

Sustainable Practices in Aquatic Sciences

Editors

Erkan CAN

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Aquatic Sciences



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PREFACE

In recent years, the growing awareness of environmental challenges and the urgent need for sustainable development have driven significant advances in aquatic sciences. This book, *Sustainable Practices in Aquatic Sciences*, brings together innovative research and cutting-edge applications that aim to harmonize aquatic resource use with ecological preservation.

The chapters included span a wide array of topics that collectively offer a comprehensive perspective on sustainability in marine and freshwater environments. Starting with the exploration of novel marine-derived biomaterials from tunicates and the role of nanotechnology in aquaculture, the book delves into biodiversity and waste upcycling with a focus on marine malacostraca in Türkiye. Further sections examine crucial topics such as reducing sodium chloride in processed seafood, the potential of cellular aquaculture to mitigate disease risks, and advancements in fish breeding for inland aquaculture.

Additionally, the book addresses natural therapeutic agents like propolis for fish health, the role of freshwater snails in sustainable wastewater treatment, and the importance of seafood in functional food production. It also explores the complexities of sex control in aquaculture and concludes with an insightful discussion on transitioning from plastic to bioplastic through the use of algae, paving the way for a more sustainable aquatic future.

This collection serves as a vital resource for researchers, practitioners, and policymakers committed to advancing sustainable aquatic sciences and fostering an eco-friendly future.

Editors

Prof. Dr. Erkan Can

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CHAPTER I

SUSTAINABLE APPLICATIONS OF TUNICATES: NOVEL MARINE-DERIVED BIOMATERIALS AND METHODS FOR AN ECO-FRIENDLY FUTURE

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1. Introduction

Oceans, covering over 70% of the Earth's surface, are essential to sustaining life on our planet and support a vast array of biodiversity worldwide. As a critical component of the Earth's life support system, oceans and coastal regions play a fundamental role in maintaining ecological balance and regulating the global climate

The percentage of the world's population that lives in oceans, coastal regions, and other water bodies is approximately 2.4 billion people – around 40% of the global population- and this number continues to increase daily (United Nations, 2017; Visbeck, 2018). As the global population continues to grow rapidly, the demand for marine resources is expected to increase significantly. In recent years, with the growing demand for alternative, renewable, and eco-friendly marine resources, the concept of sustainability has attracted significant attention in modern science, industry, innovation, and technology studies. Today, global interest in sustainability research and sustainable development continues to rise.

Marine organisms have become increasingly focal points in sustainability research, as they play vital roles in maintaining ecosystem health, regulating global biogeochemical cycles, and supporting biodiversity. Their ability to

sequester carbon, serve as bioindicators of environmental change, and provide sustainable sources for pharmaceuticals, high-quality nutrients, and biofuels underscores their importance. (Lemos, 2024; Nguyen et al., 2022; Zhivkoplis et al., 2024). They are a more recent source of bioactive natural products compared to terrestrial plants and non-marine organisms. For instance, while nearly half of the approved pharmaceuticals are based on biological compounds produced by living organisms, success rates are two to four times higher for compounds from marine organisms (Gerwick, 2012; Sigwart et al., 2021).

In this context, the oceans and waters are recognized as promising sources of sustainable and innovative bioproducts and materials utilized across various industries (Banday et al., 2024).

The marine environment, which plays a fundamental role in regulating climate, sustaining biodiversity, and providing essential resources, is under unprecedented pressure from overfishing, pollution, habitat degradation, and climate change, ocean warming, acidification, and species extinction (Souter et al., 2021).

In the face of the major environmental challenges of our time, such as climate change and biodiversity loss, understanding and protecting marine life has become more critical than ever. Ensuring the health and functionality of marine ecosystems is essential for maintaining ecological balance and the sustainability of marine resources. As awareness of these issues grows, there has been a notable increase in interest in environmentally friendly and sustainable aquaculture applications. These applications not only aim to meet the rising global demand for seafood but also seek to minimize environmental impacts, support marine conservation efforts, and contribute to climate resilience. The growing interest in developing environmentally friendly and sustainable materials has led researchers to explore the diversity of marine organisms. In this context, tunicates, a group of marine organisms, have emerged as a promising potential source for novel biomaterials.

Tunicates, marine invertebrates also known as sea squirts, possess unique biological characteristics that make them an ideal candidate for the development of sustainable biomaterials. Their unique properties can be harnessed to develop environmentally friendly products and solutions, helping to foster a more sustainable future.

They have been receiving increasing attention in recent years due to their abundant biomass, highly crystalline cellulose (~90% crystallinity), bioactive compounds, and ecological resilience. Found in oceans worldwide, tunicates grow rapidly, require minimal inputs, and often colonize artificial structures making them both a valuable resource and, in some cases, an invasive nuisance.

This dual nature positions them as a compelling candidate for sustainable exploitation, transforming a potential marine pest into a bioeconomic opportunity. The sustainable applications of tunicates span a wide range of fields, including biomedicine, biomaterials, environmental remediation, and circular economy solutions. Tunicate-derived nanocellulose, for instance, offers superior properties compared to plant-based cellulose, making it an ideal material for high-performance applications. Furthermore, their proteins and secondary metabolites show promise in pharmaceuticals, nutraceuticals, animal feed additives, and cosmetic and skincare formulations.

In view of the growing importance of sustainable resources, this study provides an overview of tunicate biology, with a particular emphasis on their utilization as sustainable resources. It aims to raise awareness of the potential utilization of tunicates in sustainability practices, with the objective of promoting the expansion of their application areas and contributing to sustainable development.

2. Tunicates and Their Biological Significance

2.1. Classification and morphological types

Tunicates (or urochordates) are marine invertebrate filter feeders classified within the phylum Chordata, which also encompasses cephalochordates and vertebrates. A defining synapomorphy of this phylum is the presence of a notochord, a structural feature shared across all chordate lineages. Phylogenetic analyses currently support tunicates as the closest extant relatives of vertebrates, positioning them as a valuable model for investigating developmental and evolutionary processes (Anselmi et al., 2025). In particular, tunicates offer significant insights into the evolution of the immune system, owing to their phylogenetic placement and relatively simple body plans (Bauermeister et al., 2019).

Tunicates exhibit a high degree of diversity in their morphology, ecology, and life cycles. They exhibit a wide range of taxonomic diversity, and they are vital to marine ecosystems. Currently, approximately 3,000 tunicate species have been documented worldwide (Shenkar et al., 2025; Shenkar & Swalla, 2011; WoRMS, 2025). They currently comprise 3,145 valid species (Shenkar et al., 2023), distributed in two classes: Ascidiacea, with 3,068 species, most of which have benthic adult forms, and Appendicularia, with 77 holoplanktonic species (Rocha et al., 2024). Three thousand species of benthic or sessile ascidians exceed the total number of species of the other two classes of pelagic tunicates by more than twenty times (Holland, 2016).

There are three classes and seven orders within the subphylum Tunicata: the pelagic Appendicularia (larvaceans) (order Copelata), the Ascidiacea (the orders Aplousobranchia, Phlebobranchia, and Stolidobranchia), commonly known as sea squirts, which is the most speciose class of tunicates, and the pelagic Thaliacea (the orders Doliolida, Pyrosomatida, and Salpida), which includes doliolids, pyrosomes, and salps as shown in Figure 1.

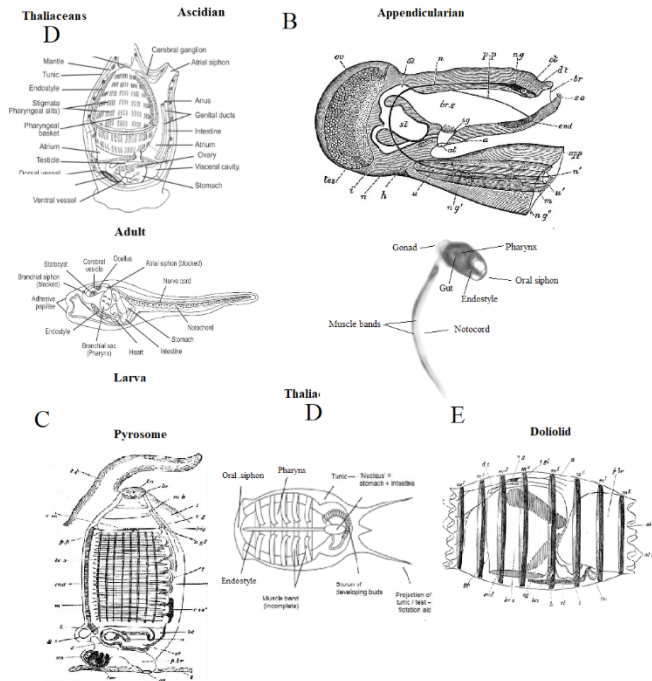


Figure 1. Diagrams of the five major groups of tunicates. (A) Class Ascidiacea, a solitary ascidian adult and larva by Jon Houseman, is licensed under CC BY-SA 3.0. (B) Class Appendicularia, an appendicularian by William Abbott Herdman and by Thomas Clarke, is licensed under CC0. (C) Class Thaliacea, the three groups of thaliaceans. Order Pyrosomatida, a Pyrosome by William Abbott Herdman, is licensed under CC0. (D) Order Salpida, a salp by Ludwik Gąsiorowski, is licensed under CC BY-SA 4.0. (E) Order Doliolida, a doliolid by Public Domain, is licensed under CC BY-SA 3.0.

Thaliaceans possess transparent, gelatinous bodies primarily composed of acidic mucopolysaccharides, with water comprising approximately 95% of their body mass. Among them, salps and doliolids are relatively small, barrel-shaped organisms that can occur either as solitary individuals or in chains. Both are characterized by their transparent morphology. Salp aggregations have been

observed in various marine environments, including both coastal and open-ocean regions, often in association with dynamic oceanographic processes such as upwelling events and slope water intrusions. In contrast, doliolids tend to exhibit a more restricted distribution, frequently occurring near shelf breaks, which may indicate specific habitat preferences or ecological constraints. Pyrosomes, another group within the Thaliacea, are notable for their bioluminescence and colonial organization (Figure 2). These colonies form large, gelatinous, tubular or conical structures that can reach lengths of up to 20 meters, making them among the largest pelagic colonial organisms (Henschke et al., 2016).



