Tubifex*'s growth.

The head-down deposit feeder can alterate substrate by burrowing into this substrate (Lopez & Levinton, 1987; Amon & Herndl, 1991; Clough & Lopez, 1993). This activity, bioturbation, impacts to water and materials exchange in the water column-substrate interface (Lagauzère et al., 2009a; Mao et al., 2020).

The bioturbation activity of tubificid worms (e.g., *Tubifex* sp.) plays an important key in processing or cycling of the organic materials and nutrients in the aquatic environment, including the involvement of microorganism processes occurring between the water column and substrate interfaces in aquatic ecosystem (Mermillod-Blondin et al., 2018) in both condition of aerobic and anaerobic (van de Bund et al., 1994; Mermillod-Blondin et al., 2001). This activity influences the modification of benthic microorganism communities' structure and diversity by influencing the biogeochemical cycle in the substrate. The bioturbation stimulates the biogeochemical processes at the water column-substrate interface and it has a significant effect on microbial communities' diversity and structures. The presence of *Tubifex* sp. and its bioturbation activity initiates to stimulate organic matter mineralization and bacterial species communities (Cariou et al., 2021).

Table 2. Studies about the ability of *Tubifex tubifex* to survive in a contaminated environment.

Medium	Cultivation periods of the research treatment	References
Tofu dregs, mustard greens, fine bran, and fish silage	33 days	Yazid et al., (2022)
Chicken manure, tofu dregs	21 days	Bonse et al., (2021)
Chicken manure	20 days	Pursetyo et al., (2011)
Soybean curd residue, chicken manure, and pig manure	21 days	Solang et al., (2014)
Feces of fish (catfish, carp, and tilapia)	45 days	Ardana et al., (2018)
Cow dung, raw fish and vegetables	145 days	Begum et al., (2014)
Palm kernel cake	50 days	Putri et al., (2018)
Soy bean and rice wine waste and chicken manure	45 days	Akbar et al., (2016)
Lemna minor and mud	50 days	Mahendra et al., (2019)
Hg ²⁺ , Cu ²⁺ , Cr ⁶⁺ , Zn ²⁺ , Ni ²⁺ , Cd ²⁺ , Fe ²⁺ , Co ²⁺ , Pb ²⁺ , and Mn ²⁺		Rathore & Khangarot (2002)
Cd, Ni, and P	24 hours	Gillis et al., (2004)

Cu, Zn, Ni, and Pb	28 days	Şimşek et al., (2023)
Cd, Fe, Pb, Zn, and Cu	-	Singh et al., (2007)
Cd	-	Kaonga et al., (2010)
Cu, Co, Ni, Zn, and Pb	28 days	Méndez-Fernández et al., (2014)
Hexachlorocyclohexane (HCH) isomers	-	Di et al., (2016)
Hexachlorobenzene	-	Egeler et al., (2001)

T. tubifex' activities in the substrate can increase the biomass and photosynthesis rate of plants in the wetland ecosystem. The *T. tubifex* demonstrates a positive impact on plant production in the substrate by increasing the mineralization of nutrients, water, and oxygen in the rhizosphere area, hormonal effects, distribution of plant-stimulating microbial, and infectious microbial reduction in the roots (Blouin et al. 2005; Eisenhauer & Scheu 2008; Mermillod-Blondin & Lemoine, 2010).

The presence of *T. tubifex* leads to positive interactions between benthic microorganisms, animals, and plants, and furthermore accelerate habitat ameliorations (Bertness & Callaway 1994; Mermillod-Blondin & Lemoine, 2010). The bioturbation ameliorates oxygen conditions and actively increases the exchanges between high- and low-oxygen level in the water by bioirrigation mechanism. Hence, the presence of *T. tubifex* results oxygen availability in substrate and it contribute to plant growth (Morard & Silvestre 1996; Jackson & Colmer 2005; Mermillod-Blondin & Lemoine, 2010).

Bioturbation has been known as an important mechanism affecting biological processing and cycling in water ecosystems (Aller, 1983; Mermillod-Blondin & Lemoine, 2010; Cariou et al., 2021). The bioturbation impacts on the dynamics of the organic matter at the water column-substrate interface in different ecosystems have been evaluated by Mermillod-Blondin (2011). *Tubifex*'s bioturbating induces biological structure building and bioirrigation that have an impact on microorganism communities and the biogeochemical mechanisms (Kristensen et al., 2012; Deng et al., 2020). This activity significantly impacts microbial diversity structures by modification of biogeochemical mechanisms and the increase in the presence of organic matter such as mucus, minerals, and distribution of organic materials in substrate (Mermillod-Blondin & Rosenberg, 2006; Papaspyrou et al., 2005; Papaspyrou et al., 2006; Lukwambe et al., 2018; Aller, R. C., & Cochran, 2019; Gonzalez et al., 2019; Deng et al., 2020; Hou et al., 2021).

