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Review

Biological and Ecological Roles of External Fish Mucus: A Review

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Abstract: Fish mucus layers are the main surface of exchange between fish and the environment, and they possess important biological and ecological functions. Fish mucus research is increasing rapidly, along with the development of high-throughput techniques, which allow the simultaneous study of numerous genes and molecules, enabling a deeper understanding of the fish mucus composition and its functions. Fish mucus plays a major role against fish infections, and research has mostly focused on the study of fish mucus bioactive molecules (e.g., antimicrobial peptides and immune-related molecules) and associated microbiota due to their potential in aquaculture and human medicine. However, external fish mucus surfaces also play important roles in social relationships between conspecifics (fish shoaling, spawning synchronisation, suitable habitat finding, or alarm signals) and in interspecific interactions such as prey-predator relationships, parasite–host interactions, and symbiosis. This article reviews the biological and ecological roles of external (gills and skin) fish mucus, discussing its importance in fish protection against pathogens and in intra and interspecific interactions. We also discuss the advances that "omics" sciences are bringing into the fish mucus research and their importance in studying the fish mucus composition and functions.

Keywords: fish mucus; mucus molecules; interspecific communication; mucus microbiome; mucus metabolome; mucus bioactivities

1. Introduction

External (skin and gill) mucus is the main surface of exchange between fish and their surrounding environment, and thus plays a key role in intra- and interspecific chemical communication [1,2]. Mucus acts as a dynamic physical and biochemical barrier, displaying numerous biological and ecological roles such as osmoregulation [3,4], protection against abrasion [5], protection against environmental toxins and heavy metal toxicity [6], parental feeding [7], protection against pathogens [8], and chemical communication [9]. Teleost mucus is similar to mammalian mucus and is mainly composed of mucins [3]. Fish mucus also contains numerous immune molecules, such as lysozymes, immunoglobulins, complements, lectins, and antimicrobial peptides (AMPs) [10], and other molecules like mycosporine-like amino acids (MAAs) [11], toxins, and kairomones—uncharacterized

semiochemicals that mediate interspecific interactions by providing information that benefits individuals of another species and harms the emitter [12,13].

Composition of fish mucus and its rheological properties are vital for the maintenance of mucus functions [14]. Mucus surfaces are dynamic matrices and their composition varies among fish species and with endogenous (sex and developmental stage) and exogenous factors (stress, water temperature, pH and infections) [15]. Stress conditions (e.g., handling stress, confinement, food deprivation, exposure to toxic substances) can change the mucous production and composition (e.g., level of proteins and immune molecules), compromising fish health and increasing the fish susceptibility to bacterial pathogens [16–19]. Mucus viscoelasticity determines its ability to block many types of motile bacteria [20], and several studies showed that fish tend to increase their mucus secretion and change their composition when exposed to pathogens [21–23], which may contribute to the defence against these pathogens. Furthermore, pathogen infections (e.g., virus, bacteria) can also alter the mucosal microbiome of fish, facilitating the increase of pathogenic bacteria [24,25].

Fish mucus research has increased in the last ten years mainly due to the discovery of numerous bioactive molecules (antibacterial, antiviral, antifungal, and antiparasitic) and their potential application in human medicine and in aquaculture [26–28]. Furthermore, the study of external fish mucus provides nonlethal alternatives for the early detection of infections [29–31] and for monitoring the impact of environmental pollutants on fish health [32–34]. To date, most research on fish mucus has focused on immune-related molecules and AMPs, but few studies have analysed other mucus molecules and their ecological roles in the environment. Secondary metabolites, for example, even though known to play a key role in the communication of a wide range of species, including plants, invertebrates, and microorganisms, have rarely been studied in fish tissues or mucus [35]. There are currently numerous research studies and review articles investigating specific components of fish mucus (e.g., immune molecules [36], antimicrobial peptides [26], and bacterial communities [37]), but to the best of our knowledge, no recent study (beyond Shepard, 1994 [4]) has investigated the importance of fish mucus for both the fish and the ecosystem. This review analyses the current state of knowledge about the roles of external fish mucus in order to highlight the importance of fish mucus in the marine ecosystem, to identify gaps in knowledge, and to provide future directions. We describe the different biological (antimicrobial, immune-related, and UV protection roles of the fish mucus components as well as the fish mucus roles in intra and interspecific interactions. We also review and evaluate the use of the different "omics" technologies (genomics, transcriptomics, proteomics, and metabolomics) on fish mucus research and their potential to discover novel mucus components and deepen the understanding of the fish mucus functions.