Figure 2. A solitary ascidian, *Ciona intestinalis* (Linnaeus, 1767) by (Gall et al., 2019) is licensed under Creative Commons CC0 BY 1.0. Universal Public Domain Dedication, (<https://creativecommons.org/licenses/by/1.0>). An appendicularian, *Oikopleura (Vexillaria) dioica* Fol, 1872 by Mowgli and three thaliaceans; a pyrosome, *Pyrosoma atlanticum* Péron, 1804 by Rhododendrites–Monterey is licensed under Creative Commons CC-BY-SA-4.0, Creative Commons Attribution-Share Alike 4.0 International (<https://creativecommons.org/licenses/by/4.0>), a solitary salp, *Cyclosalpa bakeri* Ritter, 1905 by Charlotte Seid, is licensed under Creative Commons CC0 BY 4.0. Creative Commons Attribution 4.0 International (<https://creativecommons.org/licenses/by/4.0>), a doliolid *Doliolum* sp. by Otto Larink is licensed under Creative Commons Attribution 3.0 Unported (<https://creativecommons.org/licenses/by/3.0>).

The class Ascidiacea, a major group within the subphylum Tunicata, exhibits considerable morphological and ecological diversity. Members of this class may exist in three distinct organizational forms: solitary, consisting of individual organisms; colonial, where genetically identical individuals are physically connected yet functionally independent; and compound, in which multiple zooids are embedded within a shared tunic and may share common physiological systems.

Ascidiaceans display a wide range of morphological characteristics, varying significantly in form, size, and coloration, such as translucent to blue, green, yellow, red, and brown. Their body shapes include cone-shaped, elongated, globular, and oval morphologies, reflecting adaptations to different ecological niches. Size variation is also notable, with individuals ranging in length from approximately 0.5 mm to 200 mm (Petersen, 2007; Shenkar & Swalla, 2011). Colonial forms consist of multiple genetically identical zooids, while solitary species range from 0.5 to 20 cm in length (Gasparini & Ballarin, 2018); however, some, such as *Pyura pachydermatina*, can reach lengths of up to 100 cm (Rosner & Rinkevich, 2024; Ruppert et al., 2003). This diversity underscores the ecological versatility and evolutionary success of the Ascidiacea within marine environments.

The term “Tunicata” originates from the distinctive integumentary tissue known as the “tunic,” which is the extracellular layer covering the epidermal surface. It is considered the first barrier against pathogens and predators and is composed of cellulose microfibrils, made up of polysaccharides, including a carbohydrate polymer called tunicin. Tunicin is likewise called animal cellulose. It also contains proteins such as collagen, elastin, pseudoelastin, and hydroxyproline (Hirose et al., 1999). Tunic is the outer layer of tunicates and is a hierarchical complex composed of approximately 60% cellulose (which is higher than the 45% found in wood), 40% protein, and trace amounts of lipids and ashes (Piri et al., 2022). In some tunicates, cellulose can attach to proteins alone or to both proteins and acid mucopolysaccharides (AMPS), creating a protein-polysaccharide combination (Van Daele et al., 1992). To date, cellulose microfibrils have been identified in nearly all ascidians and thaliaceans. However, appendicularians lack a tunic as their integumentary tissue. The tunic encloses the body of adult tunicates and serves as a protective exoskeleton. (Kimura & Itoh, 2001).

The tunic, a defining feature of tunicates, may exhibit a range of appearances, including translucent, brightly colored, or dull forms. Its surface is often characterized by various structural features, such as spines, calcareous

spicules (Lambert & Lambert, 1987), or a dense coating of sand grains that may provide camouflage or structural reinforcement (Young, 1989).

Appendicularians represent another distinct group within the subphylum Tunicata. Although molecular phylogenetic analyses based on 18S rDNA sequences suggest that appendicularians share a common ancestor with other tunicate groups (e.g., Ascidiacea and Thaliacea). They exhibit several distinct morphological characteristics. Notably, unlike other tunicates, appendicularians lack a tunic, the characteristic integumentary tissue composed of tunicin that gives the subphylum its name. Instead, appendicularians produce a balloon-like, gelatinous structure known as a “house”, which serves as a specialized feeding apparatus, facilitating the capture and filtration of suspended particles from the surrounding water (Kimura & Itoh, 2001).

The tunic of tunicates can be classified into two types based on its composition: rigid and soft. These types vary depending on the species of tunicate. Tunicates are categorized as either gelatinous (soft, such as *Molgula manhattensis*) or leathery (tough, like *Styela clava*). This classification affects their habitat preferences, predation risks, and efficiency in absorbing food, gases, and pollutants (Rosner & Rinkevich, 2024).

Certain ascidian taxa, particularly within the families Didemnidae, Polyclinidae, and Pyuridae (Aplousobranchia), possess calcareous spicules composed of calcium carbonate embedded within their internal tissues, most notably within the tunic of colonial forms (Monniot et al., 1995). In many didemnid ascidians, these spicules are abundant and well-developed. Given that the morphology and size range of spicules are species-specific, they are frequently utilized as key diagnostic characters in ascidian taxonomy (Hirose et al., 2010).

2.2. Biology of tunicates

Tunicates are an evolutionarily important group of cosmopolitan marine, sessile, filter-feeding animals belonging to the subphylum Tunicata (or Urochordata), a phylum within the Chordata. In their phylogenetic relationship, they are the closest living invertebrate relatives of vertebrates, possessing key vertebrate-like features, complex organs, and a diverse array of cell types. They serve as valuable model organisms in various scientific fields such as developmental and evolutionary studies (Anselmi et al., 2025; Johnson et al., 2024; Todorov et al., 2024), the immune system, embryogenesis (Bauermeister et al., 2019), regeneration, stem cell biology (Tiozzo et al., 2008; Vanni et al., 2022), and bioactive compounds (Cooreman et al., 2023; Ramesh et al.,

2021) and the production of secondary metabolites, some of which may have pharmaceutical potential (Gao et al., 2023), biological invasion (Zhan et al., 2015), allorecognition (Nydham et al., 2021), germ cell sequestration (Kassmer et al., 2016), cell fate determination (Fujii et al., 2021). These features not only make them biologically fascinating but also provide them with qualities and functions suitable for sustainable applications.

Ascidians are classified as chordates due to their short-lived (12-24 h) swimming larval stage, which exhibits key chordate features, including a notochord, dorsal tubular nerve cord, and pharyngeal gill slits. These structures are lost during metamorphosis, when the larvae settle, absorb their tails, and transform into sessile juveniles. The adult ascidian has a sac-like body with two siphons: the oral siphon, which draws water in, and the atrial siphon, which expels filtered water.

The body of an adult ascidian, or each zooid in colonial ascidians, contains several distinct organ systems. In addition to the outer tunic and reproductive organs, key internal structures include a branchial sac that occupies a significant portion of the trunk, a digestive system comprising the pharynx, stomach, and gut, a tubular heart, and an open circulatory system containing hemolymph with various blood cell types (Figure 3).

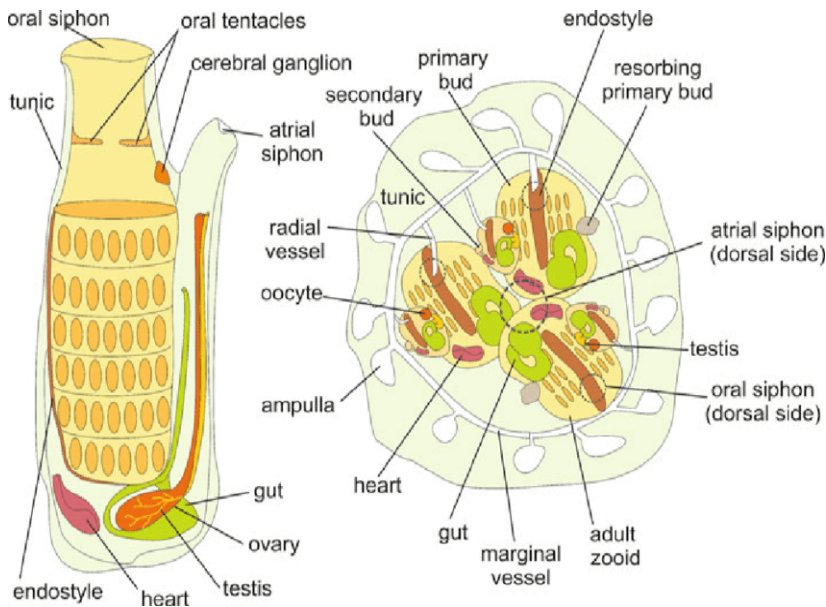


Figure 3. Illustration of a solitary ascidian (left) and a colonial ascidian (right) in ventral view. Vanni et al. (2022) via the Creative Commons Attribution 4.0 International Public License <https://creativecommons.org/licenses/by/4.0/legalcode> (accessed on 13 October 2025).

2.3. The nervous system

Ascidians possess a well-organized nervous system comprising peripheral nerve cells and a central neural tube, which includes the sensory vesicle, neck, visceral or tail ganglion, and caudal nerve cord. A prominent ganglion is situated within the connective tissue between the oral and atrial siphons, adjacent to an exocrine gland. Additional structural features include segmented muscle bands and a continuous epidermal layer enclosing the body. In colonial species, individual zooids are interconnected via blood vessels, facilitating physiological coordination across the colony (Rosner & Rinkevich, 2024).

Appendicularia, ascidians, and some thaliaceans have a tadpole-like larva with a notochord, which metamorphoses into sessile adults, particularly in the case of ascidians (Lemaire & Piette, 2015).