In principle, Oligochaeta have the ability to vermicompost organic and inorganic waste (Ratnasari et al., 2023). Soil contamination by organic and inorganic waste and the presence of heavy metals can also contribute to soil stability and decomposition process (Trentin et al., 2019; Wang et al., 2021). Consequently, this condition may occur in an aquatic environment, thus the approach of vermicomposting with earthworms may also be implemented by silkworms.

The vermicomposting process consists of two processes, i.e., (a) mechanical and physical, and (b) biochemistry and ecology. The first process involves the organic matter aeration, followed by mixing the organic matter with the worms. Meanwhile, the second process exhibits the interrelation of both the worms and

microorganisms (Ndegwa et al., 2000; Ganti, 2018). Vermicomposting includes pre-composting, composting, and separation or harvesting of the product. Vermicomposting is considered to have been successfully applied to detoxification of industrial wastes or sludges, removal of metals or metal ions from solid waste and contaminated soil, wastewater sludge treatment, etc.

Vermicomposting involves microbes in the environment. The worms promote the growth of bacteria to decompose waste, and the worms also grind, crush, and degrade waste (Binet et al., 1998; Singleton et al., 2003). The vermicomposting is organic matter stabilization through the combined activity of worms and microorganisms. The microbes are responsible for organic matter degradation, meanwhile, the worm drives the process to alter the substrate and biological condition in the environment (Suthar, 2009). The vermicomposting provides mass reduction, shorter time in processing, and produces high concentration of humic acid (humus). Vermicompost also contains high-quality of humus and enzymes (Sinha et al., 2010a, b). Humus contains organic acids that can bind to metals to form stable metals in the environment (Swati & Hait, 2017). An organic compound which has a COOH⁻ functional group can bind to ions H⁺ or cations causing an increase in pH value (Kurniawan et al., 2022).

In addition, *T. tubifex* can also directly accumulate metals in the tissues (Gillis et al., 2004; Redeker & Blust, 2005; Singh et al., 2007). The ability of *Tubifex* sp. as a bioaccumulator have been tested in laboratory and naturally found in its habitats, thus *Tubifex* can be used as a standard substrate bioassay (Reynoldson et al., 1991; Martinez-Madrid et al., (1999); Maestre et al., 2007). In the contaminated environment, organisms develop their body's defense system for survival. *Tubifex* develops biochemical mechanisms to respond to environmental stress, and these metabolic mechanisms are activated in the early stage of xenobiotic contamination.

Therefore, *Tubifex* is able to accumulate metals, pharmaceuticals, polycyclic aromatic hydrocarbons, or pesticides., *Tubifex* produces metabolic compounds, namely superoxide dismutase enzyme, catalase enzyme, carboxylesterase enzyme, and glutathione-S-transferase enzyme (Arendarczyk & Grabińska-Sota, 2020). Superoxide dismutase (SOD) is a metalloenzyme, a crucial antioxidant and detoxification enzyme in the cell. It is essential as a defense against oxygen toxicity or reactive oxygen species (ROS), and it uses an unstable free radical as its natural substrate. This enzyme catalyzes the intermediates of oxygen reduction and prevents the production, i.e., O₂⁻, H₂O₂, and OH⁻ as the main factors which cause of oxygen toxicity, and then defends against this toxicity (Fridovich, 1981; Ighodaro & Akinloye, 2018). Catalase (CAT) is an antioxidant enzyme which is crucial for

reducing oxidative stress by breaking down cellular hydrogen peroxide into water and oxygen production (Nandi et al., 2019), and it is very efficient in breaking the chemical bonds of hydrogen peroxide molecules (Ighodaro & Akinloye, 2018). Glutathione-S-transferase (GST) is a group of enzymes which play an important role in preventing lipid peroxidation and detoxifying through their Se-independent glutathione-peroxidase activity (Singhal et al., 2015). Carboxylesterase (CarE) is responsible for detoxifying organic compounds, hydrolyzing ester bonds, and amide range. This enzyme is important in hydrolytic detoxification and phosphorylation of insecticides (Galloway et al., 2002; Satoh, 2005).