2. Mucus Production

The mucus matrix is produced by goblet, club, and sacciform cells found in the fish epithelium [4,20]. In fish, few studies have examined the excretion or delivery of molecules other than mucins to the mucus layers, though it seems that the mode of delivery could be conserved [36]. Proteins could be transported to the mucus layer by the classical delivery of extracellular material, where proteins are synthesized by ribosomes on the rough endoplasmic reticulum (ER) and then delivered to the cell membrane through the Golgi complex [38]. Proteins (synthesized in the cytosol) and other molecules could also be delivered to the mucus layers via transport routes directly over the cell membrane either by transporters or through channels or other nonclassical mechanisms such as membrane vesicles like exosomes and microvesicles [39,40]. Dead epidermal cells could also be a source of mucus proteins and other molecules [36]. However, it is important to note that molecules that are released from cellular debris might still play important functions in the mucus layer. For example, it is known that proteins can have additional functions beyond their known functions [41]. The commensal microbiota community (bacteria and fungi) could also be a source of varied mucus molecules such as antimicrobial peptides and secondary metabolites [42–44].

3. Mucus Sampling and Analysis

The study of external fish mucus is becoming more popular as it provides nonlethal alternatives for both detecting fish infections and monitoring the environmental pollutants [29–34]. External fish mucus is most often sampled by gently scraping (e.g., with cell scraper or spatula) the external surfaces of a fish (body, fins, gills), avoiding ventral areas in order to avoid intestinal or sperm contaminations [45–48]. However, a recurrent issue with this method is the possible contamination of the mucus from other tissues such as blood or epithelial cells during fish mucus collection. The use of adsorbing materials like filter paper [49] or cotton swabs [50] has also been used in fish mucus collection as a strategy to avoid epithelial contamination. Raj et al. [51] showed that the use of cotton swabs removed the upper most layers of epidermis, whilst the use of filter paper allowed mucus removal without apparent damage of the epithelial cells. A recent study showed that the metabolome of mucus samples obtained through absorption displayed the highest repeatability, whilst those from scrapped mucus displayed the most variability [52]. Other strategies to collect fish mucus include rinsing the fish surfaces with different solutions [53], aspirating the fish mucus using a vacuum cleaner [54], and the use of plastic bags filled with different solutions [55,56].

Histocytochemical techniques such as histochemistry, immunohistochemistry, or even electron microscopic cytochemical methods have been traditionally used to study the distribution of molecules in fish epithelial and mucus layers [57–59]. The application of immunohistochemistry techniques provides the advantage of detecting molecules in situ while avoiding mucus contamination; however, these techniques are limited to molecules for which their antibodies have been identified [60,61]. In contrast, the recently developed matrix-assisted laser desorption/ionization–imaging mass spectrometry (MALDI–IMS) enables the study of spatial molecular arrangements in tissue sections without the need of target-specific reagents. Compared to immunochemistry where a single antigen is typically studied, imaging mass spectrometry (IMS) enables the measurement of thousands of analytes in parallel, while allowing for the study of conventional histology [62]. The main advantage of MALDI–IMS is the broad spectrum of analytes that can be studied with this in situ technique ranging from proteins and peptides to lipids and secondary metabolites [63]. Matrix-assisted laser desorption/ionization–imaging mass spectrometry (MALDI–IMS) has been successfully used to highlight the localization of saponins in the mucus layer of the sea star *Asterias rubens* [64], and it presents a great potential for the discovery of new fish mucus molecules, a means to study the spatial distribution of those molecules, and the identification of new extraction techniques.