2.4. The circulatory system

Tunicates possess an open circulatory system, characterized by few distinct blood vessels. The heart is a simple tubular structure that propels blood through peristaltic contractions. The heart consists of two tubes, the pericardium and the myocardium (Brunetti & Mastrototaro, 2017). The tunicate heart periodically reverses the direction of blood flow after approximately 100 beats in one direction, pausing briefly before contracting in the opposite direction (Shimek, 2008). Thaliaceans possess a closed circulatory system with a tubular heart that periodically reverses the direction of blood flow, enhancing the distribution of nutrients and gases throughout the body (Lemaire & Piette, 2015).

2.5. The respiration system

The branchial sac (also known as the branchial basket) functions both in respiration and the filtration of food particles. Gas exchange primarily occurs across the gill slits, but it is also facilitated through additional body surfaces, including the lining of the atrium.

The branchial sac plays a central role in filtration and bioaccumulation. Its walls are perforated by numerous stigmata and lined with ciliated epithelium. Ciliary movement generates a continuous flow of water from the oral siphon through the branchial sac, allowing for the efficient filtration of seawater. This process is vital for respiration, as it captures suspended particulate matter and directs it toward the esophagus for further processing.

Located on the ventral side of the branchial sac, the endostyle is a ciliated groove that secretes mucus essential for trapping suspended particles. It is

considered homologous to the vertebrate thyroid gland, although its function differs significantly. The branchial sac also contains various hemocytes, including granular amoebocytes that express immune-related genes such as tumor necrosis factor (*TNF- α*) and complement component *C3*, as well as stem cells. Additionally, the morphology of the branchial sac serves as a key taxonomic feature distinguishing the three orders within the class Ascidiacea: *Aplousobranchia*, characterized by a simple branchial sac; *Phlebobranchia*, possessing longitudinal blood vessels; and *Stolidobranchia*, which exhibit a folded branchial sac (Shenkar & Swalla, 2011).

Appendicularians lack specialized respiratory structures; gas exchange occurs primarily via diffusion across the thin, permeable body surface, aided by continuous water flow through the mucous house. Gas exchange primarily occurs across the numerous gill slits of the branchial sac, as well as other thin, vascularized body surfaces, such as the atrial lining, facilitating efficient oxygen uptake and carbon dioxide removal.

2.6. Digestive and excretory systems

The excretory system of tunicates is relatively simple and closely integrated with their feeding and circulatory systems. Water enters the body through the incurrent siphon and passes through the branchial basket via modified gill slits, then flows into the atrium before exiting through the excurrent (atrial) siphon. The atrium also serves as a common cavity where gametes and fecal matter accumulate, both of which are expelled through the atrial siphon (Shimek, 2008). Sea water is driven through the gill slits into the branchial basket by cilia lining its surface. Within the basket, a ventral groove known as the endostyle secretes mucus that forms a sheet lining the interior of the basket. Particulate organic matter entering through the incurrent siphon becomes trapped in this mucus. Ciliary action then transports the mucus, now containing food particles, to a dorsal food groove, which directs it to the mouth. The food-laden mucus passes through the esophagus into the stomach for digestion. Undigested material moves into the intestine and is ultimately expelled through the anus. Fecal matter accumulates in the atrium and is flushed out via the excurrent siphon with outgoing water (Shimek, 2008). This efficient filter-feeding mechanism contributes to the ecological success and invasiveness of many tunicate species (Figure 4).

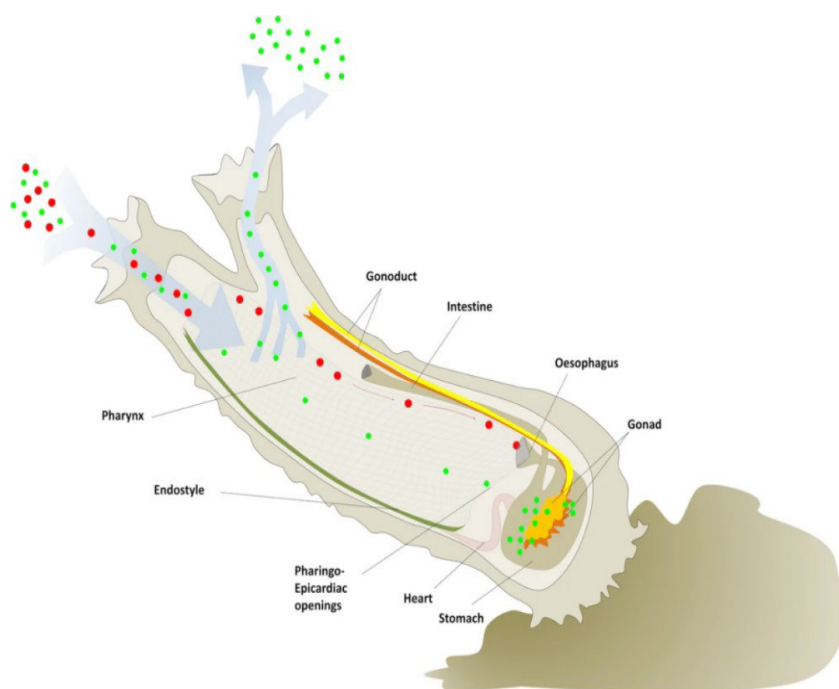


Figure 4. The water circulation within a *Ciona robusta* – red dots signify larger particles, while green dots are smaller ones. Valsesia et al. (2021) via Creative Commons (<https://creativecommons.org/licenses/by/4.0>), adapted by N. Hanacek/NIST.

In appendicularians, the mucus structure, used for feeding, is actively maintained and pumped within water containing suspended particles. This structure functions as a highly efficient filtration apparatus, wherein food particles are selectively trapped and directed toward the oral opening for ingestion. The gut includes a dorsal esophagus, a bilobed saccular stomach, and a curved intestine, divided into vertical, mid-, and distal intestine (or rectum) (Burighel et al., 2001). The feeding mechanism in appendicularians is distinct from that of other tunicates. Specialized glands on the surface of the body secrete a complex mucous structure, commonly referred to as a “house,” which envelops the animal. Rhythmic undulations of the tail generate a feeding current that draws water into the house and passes it through a finely woven mucous mesh. This mucous sheet functions as an effective filtration net, capturing suspended food particles from the surrounding water for ingestion. The digestive tract in thaliaceans includes the pharynx, esophagus, stomach, intestine, and anus. This system efficiently processes suspended particles filtered from the water column.

2.7. Life cycle and reproduction

Tunicates exhibit diverse reproductive strategies, including both asexual budding and sexual reproduction. Most tunicates are primarily hermaphroditic, possessing both male and female reproductive organs, which enables reproduction through both sexual and asexual means, thereby facilitating rapid population expansion. They can often self-fertilize or cross-fertilize and can also produce new individuals through a process known as asexual reproduction, also referred to as budding. They are releasing eggs and sperm into the water column for external fertilization. Others brood their offspring internally.

In solitary ascidians, reproduction predominantly occurs sexually, whereas colonial ascidians are capable of both sexual reproduction and asexual reproduction via budding (vegetative reproduction) and strobilation, with an extended capacity of regeneration. Most solitary ascidians are hermaphroditic and reproduce via external fertilization, and while the larval phase is absent or very reduced in the planktonic forms, the benthic adult ascidians present a planktonic stage in which the larva resembles a free-swimming tadpole that exhibits a brief pelagic phase, with an ovate trunk and an elongated tail, responsible for dispersal (Lambert, 2005a).

During this stage, the larvae actively disperse and then settle onto a wide range of substrates. Upon settlement, they undergo metamorphosis and transition into sessile adult forms (Hong et al., 2025; Shenkar & Swalla, 2011).

Notably, hermaphroditic ascidians exhibit self-incompatibility (SI) mechanisms at fertilization, which prevent self-fertilization and promote genetic diversity (Nonaka & Satake, 2010). In contrast, appendicularians, another tunicate group with a cosmopolitan distribution, possess separate sexes and reproduce exclusively through sexual reproduction, a trait considered atypical among tunicates. The asexual reproductive capacity observed in many tunicate species is also hypothesized to contribute to their regenerative abilities, potentially allowing for the replacement or regrowth of body parts through budding mechanisms (Holland, 2016).

Ascidians, or sea squirts, typically exhibit a biphasic life cycle comprising a free-swimming larval stage and a sessile adult phase. The larval form, which resembles a tadpole, possesses a notochord, a key diagnostic feature of the phylum Chordata that facilitates active dispersal. This dual-phase lifestyle enhances ecological plasticity and supports the rapid colonization of suitable substrates in marine environments. Life span among ascidian species varies considerably, generally ranging from approximately two months to one year

(Shenkar & Swalla, 2011). The solitary species reproduce through external fertilization as broadcast spawners, while colonial species are typically ovoviviparous (Gasparini & Ballarin, 2018).

Tunicate larvae bear a striking resemblance to the tadpole larvae of other chordates, possessing key features such as a notochord and a post-anal tail. It has several sensory systems, including the ocellus and otolith, which are sensitive to light and gravity, respectively. However, following retrogressive metamorphosis, these structures are lost, resulting in a sessile adult form devoid of typical chordate characteristics. Due to this transitional morphology, tunicates are often regarded as an “evolutionary connecting link” between invertebrates and vertebrate chordates (Ramesh et al., 2021).

The reproductive periodicity of ascidians varies considerably among species and remains poorly characterized for many taxa. However, it is well established that water temperature plays a key regulatory role in their reproductive cycles (Lambert, 2005a). In temperate regions, ascidian spawning typically peaks during the summer months, followed by a marked decline in reproductive activity as temperatures decrease (Stoner, 1990). Conversely, in tropical and subtropical environments, ascidian populations often exhibit continuous gamete release throughout the year (Goodbody, 1961).