T. tubifex also shows the ability to produce metallothioneins (MTs) as a non-enzymatic protein that is responsible for metal ion detoxification (Mosleh et al., 2005; Mosleh et al., 2006) or heavy

metals accumulation (absorption) (Widiastuti et al., 2019). Metallothionein has a high cysteine content, and the cysteine residues form thiol groups (-SH) that allow MTs to bind heavy metals. It has the ability to bind specific metals; each MT only binds one metal (e.g., Cd, Hg, and Pb with different MTs) (Astutik & Zulaika, 2015; Hertika et al., 2019; Hertika et al., 2023)

Thus, the presence of oligochaetes in an environment can increase organic matter (wastes) decomposition and reduction; furthermore, the active compound or functional group of organic wastes that plays an important role in increasing the pH value and binding cations including heavy metals. We presented an illustration about the role of *Tubifex* sp. In the pathway of organic matter decomposition, and heavy metals or chemical substances accumulations and detoxification (Figure 10).

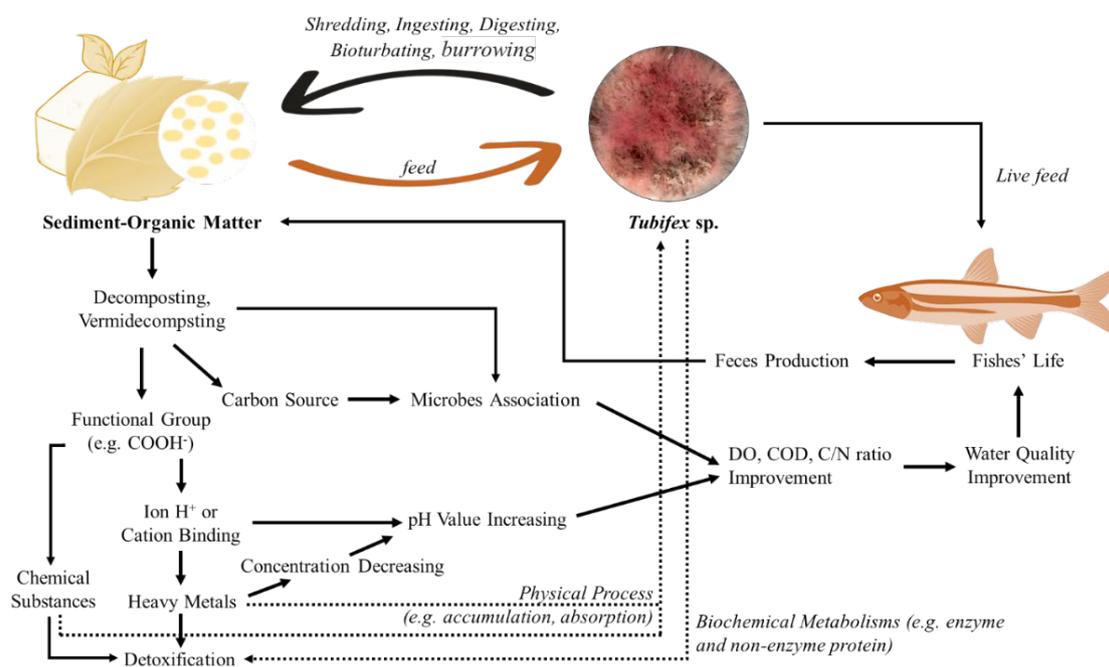


Figure 10. Schematic pathway of the role of *Tubifex* sp. in the freshwater ecosystem.

5. Conclusion

This paper has elaborated information on *Tubifex tubifex* comprehensively, about the biology as well as its role for bioremediator. *T. tubifex* has played an important role in bioremediation by physical activity (e.g., accumulation or absorption) and biochemical activity (e.g., enzyme and non-enzyme protein production). The presence of organic material in waters can be decomposed by *Tubifex*. Its bioturbation activity promoted exchange of materials between the water column-substrate interface. Decomposition of organic materials produces functional groups that are able to interact with inorganic materials through the binding of their ions. This can have a positive impact on reducing inorganic materials that pollute waters. *Tubifex* is a meiobenthic species of aquatic worm and an important key for organic matter decomposition, heavy metal, or chemical substances reduction and detoxification. It can perform

vermicomposting on organic matter and also accumulate chemical substances by its metabolic compounds, such as superoxide dismutase enzyme, catalase enzyme, carboxylesterase enzyme, glutathione-S-transferase enzyme, and metallothionein enzyme. Reducing organic and inorganic pollution in water can indirectly improve water quality. Thus, *Tubifex* can play an important role in water bioremediation.

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