4. Mucus Biological Activities

The mucus gel matrix is primarily comprised of *O*-glycosylated proteins (GPs) called mucins, but it also contains a diverse array of other molecules such as (1) proteins (structural proteins, immune-related proteins, and antimicrobial peptides and proteins)(reviewed by Brinchmann 2016 [36]), (2) lipids [65], and (3) smaller molecules such as crinotoxins [66]—fish epidermal toxins not associated with any venom apparatusand MAAs [11] that display a wide array of biological roles (Table 1). Fish mucosal surfaces also harbor a diverse community of organisms (bacteria, fungi, and viruses) that play a major part in maintaining host health and homeostasis (reviewed by Gomez et al. [8], Llewellyn et al. [67], and Kelly and Salinas [37]). Since fish mucus is the main barrier against infections, all its components (molecules and microbiota) might coordinate to block pathogen entrance by deploying different antimicrobial activities and participating in immune responses in a similar way as observed in rodents [68–70] (Table 1).

Table 1. Biological activities of fish mucus molecules. If molecules are reported in more than three fish species, species are not specified.

Activity	Molecule	Molecule Family	Fish Species	Reference
Antimicrobial	Glycoproteins	Glycoproteins	*Tinca tinca, Anguilla anguilla, Oncorhyncus mykiss*	[71]
	Keratin	Protein	Several species	[36]
	Apolipoprotein 1	Protein	Several species	[36]
	Piscidins	α-Helical AMP	Several species	[72,73]
	Pleurocidins	α-Helical AMP	*Pleuronectes americanus*	[74]
	Dicentracins	α-Helical AMP	*Dicentrarchus labrax*	[75]
	Chyrsopsins	α-Helical AMP	*Sparus aurata*	[72,73]
	Moronecidins	α-Helical AMP	*Morone saxatilis x chrysops*	[76]
	Pardaxin	AMP	*Pardachirus marmoratus*	[77]
	Pelteobagrin	AMP	*Pelteobagrus fulvidraco*	[78]
	β-defensin	Cysteine-rich AMP	Several species	[79–81]
	AJN-10	Cysteine-rich AMP	*Anguilla japonica*	[48]
	Parasin-1	Histone-derived AMP	*Parasilurus asotus*	[82]
	Hipposin	Histone-derived AMP	*Hippoglossus hippoglossus*	[83]
	SAMP H1	Histone-derived AMP	*Salmo salar*	[84]
	Onchorrhycin II	Histone-derived AMP	*Onchorhyncus mykiss*	[85]
	β-chain of hemoglobin	Protein	*Pomatotrygon* cf. *henlei*	[86]
	Histones (H1, H2A, H2B)	Protein	Several species	[87,88]
	L-amino oxidase (LAOs)	Protein	*Sebastes schlegeli*	[46]
	L40, L36A, L35, S30	Ribosomal protein	*Gadus morhua*	[45]
	Hemoglobin-like protein	Protein	*Ictalurus punctatus*	[89]
	Chitinase	Hydrolitic enzyme	Dasyatis pastinaca	[90]
Immune-related	Transferrin	Glycoprotein	Several species	[36]
	Misgurnan	Polysaccharide	*Misgurnus anguillicaudatus*	[91,92]
	HSC70, HSP60, HSP90	Heat shock protein	Several species	[36]
	Peroxiredoxins		Several species	[36]
	FK-506 binding protein	Immunophilin/Protein	*Gadus morhua*	[93]
	Cyclophilin A	Immunophilin/Protein	*Gadus morhua*	[93]
	Cystatin B	protein	*Gadus morhua*	[93]
	Mannan binding lectin	Lectin/protein	*Gadus morhua*	[93]
	Galectins	Lectin/protein	*Gadus morhua*	[93]
	Concavalin A	Lectin/protein	*Gadus morhua*	[93]
	Congerins	Lectin/protein	*Congus myriaster*	[94]
	AJL-1, AJL-2	Lectin/protein	*Anguilla japonica*	[95]
	Pufflectin	Lectin/protein	*Takifugu rubripes*	[96]
	Intelectin	Lectin/protein	*Silurus asotus*	[97]
	G-type lysozyme	Lysozyme/protein	Several species	[15,98]
	C-type lysozyme	Lysozyme/protein	Several species	[15,98]
	Tumor necrosis factor α	Cytokine, protein	Several species	[15,98]
	Acid and alkaline phosphatases	Enzyme/proteins	Several species	[98,99]
	C1q, C3, C5, C6, C9, Complement factor B	Complement/protein	Several species	[36]
	Ig M, Ig T	Immunoglobulins/Protein	Several species	[100]
	Interleukins (IL-1β, IL-8, IL-10)	Cytokine, protein	Several species	[15]
	Calpain	Protein	*Gadus morhua*	[22]
	Trypsin	Serine protease/protein	Several species	[15]
	Metalloproteases	Protease/protein	Several species	[15]
	Cathepsin B and L	Cysteine protease/protein	Several species	[15]
	Cathepsin D	Aspartic protease/protein	Several species	[15]
	Aminopeptidases	Protease/protein	Several species	[15]
Cellular metabolism	Ubiquitin		*Gadus morhua*	[93]
	Gluthathione hydrolase and transferase		*Gadus morhua*	[93]
	Calreticulin		*Gadus morhua*	[93]
Carbohydrate metabolism	Citrate synthase		*Gadus morhua*	[93]
	Enolase andglyceraldehyde-3-phosphate dehydrogenase		*Gadus morhua*	[93]
Lipid metabolism	Preapolipoprotein A	Protein	*Gadus morhua*	[93]
	Fatty-acid binding protein	Protein	*Gadus morhua*	[93]
UV protection	Palythene	MAAs	Several species	[93]
	Asterina-33	MAAs	Several species	[93]
	Mycosporine-N-methylamine serine	MAAs	Several species	[101]