Gonads develop on either side of the zooid, with the ovary positioned posterior to the testis. During reproduction, the ovulated egg is released into a sac-like brood pouch, which is formed as an outgrowth of the body wall, where internal fertilization occurs. Embryonic development occurs within this pouch, and gestation typically lasts more than one month. Metamorphosis begins shortly after larval release, during which two buds form on the right side of the oozoid and one on the left. Individuals of the same generation tend to develop and senesce synchronously. Notably, sexual reproduction does not interfere with asexual reproduction, which occurs via self-division or budding. This process results in colonies of genetically identical zooids (Rodriguez et al., 2017).

3. Tunicates in Marine Ecosystems

3.1. Ecological role of tunicates

Tunicates play a crucial ecological role, contributing to the stability of marine ecosystems through their filter-feeding behavior, which is vital in nutrient cycling and maintaining water quality (Bone et al., 2003; Piri et al., 2022).

Ascidians are benthic and pelagic filter feeders; they're commonly called sea squirts and derive their name from their ability to contract and expel water through siphons upon contact. Sea water enters the pharynx through an oral siphon (branchial, inhalant, incurrent siphon), which is typically moved by ciliary beating. Food particles are trapped on a mucous net secreted by the endostyle, while water and waste exit the body through an atrial siphon or exhalant (excurrent siphon) (Petersen, 2007).

Tunicates play a critical role in marine biogeochemical cycles through their unique feeding and waste production mechanisms. By producing mucus-rich organic matter, fecal pellets, and trapping themselves within sinking particulate organic matter, tunicates contribute significantly to vertical carbon fluxes, transporting organic carbon and associated minerals from surface waters to the deep ocean (Ramesh et al., 2021). Their filtration capacity enables them to remove large volumes of suspended particles from the water column, thereby enhancing water clarity, influencing nutrient dynamics, and controlling plankton populations. In addition to their role in nutrient cycling, tunicates play a crucial part in the marine food web by transferring energy to various compartments, serving as prey for various fish and invertebrate species (Luo et al., 2022).

Photosymbiosis has been documented in several colonial ascidians of the family Didemnidae, predominantly inhabiting tropical and subtropical marine environments. These ascidians host cyanobacterial symbionts, primarily species of *Prochloron* and *Synechocystis*. Approximately 30 species across four didemnid genera have been described as hosts to these photosymbionts. While many of these ascidian species maintain obligate symbioses, consistently harboring specific cyanobacterial partners, others engage in facultative associations, where the presence of photosymbionts is occasional or environmentally dependent (Su et al., 2013).

Some tunicate species also exhibit obligate photosymbiosis and have been proposed as potential bioindicators of environmental stress, providing insight into the health of marine ecosystems. Despite these known ecological functions, the roles of certain tunicate-derived marine natural products (MNPs) remain poorly understood, and further research is necessary to elucidate their ecological and functional significance (Ramesh et al., 2021).

Ascidians represent a crucial component of marine benthic communities and are also recognized as significant contributors to marine biofouling, particularly through their colonization of artificial substrates such as ship hulls, buoys, and

fishing nets. Despite extensive research on the chemical cues mediating larval settlement and metamorphosis in ascidians, the role of physical environmental factors in these processes remains relatively underexplored. To date, only a limited number of studies have investigated the influence of physical parameters on the behavior and developmental transitions of ascidian larvae, underscoring the need for further research in this area (Feng et al., 2010).

Tunicates are sciaphilous species (Brunetti & Mastrototaro, 2017). Light is widely recognized as an important environmental cue influencing the settlement behavior of tadpole larvae in many ascidian species (Svane & Young, 1989). Numerous species exhibit photonegative behavior during settlement, a trait believed to facilitate their preferential colonization of cryptic or shaded habitats (Manríquez & Castilla, 2007). In addition to light, water temperature has been identified as a key environmental factor influencing the distribution and occurrence of ascidian populations (Lambert, 2005b). Water salinity is a critical abiotic factor that significantly influences both the development and distribution of ascidian species. Variations in salinity can affect larval settlement, growth rates, reproductive success, and overall physiological tolerance, thereby shaping their ecological niches and biogeographic patterns (Nagar & Shenkar, 2016). Furthermore, the physical characteristics of the substratum, including its lithological properties, have been shown to affect larval settlement. For example, Groppelli et al. (2003) demonstrated that the mineral composition of the substratum significantly influences the settlement of *Phallusia mammillata* larvae, suggesting that substratum composition may play a regulatory role in the spatial distribution of tunicate communities.

Ascidians are sessile marine invertebrates belonging to the subphylum Tunicata and are considered the closest living relatives of vertebrates within the phylum Chordata. They have garnered considerable scientific interest for their utility in addressing complex biological processes, particularly in the fields of developmental biology and immune system evolution.

As holobionts, ascidians maintain intricate associations with diverse microbial communities, which play a key role in enhancing host adaptability to varying environmental conditions. This symbiotic relationship contributes to their ecological success, enabling ascidians to act as both dominant components of benthic communities and effective marine invaders.

As filter feeders, ascidians are continuously exposed to environmental microorganisms, including potential pathogens, necessitating a highly responsive and organized immune system. The tunic, a metabolically active

outer tissue composed primarily of a cellulosic matrix, serves as the first line of defense against both pathogens and predators. Internally, the oral siphon and pharynx play central roles in immunological surveillance, functioning as primary immune organs that can detect foreign material and initiate appropriate immune responses (Bauermeister et al., 2019).

In contrast, the introduction of non-native ascidian species has been linked to negative ecological impacts, particularly within benthic communities where they often outcompete native species. Additionally, invasive ascidians pose a threat to the aquaculture industry by fouling infrastructure and reducing productivity (Topić Popović et al., 2025).

Adult ascidians exhibit a significant reduction in chordate features, with their bodies encased in a protective outer tunic composed of a unique cellulose-like polysaccharide known as tunicin. This extracellular matrix often contains vacuolated cells that may store sulfuric acid, potentially serving as a defense mechanism against predation. Additionally, several ascidian species demonstrate the ability to bioaccumulate trace metals such as vanadium, chromium, and molybdenum within specialized vanadocytes or other hemolymphatic cells, a phenomenon that remains of interest in both ecotoxicology and marine biochemistry (Bauermeister et al., 2019; Goodbody, 1974).

3.2. Habitat and global distribution

Tunicates appear to have undergone particularly rapid evolutionary diversification, remaining restricted to exclusively marine environments while radiating to occupy a broad range of habitats.

Most ascidian species are ecologically significant benthic organisms, predominantly inhabiting shallow coastal waters and substrates. However, tunicates as a whole are found across diverse marine environments, including pelagic zones of the open ocean and deep-sea habitats, demonstrating their extensive ecological adaptability (Shenkar & Swalla, 2011).

Tunicates are exclusively marine organisms, exhibiting an inability to survive in environments with low salinity. They are widely distributed across diverse marine habitats, ranging from tropical to polar regions. These organisms inhabit a variety of ecological niches, including shallow coastal zones, the open ocean, and depths extending from the intertidal zone to hadal depths (Holland, 2016).

They are cosmopolitan and commonly found in coastal waters and coral reefs, inhabiting the littoral zone, which extends from the intertidal to the subtidal zones, where they attach to rocks, shells, seaweeds, mangrove roots,

coral reef substrates, docks, or a ship's hull, as well as man-made structures like piers, artificial structures, and aquaculture cages.

Other free-swimming tunicates are found throughout the oceans as plankton and inhabit pelagic zones. Fewer than 100 species of sea squirts are found at depths of more than 200 m (660 ft), and other hard surfaces (Tahir, 2016).

They are predominantly abundant in anthropogenically influenced areas, such as ports, harbors, and industrial coastal zones, where environmental conditions are often altered by pollution and habitat disturbance. While the majority of ascidians are found in shallow waters, a limited number of species, such as *Dicopia antirrhinum*, inhabit the deep sea, occurring at depths of up to 4,500 meters, where they adopt a macrophagous feeding strategy. Furthermore, a considerable number of ascidian species are recognized as invasive, a status frequently attributed to their fouling capabilities and rapid colonization of artificial substrates (Piri et al., 2022; Rosner & Rinkevich, 2024).

3.3. Role in nutrient cycling and carbon flux

Tunicates are widely distributed marine invertebrates whose filter-feeding behavior plays a vital role in nutrient cycling and water quality regulation in marine ecosystems. By removing suspended particles such as plankton and organic detritus from the water column, they contribute to water clarity and ecological balance (Luo et al., 2022). This high filtration efficiency also presents potential for applications in biofiltration and environmental remediation (Gao et al., 2023).

They serve as prey for various marine species and host a diverse array of symbiotic and commensal organisms, further emphasizing their ecological significance. (Shenkar & Swalla, 2011). Notably, certain species within the family *Didemnidae* harbor symbiotic algae within their tunic, enhancing their role in primary production and nutrient dynamics (Matos & Antunes, 2021). These interactions highlight the importance of sustainably managing tunicate populations and their associated biomass.

Sustainable development demands the use of renewable, low-impact biomass sources that do not compete with food crops or deplete terrestrial ecosystems. In this context, tunicates offer a promising marine-based biomass that aligns with the principles of ecological sustainability, resource efficiency, and circular economy. Their high productivity, low ecological footprint, and versatile biochemical composition make them an appealing candidate for biotechnological applications.

The three primary nutrients influencing ocean productivity, carbon, nitrogen, and phosphorus, are processed differently by benthic tunicates. These organisms extract nutrients from the water column during filter feeding and contribute to nutrient sequestration by transferring organic matter to the benthos. For instance, a substantial portion of filtered carbon is likely to sink and become incorporated into sedimentary reservoirs, reducing its availability in the pelagic zone. Similarly, nitrogen is removed from the water column during feeding, potentially decreasing its availability for other pelagic organisms. Phosphorus, released as waste or upon the death of tunicates, accumulates on the substrate, further reducing the concentration of this essential nutrient in the water column (Luo et al., 2022).