AMPs: Antimicrobial peptides; MAAs: Mycosporine-like amino acids.

4.1. Antimicrobial Components

Many antimicrobial molecules have been found in fish external mucus including pore-forming glycoproteins [71], enzymes (e.g., chitinases with antifungal activity) [90], proteins (e.g., apoliporotein-1, warm temperature acclimation protein WAP65) [74,99,102], and several crinotoxins [13,103] (Table 1). Antibacterial peptides (AMPs), which are one of the main molecules to fight pathogens, have also been observed in fish mucus [72] (Table 1). Conventional AMPs found in fish mucus include the α-helical peptides piscidins (moronecidins, pleurocidins, dicentracins, and chrysopsins [73–75]), other linear peptides like pardaxin and pelteobagrin [77,78], and the cysteine-rich AMPs defensins [79,80] (reviewed by Smith et al. [81] and Masso-Silva and Diamond [72]).

While most AMPs are derived from a biologically inactive proprotein that is processed to the active form, some AMPs are derived from larger, functional proteins that have primary functions other than antibacterial activities [88]. Several histones (H1, H2A, H2B) with antibacterial, antifungal and antiparasitic activities have been identified in skin mucus of fish [45,87], and several AMPs derived from terminal parts of histones have been described including parasin-I, hipposin, salmon antimicrobial peptide SAMP H1, and oncorhyncin II [82–85]. Other antibacterial proteins found in fish mucus include L-amino acid oxidases (LAOs) such as *Sebastes schlegeli* antibacterial protein (SSAP), ribosomal proteins such as L40, L36A, L35, and S30, and hemoglobin-like proteins (Hb-β) [45,46,88,89].

Mucus commensal microbiota play a key role in controlling opportunistic pathogens; however, the mechanisms involved are not fully understood yet [104,105]. Until recently, the control of pathogens by commensal bacteria was thought to be a result of mutually competitive relationships [106]; however, recent studies are showing specific mechanisms by which commensal bacteria could be recognized by the host and control pathogen proliferation [37,107]. For example, Sepahi et al. [108] found that *Flectobacillus major* from external mucosal surfaces of rainbow trout produced sphingolipids that induced immunoglobulin T (IgT) production, shaped teleost B cells and antibody responses, and were able to control the growth of other symbionts. Other studies found antibacterial and antifungal activities in bacterial strains isolated from fish mucus, suggesting microorganism production of specialized metabolites that could control host pathogens development [109,110].

4.2. Immune-Related Components

Teleost fish possess an active mucosal immune system (reviewed by Esteban et al. [15] and Salinas, 2015 [10]). The external mucosa-associated lymphoid tissues (MALT) found in fish include skin-associated lymphoid tissue (SALT), the gill-associated lymphoid tissue (GIALT), and the nasopharynx-associated lymphoid tissue (NALT) [10]. The main cellular components of fish innate immunity observed in mucosal surfaces are leukocytes, mast/eosinophilic granule cells (EGCs), mucosal dendritic cells (DSs), macrophages, and granulocytes [8].