Tunicates are benthic suspension feeders that extract particulate organic matter (POM) from the water column by drawing water through an oral siphon, filtering it internally via a mucous net, and expelling the filtered water through an atrial siphon (Jørgensen & Goldberg, 1953). This efficient filter-feeding mechanism plays a crucial role in nutrient cycling and carbon flow, contributing to water clarity in marine ecosystems by reducing suspended particulate loads. In contrast, invasive tunicate species of concern in coastal communities are primarily benthic, attaching to submerged surfaces and filtering nutrients from the water column before depositing waste and biomass onto the ocean floor (Ramesh et al., 2021).

Salps are highly efficient pelagic filter feeders whose biological activity significantly contributes to deep-ocean remineralization processes through the production of organic waste. As they inhabit the water column, salps play a crucial role in marine food webs and nutrient cycling, particularly through the biogeochemical redistribution of carbon, nitrogen, and phosphorus (Sutherland et al., 2010).

While both salps and benthic tunicates share similarities in terms of filtration efficiency, dietary composition, and fecal pellet production, their ecological impacts differ markedly due to their respective habitats. The cumulative effect of invasive benthic tunicates may therefore result in the localized depletion of key nutrients from the pelagic zone, ultimately limiting primary productivity and altering the structure of marine food webs. Unlike salps, which contribute to nutrient cycling within the water column, benthic tunicates act as sinks, diverting essential elements to the ocean floor and thereby disrupting the natural nutrient balance of coastal marine systems (Sutherland et al., 2010).

Pelagic tunicates possess the ability to filter particles at the micrometre scale, enabling them to feed on picoplankton, a component of primary production that is largely inaccessible to most marine herbivores. This highly efficient filter-feeding strategy allows them to consume a substantial fraction of oceanic primary productivity. Upon death, their carbon-rich bodies and fecal pellets sink rapidly to the seafloor, facilitating the vertical transport of organic matter and delivering significant quantities of particulate organic carbon to deep-sea ecosystems. These sinking materials not only sustain benthic communities but also contribute to the enrichment of nutrient-rich water masses, which are eventually brought back to the surface through upwelling processes. Despite their crucial role in marine biogeochemical cycles, including the global carbon cycle, pelagic tunicates remain understudied and poorly understood, underscoring the need for further ecological and physiological research (Luo et al., 2024).

Alongside appendicularians and doliolids, they facilitate the downward transport of organic matter through the water column by consuming phytoplankton in surface waters and producing carbon-rich fecal pellets. The sinking of these pellets, together with their deceased bodies, plays a critical role in carbon cycling and nutrient transfer to deeper ocean layers (Luo et al., 2022).

3.4. Contributions to food webs

Ascidians and other tunicates form complex benthic structures that provide habitat and shelter for various marine organisms, including small invertebrates and juvenile fish. Although tunicates are rarely consumed by humans, they occupy a significant position in marine food webs, indirectly supporting fisheries by serving as prey for ecologically and commercially important species. From an evolutionary perspective, tunicates are of particular scientific importance as they offer critical insights into the ancestry of vertebrates, sharing key developmental and genetic features that illuminate the early evolution of chordates (Rosner & Rinkevich, 2024).

As highly efficient filter feeders, tunicates consume vast quantities of nanoplankton and picoplankton, which are foundational to marine productivity and biomass and form the base of the oceanic food web. A substantial increase in tunicate abundance would reduce the availability of these essential planktonic resources for other filter feeders such as mussels, clams, and fish (Luo et al., 2022).

The reduction of plankton also intensifies competition for food among marine organisms. Moreover, the removal of plankton affects meroplanktonic

larvae, including shrimp and crabs, thereby impacting populations of predators that rely on the adult forms of these species. Excessive tunicate filtration could overwhelm native plankton populations, destabilizing the entire marine food web (Colarusso et al., 2016).

Additionally, tunicates deposit organic waste on the seafloor, which removes organic carbon from the water column, a crucial resource for planktonic communities. The combined effect of filtration and organic matter removal poses a threat to plankton survival, with potentially severe consequences for marine biodiversity and ecosystem services (Elvines et al., 2024).

Tunicates also pose direct threats to shellfish aquaculture by fouling farms and suffocating shellfish through the obstruction of their feeding structures, thereby limiting access to nutrients and oxygen (Carman et al., 2010).

They play a fundamental role in marine ecosystems, serving as both filter feeders and integral components of marine food webs. Their filtration activity regulates planktonic populations, helping to control excess particles and nutrients and thereby maintaining ecosystem balance. Sessile ascidians, in particular, play a significant role in benthic habitats by providing shelter and substrate for a diverse array of marine organisms. Moreover, tunicates serve as a significant food source for numerous predators, including fish, starfish, and sea turtles (Ramesh et al., 2021).

Alongside appendicularians and doliolids, tunicates are key mediators in the transfer of energy within marine food webs.

3.5. Tunicates as bioindicators of ocean health

Due to their widespread global distribution, high sensitivity to pollutants, abundant populations, mass sexual reproduction, and prevalence in coastal areas affected by anthropogenic pollution, ascidians are excellent bioindicators for monitoring marine pollution such as heavy metals (Aydın-Önen, 2016; Tzafriri-Milo et al., 2019), pharmaceutically-active compounds (PhACs) (Navon et al., 2020), organic pollution (Terlizzi et al., 2002), microplastic (Harel et al., 2024; Li et al., 2021; Vered et al., 2019) and global environmental changes (Rosner & Rinkevich, 2024).

Gallo et al (2018) evaluated the adverse effects of various xenobiotics, specifically lead, zinc, an organic tin compound, and a phenylurea herbicide, on the reproductive processes of the marine invertebrate (ascidian). The findings demonstrate that the reproductive functions of *Ciona intestinalis* are highly sensitive to these xenobiotics, indicating their potential as reliable biomarkers

for assessing the quality of marine environments. These results further support the use of ascidians as suitable model organisms for reprotoxicological bioassays. As sessile filter feeders, ascidians have the capacity to accumulate and concentrate contaminants present in seawater within their tissues, thereby enhancing their utility in monitoring marine pollution.

As filter feeders and suspension feeders, they can filter large volumes of seawater to extract plankton and organic particles, bioaccumulate pollutants from the water column, exhibiting heightened sensitivity to toxins while tolerating fluctuations in abiotic conditions, such as temperature, pH, and salinity, offering insight into climate-related stressors like ocean acidification and warming. Being stationary, tunicates reflect the local conditions of their habitat, making them good indicators of site-specific changes in water quality. Their abundance and ease of sampling, coupled with high regenerative capacity, enhance their utility in toxicological assessments (Thiyagarajan & Qian, 2003).

As members of the epifaunal community, ascidians often form dense colonies on various submerged surfaces, creating complex biogenic habitats that provide shelter and protection to a diverse array of marine organisms, including small fish, crustaceans, and other invertebrates. Certain specialized taxa, such as ascidicolous copepods, exhibit unique ecological associations with these ascidian structures. However, these biogenic habitats are also highly susceptible to environmental contamination, frequently accumulating hazardous substances such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), and chlorinated organic compounds, which may pose significant risks to the integrity and health of the resident biological communities (Rosner et al., 2024).

4. Economic Importance of Tunicates in Marine Ecosystem

Tunicates, a diverse group of sessile marine invertebrates, possess considerable economic and scientific value. In various regions, particularly in parts of Asia and the Mediterranean, certain species are harvested for human consumption, valued both as a source of high-quality protein and as local delicacies. Beyond their nutritional importance, tunicates have garnered significant attention for their biomedical and biotechnological potential. Numerous species produce structurally diverse bioactive compounds with a wide range of pharmacological properties, including anti-cancer, anti-inflammatory, and antimicrobial activities (Gao et al., 2023). To date, over 1,000 such molecules have been identified from tunicates and their associated microbiota, highlighting their immense promise in drug discovery and marine biotechnology.

Tunicates also serve as model organisms in multiple fields of biological research. Ascidians, in particular, are widely used in studies addressing biological invasions, allorecognition, innate immunity, regeneration, aging, embryonic development, germ cell dynamics, and the biosynthesis of bioactive secondary metabolites (Zhan et al., 2015). Their relatively simple body plan, coupled with their phylogenetic proximity to vertebrates, renders them especially valuable in the field of evolutionary developmental biology (Anselmi et al., 2025).

From a sustainability perspective, tunicates offer opportunities for integrated resource management. Addressing the dual challenge of increasing demand for sustainable marine biomaterials and mitigating the spread of invasive tunicate species presents a unique entrepreneurial avenue. Such approaches aim to capitalize on the ecological and economic potential of tunicates while contributing to environmental management through the control and utilization of invasive biomass.

Nevertheless, certain tunicate species also pose significant challenges as biofouling organisms. Their propensity to colonize ship hulls, aquaculture infrastructure, and other submerged surfaces imposes considerable maintenance and economic burdens on the maritime and aquaculture industries.

In conclusion, tunicates are not only integral to the functioning of marine ecosystems but also present a valuable resource for biotechnology, medicine, and sustainable aquaculture.

4.1. Biochemical composition and nutritional importance of tunicates

Seafood is a highly valuable nutrient source, characterized by its high protein content, rich amino acid profile, vitamins, micronutrients, and polyunsaturated fatty acids, including essential omega-3 fatty acids that must be obtained through diet for optimal human health. It is also notable for its low cholesterol and calorie content, making it beneficial for maintaining a healthy diet. Beyond its nutritional value, seafood has been shown to support health maintenance and aid in the treatment of various diseases. Due to its low connective tissue content, seafood is highly digestible and is often preferred in the diets of children and the elderly.

Recent studies have increasingly highlighted ascidians, particularly the solitary species *Ciona intestinalis*, as promising marine bioresources for n-3 polyunsaturated fatty acids (PUFAs), including essential fatty acids such as EPA and DHA, and *Styela clava* Herdman, 1881, contains important compounds with varying properties, including ACE (angiotensin-converting enzyme)

inhibition. *Botrylloides niger*, an alien ascidian species, represents a promising source of bioactive compounds likely developed for ecological adaptation and competition. It is notably rich in diverse lipid species beneficial to human health, including polyunsaturated fatty acids (PUFAs) that are uncommon in most foods. These biochemical properties highlight its potential to transition from an ecological threat to a valuable and accessible bioresource. Consequently, *B. niger* and other invasive ascidians may offer significant economic potential, particularly in the food and pharmaceutical sectors, through the development of functional products derived from these organisms. (Hassanzadeh, 2014).

The lipid profile of ascidians has been found to resemble that of fish oil (Hassanzadeh, 2014), suggesting their potential as alternative sources of marine lipids for aquaculture. Additionally, their amino acid composition is comparable to egg albumin, further supporting their use as a high-quality feed ingredient. Combined with their high protein content and low caloric value. Ascidians represent a valuable and underutilized source of marine-derived nutrition (Hassanzadeh, 2014).

Despite these benefits, the consumption of aquatic products in some countries remains significantly lower than the global average. Consequently, aquatic products represent a critical component of a balanced and healthy diet and should be better utilized and promoted. Increasing public awareness about the nutritional and sensory qualities of seafood can encourage greater consumption over time.

Ascidians serve as a crucial food source for a range of marine predators, including fish, sea stars, and specific crab species. Additionally, some ascidian species, such as *Halocynthia roretzi* and *Styela clava*, are consumed by humans (Gao et al., 2023; Lambert et al., 2016). In recent decades, ascidians have garnered increasing interest as potential alternative food sources due to their high nutritional value, which includes proteins, amino acids, lipids, and bioactive secondary metabolites (Palanisamy et al., 2017).

Moreover, ascidians are recognized as a rich source of diverse bioactive chemical compounds, including peptides, alkaloids, polyethers, macrolides, terpenes, and polysulfides (Palanisamy et al., 2017). In addition to their chemical potential, ascidians are increasingly being utilized as model organisms in various fields of biological research, including immunobiology, allorecognition, angiogenesis, and whole-body regeneration. Their remarkable regenerative abilities have garnered significant attention in the fields of regenerative medicine and ageing research (Della Sala et al., 2022).

4.2. Cellulose content (*tunicate cellulose = highly crystalline nanocellulose*)

Tunicates have a gelatinous outer “tunic” composed primarily of cellulose-like compounds, including *tunicin*. This natural polymer shares structural similarities with plant cellulose (with longer polymer chains) but is biologically derived, making it a unique and renewable resource for material applications. The tunic, a key component of the tunicate’s defense mechanism, offers both durability and flexibility, enabling tunicates to thrive in a wide range of marine environments. This remarkable combination of strength and biodegradability positions as a compelling alternative to synthetic, petroleum-based materials (Jiang et al., 2023).

5. Threats to Tunicate Populations

5.1. Climate change effects (*temperature, ocean acidification*)

Climate change poses a significant threat to tunicate populations through rising sea temperatures, ocean acidification, and shifting oceanographic conditions. Elevated temperatures can alter metabolic rates, reproductive cycles, and larval development in tunicates, potentially leading to population declines or shifts in distribution. Ocean acidification, caused by increased CO₂ absorption, may also affect the structural integrity of tunicate tunics and disrupt microbial symbioses essential for nutrient cycling. Moreover, changes in current patterns and salinity can impact larval dispersal and settlement, further influencing population dynamics (Gallo et al., 2019).

5.2. Pollution and its impact on marine biodiversity

Tunicates are highly susceptible to pollutants due to their filter-feeding behavior and sedentary lifestyle. They can accumulate a wide range of contaminants, including heavy metals, microplastics, polycyclic aromatic hydrocarbons (PAHs), and chlorinated compounds, such as tributyltin (TBT) and copper, both of which are components of marine antifouling paints. This not only affects their own physiology but also poses a risk to the broader marine food web. The degradation of tunicate habitats through chemical pollution and nutrient overloading can lead to a decline in biodiversity, particularly among species that rely on tunicates as shelter or food. Long-term exposure to pollutants may also alter species composition within tunicate communities, favoring more tolerant invasive or opportunistic species over native biodiversity (Radford et al., 2000).

5.3. Invasive Species and Habitat Competition

Bioinvasions pose serious threats to marine biodiversity and ecosystem services, with marine non-indigenous species (NIS), particularly invasive species, imposing substantial ecological and economic costs globally. These impacts extend beyond direct market losses to include damage to biodiversity, public health, and nonmarket environmental assets.

Marine biological invasions are primarily driven by global shipping activities, particularly through the discharge of ballast water and biofouling on vessel surfaces. However, most NIS risk assessments have focused predominantly on ballast water, emphasizing three stages: uptake, survival during transit, and discharge. Given limited management resources, it is increasingly recommended that high-risk invasive species be prioritized for targeted intervention and control. (Andersen, et al., 2004).

Ascidians are widely regarded as a model taxon for studying biological invasions due to their prominent presence in reports of bioinvasions. They represent one of the most active groups in such studies and have successfully invaded coastal regions across all continents except Antarctica, spanning from the northern to the southern hemisphere.

Solitary ascidians typically inhabit deeper and more stable habitats, including rocky substrates, coral reefs, and submerged artificial structures. In contrast, colonial ascidians are predominantly found in more dynamic and shallow environments such as coastal zones, estuaries, shallow reefs, and rocky shorelines. This broad ecological distribution allows both solitary and colonial ascidians to fulfill important functional roles within marine ecosystems. However, when introduced to non-native regions primarily via human-mediated vectors such as shipping and aquaculture, they can become invasive species, posing threats to native biodiversity and ecosystem stability (Rosner & Rinkevich, 2024; Shenkar & Swalla, 2011).

The abundance, accessibility, and filter-feeding behavior of ascidians, characterized by their reproductive strategy of simultaneously releasing large quantities of gametes and undergoing rapid larval development, along with their high sensitivity to a wide range of toxins, make them particularly responsive to environmental fluctuations. This pronounced sensitivity positions ascidians as valuable bioindicators for assessing environmental health, rendering them well-suited for investigating the impacts of pollutants and stressors on marine ecosystems. Additionally, their adaptability to unstable and variable environmental conditions, alongside the highly invasive nature of certain species, positions ascidians as excellent model organisms for studying species

invasions and biodiversity dynamics (Corbo et al., 2001; Rosner & Rinkevich, 2024).

As omnivorous filter feeders with rapid growth and reproduction rates, tunicates aggressively compete for habitat and food resources. Their relatively simple feeding strategy, combined with effective anti-predator defenses, enables them to spread readily and rapidly colonize available surfaces. Because of these traits, tunicates pose a significant ecological threat as invasive species (Topić Popović et al., 2025).

Ascidians are conspicuous invasive species in both tropical and temperate waters (Rocha et al., 2012). They are known to be strong competitors. These traits make ascidians highly successful invaders that can create major economic expenses by heavily fouling marine vessels and man-made structures, as well as by overgrowing aquaculture equipment and organisms (Aldred & Clare, 2014).

Tunicates exhibit a highly efficient and generalized filter-feeding strategy that allows them to exploit a wide range of particle sizes, resulting in direct competition with native filter-feeding species in invaded ecosystems. Their success as invaders is further facilitated by the absence or scarcity of natural predators in non-native environments, coupled with a remarkable tolerance to a broad range of environmental conditions, including fluctuations in salinity and temperature. Although the natural dispersal potential of benthic tunicates is constrained by a short-lived larval stage, typically lasting only a few hours to several days, their high reproductive capacity and survival rates enable them to colonize rapidly. When combined with anthropogenic vectors such as global shipping, aquaculture operations, aquariums, Lessepsian migrants via Suez and Gibraltar Canals (e.g., rabbitfish), and ballast water and hull fouling, the fouling of artificial structures significantly enhances their capacity for long-distance dispersal and establishment in new habitats, contributing to their widespread invasive success (Hong et al., 2025).

6. Tunicates: Applications in Sustainable Materials

6.1. Nanocellulose and biopolymers of tunicates

Tunicate-derived nanocellulose exhibits exceptional properties, including high mechanical strength, remarkable purity, and an elevated Young's modulus. Additionally, it possesses unique optical, electrical, magneto-mechanical, and rheological characteristics, making it highly versatile material for advanced applications (Lv et al., 2023).

Additionally, tunicate cellulose can be processed into nanocellulose, a high-performance biomaterial with a wide range of potential applications. These applications demonstrate significant promise in both food technology and materials science (Gao et al., 2032; Zhao et al., 2015).

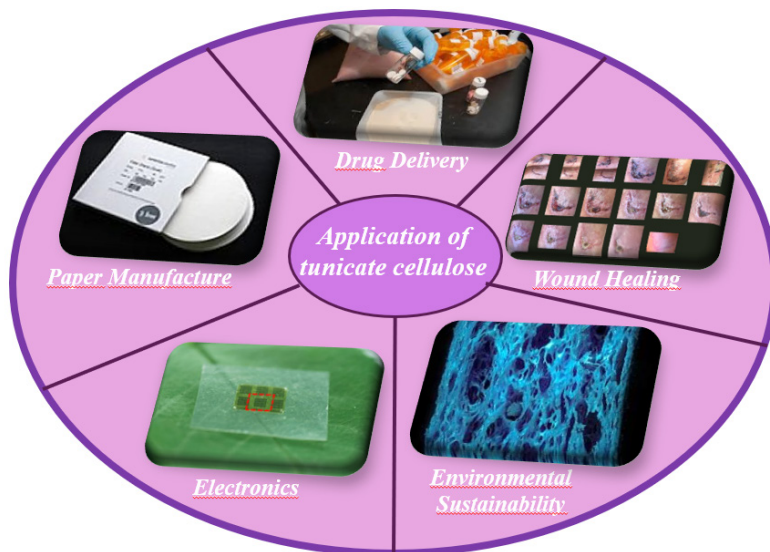


Figure 5. Application of tunicate cellulose in different fields. Drug delivery by Public Domain is licenced under CC BY-SA 4.0, wound healing by Raquel Baranow, environmental sustainability by Alexander Klepnev, and electronics by Yei Hwan Jung et al. (2015) are licenced under CC BY-4.0, and paper manufacture by KOchstudiO is licenced under CC BY-SA 3.0. (<https://creativecommons.org/licenses/>).