Fish mucus is also enriched with a multitude of immune-related proteins such as lysozymes, phosphatases, esterases, proteolytic enzymes, complement factors, lectins, immunoglobulins, and C-reactive proteins that attempt to eliminate pathogens and launch the immune cascade when an infection occurs [111] (Table 1). Two isoforms of lysozymes (bacteriolytic enzyme) that are similar to goose (g)- and chicken (c)-type in vertebrates have been detected in fish mucus [98]. Acid and alkaline phosphatases and esterases are important enzymes found in fish mucus that act as antibacterial agents and can be potential stress indicators in fish skin mucus [55,98]. Different types of proteases (trypsins, metalloproteases, cathepsins, and aminopeptidases) have been identified in fish skin mucus, with serine and metalloproteases being the most predominant [15]. Several complement components such as C7, C3, and C1q have been identified in skin and intestine mucus of several fish species [112,113]. Immunoglobulins (IgM and IgT/IgZ) are major components in fish mucus, with IgT/IgZ playing major roles in fish mucosal immunity [100,114,115].

Other molecules involved in innate immunity such as glycoproteins like transferrin [116] or immunomodulating carbohydrates such as misgurnan [91,92] have been described in fish skin mucus. Finally, is it worth mentioning that despite the main antibacterial activity of antimicrobial peptides, some fish mucus AMPs have also been found to modulate B cell functions, and thus play an important role in the innate immune system [117].

4.3. Other Activities

Fish mucus contains other MAAs that perform a photo protective function against solar radiation [11] (Table 1). To date, three different MAAs have been identified in fish mucus: palythene, asterina-33, and mycosporine-*N*-methylamine serine, with different species of fish presenting different combinations of MAAs [101].

Furthermore, some studies have also shown cytotoxic activities of external fish mucus against specific cancer cell lines, indicating the potential of fish mucus in the development of new pharmacological antitumoral strategies [68,118].

Specialized metabolites have scarcely been studied in fish mucus; however, with the development of high-throughput techniques such as metabolomics this will likely change in the upcoming years, which might reveal new biological activities of fish mucus.

5. Mucus Roles in Ecological Interactions

External fish mucus is involved in very important ecological roles, in both intra and interspecific communication (Table 2).

Table 2. Fish mucus molecules involved in ecological interactions.

Activity	Molecule Family	Producer Species	Receptor Species	Reference
		Intra-Specific Interactions		
Fish shoaling	Phosphatidylcholines	*Plosotus lineatus*	*Plosotus lineatus*	[119,120]
Reproduction	Aminoacids	*Carassius auratus, Anguilla anguilla*	*Carassius auratus, Anguilla anguilla*	[121,122]
	Apolar metabolites (prostaglandin-like)	*Anguilla anguilla*	*Anguilla anguilla*	[123]
	Tetrodotoxin	*Takifugu niphobles*	*Takifugu niphobles*	[124]
Alarm signaling	Chondroitins (glycosaminoglycan)	*Danio rerio*	*Danio rerio*	[125]
		Inter-specific interactions		
Microbial chemotaxis	Aminoacids and carbohydrates	*Onchorhyncus mykiss*	*Vibrio anguillarum*	[126]
	Lectin-like	*Ictalurus punctatus*	*Flavobacterium columnare*	[127]
	Free nucleosides	*Onchorhyncus mykiss*	*Myxobolus cerebralis, Myxobolus pseudodispar, Henneguya nuesslini*	[128]
	Glycoprotein	*Takifugu rubripes*	*Neobenedenia girellae*	[129]
	Tetradotoxin	*Takifugu rubripes*	*Pseudocaligus fugu*	[130]
	Cathelicidins (peptides)	*Salmo salar*	*Caligus rogercresseyi*	[131]
Predator repulsion	Pardaxin (AMP)	*Pardachirus marmoratus*	*Squalus acanthias*	[132]
	Pavoninin (monoglycosidic cholestanoid)	*Pardachirus pavoninus*	*Mustelus griseus*	[133]
	Mosesin (monoglycosidic cholestanoid)	*Pardachirus marmoratus*	*Negaprion brevirostris*	[134]
	Grammistins (AMP)	*Grammistes sexlineatus Pogonoperca punctata*	-	[13]

5.1. Intra-Specific Communication

Mucus cues serve in communication among conspecifics, either as attracting cues enabling the finding of suitable habitats or partners or as alarm cues, alerting to danger [9,135,136] (Table 2). Although most of the studies on conspecific cues have focused on fish odor, with no particular attention to fish mucus, it is logical to think that skin odorant cues are probably excreted through fish mucus. Conspecific cues are known to enable different migratory fish species to find their habitats [136,137]. Leonard et al. [9] found that conspecific mucus trails enable suitable habitat finding of the waterfall climbing Hawaiian gobioid *Sicyopterus stimpsoni.* Chemical cues from conspecifics also play an important role in fish shoaling [138,139]. For example, phosphatidylcholines from skin mucus were found to induce school forming in young catfish (*Plosotus lineatus*) [119,120]. Reproduction and male and female synchronisation of spawning relies in the release of pheromones in the water [140,141]. Although pheromones are often released through urine, gill diffusion, or bile salts [142–144], some studies found several attractants in fish skin mucus [136–138]. For example, Salgio and Fauconneau [121,122] suggested that skin mucus amino acids from catfish, goldfish, and European eel could have a role in the social relations of these species. In contrast, Huertas et al. [123] found a

high concentration of apolar odorants in skin mucus of European eel (*Anguilla anguilla*), which were not characterized, but their polarities suggest they could be sex steroids, prostaglandins, or related metabolites, indicating a possible role of fish mucus in the chemical communication of eel reproduction. Tetrodotoxin (TTX), which has also been found in fish mucus, is also known to act as a sex pheromone that attracts males towards fertile females [124,135].

The release of alarm signals after injury is a widely reported mechanism in fish that produces an alarm response in conspecifics with the ultimate objective of avoiding the source of danger [145]. Several studies have shown that conspecific skin extracts can elicit alarm responses, suggesting the release of alarm cues from club cells through the fish external mucous layers [146,147]. In a recent study, chondroitins—linear, heterogeneous polymers, made of disaccharides variably sulfated, which have been previously identified in fish mucus [148]—were identified as fish odorants triggering fear responses in zebrafish [125]. Zebrafish exposed to purified chondroitin exhibited alarm behaviour, darting, slow swimming, and bottom dwelling [125].

5.2. Interspecific Communication

Studies have also shown that epidermal and gill mucus substances can act as infochemicals in different interspecific interactions, such as in prey–predator relationships, parasite–host interactions, and symbioses (Table 2). Fish mucus molecules can be detected by a wide range of organisms and can generate different types of responses. For example, a recent study found that the absence of *N*-acetylneuraminic acid (Neu5Ac) in skin mucus of clownfish (*Amphiprion ocellaris*), protect the fish from being stung by the anemone *Hetractis magnifica*, whose toxin release is triggered by the detection of Neu5Ac [149]. Several studies have shown that different pathogenic bacteria exhibit positive chemotaxis towards their hosts' mucus [150,151]. Although specific molecules responsible for this activity have not yet been identified, O'toole et al. [126] suggested that free amino acids and carbohydrates could act as chemoattractant molecules in trout mucus (skin and intestinal) while Klesius et al. [127] suggested that a lectin-like substance might be responsible for bacterial chemotaxis in catfish (*Ictalurus punctatus*). In recent studies, Padra and colleagues found that fish mucosal sialic acids and specifically *N*-acetylglucosamine (GlcNac) play an important role in the pathogenic bacteria *Aeromonas salmonicida* growth and binding to its host [152,153].

Chemodetection of fish mucus and behavior modification have also been reported in other parasites such as the actinospores from oligochaetes, myxozoans, and ectoparasites like copepods [127–131,154–156]. Kallert et al. [128] found that free nucleosides, which are continuously released in trout mucus (inosine, 2′-deoxyinosine and guanosine), stimulated myxozoan attachment. Another study showed that WAP65-2 glycoprotein from tiger pufferfish (*Takifugu rubripes*, Tetradontidae) skin mucus induced attachment of *Neobenedenia girellae* oncoramicidia (Monogenea, capsalidae) [129]. Tetrodotoxin from grass pufferfish (*Takifugu niphobles*) could also act as an attractant for the infective copepodids stages of *Pseudocaligus fugu* [155]. Similarly, it is known that sea lice specifically locate and recognize their salmonid hosts by chemodetection [131], and a recent study has shown that cathelicidin peptides isolated from salmon mucus promoted the development of the frontal filament of the sea lice *Caligus rogercresseyi* [156]. A rather curious case is the protective effect of mucous cocoons of parrotfishes against gnathiid parasites, although it is yet unknown whether this protective effect is due to the chemical or physical properties of the mucous barrier [157].