Cellulose extracted from the outer capsule of tunicates has garnered significant attention from researchers due to its excellent physicochemical properties. In addition, its nanocellulose exhibits high strength, purity, modulus, and versatile photoelectromagnetic properties, and is gradually becoming a novel biomaterial. However, the utilization of tunicates is extremely low, and their discarded outer capsules accumulate in large quantities, resulting in a significant environmental burden and waste of resources. Therefore, it has become imperative to fully exploit and reuse tunicates.

Among these, ascidians are the most extensively researched for sustainable material applications due to their high nanocellulose content and stationary lifestyle, which facilitates aquaculture and harvesting (Lv et al., 2023). The

applications of tunicate and its composites in various fields (e.g., biomedical field, energy and electronics, polymer reinforcement, food packaging field, paper industry and environmental materials, luminescent fibers, high-strength composites, biodegradable sensors, bone tissue engineering, wound healing, food industry, polymer reinforced materials, paper manufacture, energy and electronics, environmental protection material, drug delivery, myocardial tissue engineering etc.) are also discussed (Figure 5) (Lv et al., 2023).

The upcycling of ascidians into nanocellulose networks presents an innovative approach to ecosystem preservation and resource valorization, with potential applications in managing both native and invasive ascidian species. This strategy introduces a novel, animal-derived class of cellulosic nanonetworks, offering a sustainable platform for the development of advanced biomaterials within the framework of a coastal blue bioeconomy.

Beyond the introduction of these unique nanocellulosics, the valorization of ascidian biomass supports the expansion of integrated biorefineries in coastal regions. These biorefineries may operate through the controlled cultivation of native species or through the ecological management of invasive populations. In both cases, the extraction and utilization of tunicate-derived nanocellulose enable circular and regenerative production models. The ease with which high-performance materials can be derived from ascidian biomass underscores their considerable potential to contribute to the global bioeconomy, particularly in the context of sustainable coastal development and marine resource management (Govindharaj et al., 2025).

Tunicates are also novel biomedical substances in the field of tissue engineering, wound dressing, and drug delivery systems (Seddiqi et al., 2021).

The use of nanocellulose fibre in the textile manufacturing sector has the potential to mitigate the environmental consequences associated with the industry by utilizing sustainable resources and reducing waste (Cho et al., 2019).

6.2. Biodegradable plastics and sustainable polymer alternatives

Ascidians produce a unique extracellular matrix known as the tunic, which contains tunicin, a cellulose-like biopolymer. This natural polymer exhibits biodegradability and renewable characteristics, making it a promising candidate for the development of biodegradable plastics and sustainable polymer alternatives. Utilizing ascidian-derived tunicin could reduce reliance on petroleum-based plastics, thereby contributing to environmentally friendly materials with lower ecological footprints (Hong et al., 2025; Kononova et al., 2022; Zhao & Li, 2014).

6.3. Biomedical applications of tunicates

Ascidians harbour a great microbial community (including bacteria, actinobacteria, cyanobacteria, and fungi). The unique chemical class of ascidians' antimicrobial metabolites, including sulfur-containing compounds, meroterpenes, alkaloids, peptides, furanones, and other aromatic derivatives, as well as their antimicrobial effects, undoubtedly could provide lead candidates for drug discovery programs in the anti-infective area of interest (Casertano et al., 2020).

Tunicates contain remarkable compounds and are a rich source of valuable marine natural products (MNPs) for potential pharmaceutical applications (Palanisamy et al., 2017). The biocompatibility and bioactivity of materials derived from tunicates make them excellent candidates for medical applications. Their low immunogenicity and natural origin facilitate their integration into human tissues and biological systems.

To date, more than 1000 chemical structures isolated from these organisms have been described (Carroll et al., 2019). Over 70% of these are alkaloids, including indole alkaloids, followed by pyrocridine, beta-carboline, and indolocarbazole (staurosporine). Several alkaloids contained in tunicates have been shown to have anti-cancer properties. Another interesting class of molecules they contain is peptides. Tunicates are also a rich source of compounds with antibacterial, antifungal, antidiabetic, anti-inflammatory, antiprotozoal, antitumoral, antiviral, anti-cancer, and immunosuppressive activities (Bauermeister et al., 2019; Miranda, 2023).

Moreover, natural products (NPs) derived from ascidians have contributed to the development of promising pharmaceuticals, including marketed drugs for the treatment of specific cancers, such as Ecteinascidin 743 or Trabectedin (Yondelis®) from *Ecteinascidia turbinata* Herdman, 1880, and the peptide dehydrodidemnin B or Plitidepsin (Aplidin®) from *Aplidium albicans* (Milne Edwards, 1841) (Ramesh et al., 2021).

Numerous compounds derived from ascidians have demonstrated significant therapeutic potential, particularly as anti-cancer and anti-viral agents. Notable examples include Didemnins, Aplidine, Trabectedin, and Palmerolide. For instance, Palmerolide, isolated from the Antarctic tunicate *Synoicum adareanum*, has shown promising efficacy against melanoma, a highly aggressive form of skin cancer (Avalon et al., 2021).

Another biologically active molecule, the relaxin hormone, has been identified in the ovarian cells of tunicates such as *Ciona intestinalis* and is noted

for its potential to enhance sperm motility. Additionally, marine invertebrates, including ascidians such as *Styela plicata*, are sources of sulfated polysaccharides, including sulfated fucans and galactans. These compounds, along with highly sulfated polysaccharides like heparin, known for its antithrombin (AT) activity, have been utilized in the treatment of arterial thrombosis (Hassanzadeh, 2011). With over 1,000 isolated bioactive compounds and a wide range of reported applications (Chen et al., 2019), tunicates represent a promising source of marine natural products (MNPs) with significant pharmaceutical potential. These compounds have demonstrated diverse biological activities, including antitumoral, antiviral, antifungal, anti-inflammatory, and antibacterial properties (Palanisamy et al., 2017).

Recent studies have identified a variety of tunicate-associated bacteria, including *Bacillus*, *Pantoea*, *Pseudoalteromonas*, *Salinicola*, *Streptomyces*, *Vibrio*, and *Virgibacillus*, as potential producers of antimicrobial compounds, bioactive secondary metabolites, and biosurfactants (Marques et al., 2022). These microbial symbionts contribute to the defensive mechanisms of their tunicate hosts and represent a promising source of novel bioactive agents for pharmaceutical applications (Ramesh et al., 2021).

The robust immune defense system of tunicates, combined with their association with symbiotic microorganisms that possess bioactive properties, makes them highly attractive candidates for marine drug discovery. A recent review by Ramesh et al. (2021) emphasized the diversity of microbial symbionts associated with tunicates, including bacteria, actinomycetes, fungi, and cyanobacteria. Among these, actinomycetes, fungi, and bacteria were identified as the predominant groups, many of which exhibit notable cytotoxic and antimicrobial activities. These microbial communities are responsible for the production of a wide array of secondary metabolites, with alkaloids representing a major class of marine natural products (MNPs) derived from tunicate-associated microorganisms.

6.4. Nutritional applications of tunicates

Although most tunicate species are not considered edible, several solitary stolidobranchs from the families Styelidae and Pyuridae are either wild-harvested or cultured for human consumption. Notable edible species include *Boltenia ovifera*, *Halocynthia aurantium*, *Halocynthia roretzi*, *Microcosmus hartmeyeri*, *Microcosmus sabatieri*, *Microcosmus vulgaris*, *Polycarpa pomaria*, *Pyura chilensis*, *Pyura pachydermatina*, *Styela clava*, and *Styela plicata*.

These species are generally consumed in Asia, Chile, Japan, Korea, and the Mediterranean, in various forms, including raw, cooked, dried, or pickled, depending on regional culinary traditions. Countries are highlighted for their potential role in food security and sustainable aquaculture (Table 1) (Gao et al., 2023; Lambert et al., 2016).

Table 1. Wild-harvested and cultured tunicates for human consumption (Lambert et al., 2016).

Wild-harvested species		
Species	Common names	Where collected
<i>Boltenia ovifera</i> (Linnaeus, 1767)		Russia
<i>Halocynthia aurantium</i> (Pallas, 1787)	bee-dahn-mung-geh (Korea)	Korea, Russia
<i>Microcosmus hartmeyer</i> Oka, 1906	harutoboya (Japan)	Japan
<i>Microcosmus sabatieri</i> Roule, 1885		Mediterranean
<i>Microcosmus vulgaris</i> Heller, 1877	sea violet	Mediterranean
<i>Polycarpa pomaria</i> (Savigny, 1816)		Mediterranean
<i>Pyura chilensis</i> Molina, 1782	piure	Chile
<i>Pyura pachydermatina</i> (Herdman, 1881)	sea tulip	New Zealand (historically)
<i>Pyura praeputialis</i> (Heller, 1878)	cunjevoi	Australia (historically), Chile
<i>Pyura vittata</i> (Stimpson, 1852)	karasuboya (Japan) dohl-mung-geh or kkeun-mung-geh (Korea)	Japan, Korea
Cultured species (all are also wild-collected)		
Species	Common names	Where collected
<i>Halocynthia aurantium</i> (Pallas, 1787)	sea peach, ice floe tunicate, akaboya (Japan)	Japan
<i>Halocynthia roretzi</i> (Drasche, 1884)	sea pineapple, mung- geh or kkoht-mung- geh (Korea), hoyo or maboya (Japan)	Japan, Korea
<i>Styela clava</i> Herdman, 1881	mee-duh-duck (Korea)	Korea
<i>Styela plicata</i> (Lesueur, 1823)	o-mahn-doong-yeo or o-mahn-dee (Korea)	Korea

Additionally, *Ciona intestinalis* has been developed as a sustainable protein source for use in animal feed formulations (Samuelsen et al., 2022). Overall, these tunicates have been cultivated and consumed as a delicacy in many countries.