Fish mucus substances often act as semiochemicals in predator-prey relationships, either as predator deterrents or as signals for both predators and prey. For example, it is known that pardaxin (antimicrobial peptide), pavoninins, and mosesins (monoglycosidic cholestanoids) secreted by several species of soles repel sharks by acting on their olfactory senses [133]. Tetrodotoxin from puffer fish has also been reported to repel predation by groupers [134,158]. Purcell and Anderson [12] found that *Physalia physalis* could identify its prey using chemical cues from fish epidermal mucus, although the chemical nature of these kairomones remains unknown. Several organisms are also able to identify chemicals in fish mucus to detect predators and to avoid predation. Several studies

indicate that nocturnal dial vertical migration (DVM) in zooplankton is affected by uncharacterized kairomones present in mucus of planktivorous fish [2,159]. Forward and Rittschof [159] proposed that disaccharide degradation products of predator mucus containing sulfated and acetylated amines can serve as kairomones, whereas Beklioglu et al. [2] suggested that both fish and mucus-dwelling bacteria interact in the release of kairomones. Recent research showed that the polychaete *Nereis* developed chemosensory mechanisms for predator detection (chemical cues from fish mucus) to minimize predation risks [160].

Goby epidermal toxins and their ecological implications are well studied and provide a good example of the importance and complexity of fish mucus molecules in ecosystem dynamics. Coral gobies are known to possess different epidermal mucus toxins that exhibit predator deterrence and parasite avoidance [66,161]. Recent studies have shown how goby mucus substances play a key role in the mutualistic association between gobies and corals [162,163]. *Acropora* corals use chemicals to attract gobies when attacked by toxic seaweeds, and in turn, gobies trim the seaweed and increase their own mucus toxicity, protecting them against predators and parasites [163]. At the same time, goby skin mucus repels corallivores, protecting corals from predation [161]. However, even though the ecological role of goby epidermal mucus toxins is well studied, their chemical structures are still unknown.

The study of chemical mediation in marine ecosystems is vital to understanding ecosystem dynamics and to understanding how ecosystems can respond and adapt to changing conditions. Fish chemical mediation is still poorly understood, with very few examples where fish mucus molecules have been elucidated and their ecological roles identified. As previously shown for TTX, a single molecule can have multifunctional properties. Species, sex, and tissue specific differences in the distribution of TTX render unclear the exact function of this molecule in pufferfish. Depending on the concentration, TTX may function as a chemical defence against predators, as an attractant for parasites, or as a chemical pheromone during spawning. These mucus molecules of keystone significance are vital in structuring ecological communities and, therefore, a deeper understanding is needed [164]. Research on fish mucus, coupled with the development of new technologies will enhance our collective understanding of how fish interact with and respond to their environment.

6. Use of "Omics" in Fish Mucus Research

The field of fish mucus research is growing rapidly [36,37,165]. Advances in high-throughput "omics" technologies such as genomics, transcriptomics, proteomics, and metabolomics allow the simultaneous study and identification of numerous genes and molecules and has, therefore, a huge potential for discovering unreported molecules and functions in fish mucus [166,167]. Developments in the field of genomics facilitate the study of genes in fish mucus, and are allowing the rapid expansion of microbiomics (microbiome characterisation) [37,168]. A recent study published by Carda-Diéguez et al. [169] sequenced the DNA of European eel skin mucus and found evidence of the role of fish mucus surfaces as natural niches for aquatic mucosal pathogen evolution.