6.5. Environmental and Industrial Applications

The ecological functions of tunicates, particularly their role as efficient filter feeders, offer promising avenues for environmentally sustainable industrial applications.

Tunicates can be used in aquaculture systems to reduce organic waste and improve water quality. Their filter-feeding abilities allow them to function as natural purifiers, reducing the environmental footprint of intensive aquaculture practices (Hong et al., 2025).

Certain tunicate species possess the ability to accumulate heavy metals and other contaminants from seawater. This trait makes them potential tools for bioremediation in polluted coastal and marine ecosystems (Aydın-Önen, 2016; Navon et al., 2020; Tamilsevi et al., 2015; Tzafiriri-Milo et al., 2019). The tunic of tunicates, primarily made of cellulose-like materials with a high carbon content, offers potential for carbon sequestration. Additionally, this biomass can be transformed into value-added products, such as biochar or advanced carbon-based nanomaterials (Zhao et al., 2015).

Recent research is investigating the lipid content of tunicates as a potential feedstock for renewable energy. Although still in the early stages, this area of study indicates possible applications in producing biolubricants and second-generation biofuels (Gao et al., 2023; Santhanam, 2025).

6.6. Exploring New Applications

Emerging applications of tunicate-derived materials are advancing the frontiers of sustainable technologies and biomedical innovation. Notably, tunicate-derived nanocellulose is being explored for integration into next-generation energy storage systems, including high-performance supercapacitors and batteries, due to its unique mechanical strength, high surface area, and electrical tunability (Zhao et al., 2015). Biodegradable electronics (Zhu et al., 2018) and smart packaging features (Zhao & Li, 2014), as well as antimicrobial properties (Casertano et al., 2020; Ramesh et al., 2021), and moisture responsiveness (Lüskow et al., 2024) are being explored (Hong et al., 2025; Lv et al., 2023). Additionally, bioinks for 3D bioprinting (Govindharaj et al., 2022)

are being investigated, particularly in the field of regenerative medicine (Gao et al., 2023; Ramesh et al., 2021; Palanisamy et al., 2017). In parallel, ongoing pharmaceutical research continues to explore the rich biochemical diversity of tunicates and their symbiotic microorganisms, which may lead to the discovery of novel marine-derived therapeutics from previously uncharacterized species (Bauermeister et al., 2019; Ramesh et al., 2021). Together, these innovations underscore the multifaceted potential of tunicates as a valuable resource in materials science, bioengineering, and drug discovery.

7. Threats to Tunicate Populations

Climate change-driven alterations in ocean conditions, such as increased water temperature and decreased pH due to ocean acidification, posing significant stressors to marine ecosystems. These environmental changes may disproportionately affect native species, potentially reducing their resilience and ability to adapt to changing conditions. As a result, certain invasive marine species may gain a competitive advantage under these shifting conditions, leading to increased establishment and spread in non-native regions. As a result of increased sedimentation, sea squirts face a double threat: their food supply is affected, and their gills become clogged. Such dynamics represent a growing concern for biodiversity conservation and the stability of marine communities.

8. Challenges and Limitations

A major challenge for future research lies in identifying novel pharmacological or commercially valuable compounds derived from tunicates. Polysaccharide-based biomaterials extracted from tunicates represent a promising and emerging area within various biomedical applications, including tissue regeneration and drug delivery systems. The intrinsic properties of tunicate-derived polysaccharides, including their biological activity, biodegradability, and capacity to form hydrogels, warrant further investigation for their potential therapeutic applications. Additionally, the quantification of metal ion concentrations within tunicate samples offers an intriguing avenue for both assessing their nutritional value and utilizing them as bioindicators for monitoring trace element pollution in marine environments (Hassanzadeh, 2011).

Despite the considerable pharmacological potential of ascidian-derived bioactive compounds, their commercialization faces several significant

challenges. The inherently low natural abundance of these metabolites, coupled with the difficulties associated with sustainable harvesting from wild populations, limits their availability. Logistical complexity, high water content, rapid decomposition, and high production costs present significant challenges.

Furthermore, the complex biosynthetic pathways involved in producing these compounds present additional barriers to large-scale synthesis and production. Recent advances in marine biotechnology, synthetic biology, and microbial fermentation provide promising avenues to address these limitations by enabling sustainable and scalable production of ascidian bioactive metabolites.

9. Management Plan for Invasive Tunicates

Effective management of invasive tunicate species requires a comprehensive strategy focused on detection, monitoring, control, and, where necessary, eradication. Regular monitoring efforts should target common fouling surfaces such as docks, boat hulls, aquaculture equipment, and other submerged structures to detect early infestations.

Public education plays a vital role in managing the spread of tunicates. Raising awareness about the ecological and economic impacts of these fouling organisms will support community involvement in prevention and reporting efforts.

To prevent future invasions, it is essential to conduct targeted research on the dispersal mechanisms, colonization rates, impacts on human health, and ecological and economic consequences of tunicate proliferation. These insights will inform risk assessments and help guide responsive strategies.

Regulation and policy enforcement are critical components in controlling invasive populations. This includes managing ballast water discharge, implementing biosecurity measures in aquaculture, and enforcing cleaning protocols for marine vessels and infrastructure.

10. Future Prospects and Research Directions

Ascidians have emerged as promising model organisms and bioresources due to their ecological adaptability, evolutionary significance, and capacity to produce valuable bioactive compounds. Future research directions focus on unlocking their potential through multidisciplinary approaches: With the availability of sequenced genomes, most notably that of *Ciona intestinalis*, ascidians provide a tractable system for genetic manipulation. These genomic

resources lay a critical foundation for the application of advanced genome editing tools, such as CRISPR-Cas9, as well as for optimizing metabolic pathways. As a result, ascidians are increasingly positioned as valuable model organisms in developmental biology, synthetic biology, and marine biotechnology.

Ascidians contain cellulose-rich tunics and bioactive compounds suitable for pharmaceutical, nutraceutical, and biofuel applications. Research is moving toward optimizing biomass processing and developing sustainable biorefinery models to enable commercial-scale extraction and valorization of ascidian-derived products.

The incorporation of ascidian cultivation into integrated multi-trophic aquaculture (IMTA) systems presents an opportunity to improve resource efficiency and environmental sustainability. Their natural filtration capacity can help maintain water quality in fish farms, while co-cultivation with algae or shellfish may lead to synergistic production outcomes. Further research on the potential use of ascidians in IMTA frameworks should focus on systems' carrying capacity (Marques et al., 2022).

Recent studies suggest that ascidians exhibit enhanced growth performance within Integrated Multi-Trophic Aquaculture (IMTA) systems, particularly when co-cultured with species such as sea cucumbers and finfish. The continued development and optimization of innovative IMTA frameworks are critical to maximizing system carrying capacity and promoting sustainable aquaculture practices.

In addition to their ecological role, ascidians are a valuable source of bioactive compounds, including the essential long-chain polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are vital for human health, as well as for the nutrition of marine fish and shrimp. Although cultural acceptance remains a limiting factor in some Western countries, ascidians are increasingly recognized as a nutritious and sustainable seafood option, rich in essential amino and fatty acids. Moreover, the incorporation of ascidian-derived materials into aquafeeds presents a promising alternative for enhancing feed formulation in aquaculture (Marques et al., 2022).

Tunicates have long been recognized as a valuable marine reservoir for the discovery of bioactive compounds, particularly in the treatment of diseases such as cancer. The continued exploration and utilization of tunicate species, ranging from coastal waters to the deep sea and across tropical to polar regions, hold significant potential for advancing drug discovery and enhancing our understanding of evolutionary lineages. To support sustainable use

and conservation, regular biodiversity monitoring and population dynamics assessments of tunicate species should be conducted to ensure their continued health. These efforts are essential for elucidating distribution patterns and evaluating their ecological impact on coastal marine resources.

11. Conclusion

Achieving a sustainable blue economy requires harnessing the potential of science and innovation to develop innovative solutions for ocean sustainability. From this perspective, tunicates offer tremendous potential and represent a highly promising yet underutilized marine resource with substantial potential to contribute to sustainable development, bioinnovation, and the circular bioeconomy. Their unique biochemical and structural properties, particularly the production of high-purity cellulose and bioactive compounds, as well as their role as natural biofilters, position them as a versatile feedstock for a broad range of applications, including biodegradable materials, advanced pharmaceuticals, environmental remediation, and functional textiles.

As outlined in this chapter, tunicates offer key advantages for sustainable material development, notably through their applicability in nanocellulose production, biomedical engineering, and eco-friendly product design. Furthermore, their integration into circular and blue bioeconomy strategies highlights their relevance in addressing global environmental and economic challenges.

However, realizing the full potential of tunicates will require overcoming several technical, ecological, economic, and regulatory hurdles. Scaling up aquaculture practices, advancing bioprocessing technologies, and fostering interdisciplinary research, particularly in synthetic biology and materials science, will be essential steps toward commercialization and wider adoption.

In parallel, the expanding field of marine bioprospecting continues to reveal the medicinal value of ocean-derived organisms, particularly tunicates, which hold promise for oncology and drug discovery. This underscores the dual importance of these organisms as both material and medicinal resources. Nonetheless, increased utilization must be balanced with robust conservation strategies to prevent undue pressure on marine ecosystems.

Future studies should prioritize the elucidation of biosynthetic pathways, the optimization of compound extraction methods, and the development of structural modifications that enhance bioactivity while minimizing toxicity.

Integrating cutting-edge approaches such as omics technologies, computational modeling, and high-throughput screening will be critical in unlocking the full pharmacological potential of ascidian-derived compounds. These interdisciplinary strategies will not only accelerate drug discovery but also support the development of novel therapeutics with significant implications for modern medicine.

As the global community seeks renewable, biodegradable alternatives to fossil-based products, tunicates stand out as a viable and ecologically responsible solution. Continued investment in scientific research, industry collaboration, and policy support will be critical to transitioning these marine invertebrates from niche research subjects into mainstream contributors to a sustainable, resilient, and bio-based future.

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