Transcriptomics, which is the study of the complete set of RNA transcripts that are produced by the genome, the transcriptome, is also a powerful tool for the discovery of new genes involved in mucosal immunity and can reveal important aspects of mucosal functions such as secretion, microbial pathogenesis, host immune responses, as well as the kinetics for these responses [166,170–172]. The biological interpretation of RNA-seq (RNA sequencing) (whole transcriptome sequencing) remains challenging and depends on availability of well-annotated genomes, which remain uncommon in nonmodel and noncommercial fish species [166]. Gene expression and transcriptomics have been successfully used to characterize the mucosal responses following bacterial infections in fish and after salmon smoltification [173–176]. A recent genome study of Atlantic salmon found seven putative mucin genes with tissue-specific transcription patterns, and it revealed that mucin transcription is regulated differently by different aquaculture stressors, providing new insights into mucosal health [177].

Proteomics, which is the study of the proteins expressed by a biological entity, has been the most used high-throughput tool in fish mucus research so far [36]. Several reference skin

mucus proteomes have been published for commercial fish species such as discus fish (*Symphysodon aequifasciata*) [178], Atlantic cod [93], European seabass (*Dicentrarchus labrax*) [179,180], gilthead seabream (*Sparus aurata*) [102], and lumpsucker (*Cyclopterus lumpus*) [167]. The use of high-throughput techniques such as proteomics allows for the pursuit of comprehensive comparative studies to better understand fish mucus dynamics. Proteomics studies have cast light on fish mucus dynamics of infected fish [181–183], overcrowding [184], chronic stress [185], and fish exposed to different diets [186].

Metabolomics is the comprehensive study of the small molecules of an organism (metabolome), and provides a snapshot of the state of an organism at a certain time under specific conditions [187]. Since metabolites are the end products of regulatory processes, their levels can be regarded as the ultimate response of biological systems to genetic or environmental changes, making metabolomics extremely useful to understanding organism responses and for biomarker discovery [188]. The study of the fish mucus metabolome can assist in the discovery of mucus molecules, but also in gaining understanding on the processes and functions in which fish mucus is involved. However, the fish mucus metabolome remains largely unexplored, with currently only three published studies [49,52,189]. Ekman et al. [49] studied the skin mucus metabolome of fathead minnow (*Pimephales promelas*) males and females exposed to bisphenol A and found that it was highly sexually dimorphic. Reverter et al. [189] studied the gill mucus metabolome of several butterflyfishes in relation to different fish traits (geographic site, type of habitat, species taxonomy, phylogeny, diet, and parasitism level) and found that diet was the main factor influencing the gill mucus metabolome. Finally, similar to other omics technologies, fish mucus metabolomics studies remain challenging due to the high amount of data obtained and the lack of information on fish metabolites and specialized databases, which render metabolite identification extremely difficult.

Finally, the integration of different omics techniques, although it is currently a major challenge, will contribute greatly to the advancement of fish mucus research and the understanding of the fish mucus system as a whole. Omics integration provides new opportunities to uncover pathways and processes that otherwise would remain undetected [190].

7. Summary and Conclusions

In summary, fish mucus surfaces are dynamic layers that display important functions in fish, playing major roles in physiological functions such as osmoregulation and protection against infections, but also in intra- and interspecific communication [2,4]. Mucus contains a wide variety of biologically active molecules that take part in numerous roles and biological interactions, some of which have drawn attention as potential candidates for drug development [27]. The study of fish mucus components has exploded in recent years [36,37], but there are still numerous mucus components that need further research in order to better characterize the molecules present, and to clarify their roles. We have discussed the multiple roles of mucus in organism interactions in nature (predator–prey, parasite–host), highlighting the ecological importance of mucus and the need to identify the molecules responsible for these interactions. Identifying the molecules responsible for the chemical mediation between fish mucus and other organisms will allow a deeper understanding of marine ecosystem dynamics and thus will give new insights in the management of parasites in aquaculture. In addition, a better understanding of the shifts and changes in mucus due to biological and physical stressors could help to prevent fish disease outbreaks and to monitor the health of the marine environment. To date, nearly all studies have focused on the study of macromolecules, via multiple techniques such as proteomics, but few studies have tried to elucidate smaller specialized metabolites. We would like to draw attention to the importance of secondary metabolites and their varied bioactivities in better studied species such as marine invertebrates and their potential presence and importance in fish mucus. Furthermore, the development of new disciplines such as metabolomics will allow for the more efficient study of secondary fish metabolites. Recent studies have shown the importance of the microbiome, and its capacity to synthesize bioactive molecules [44,99]. Therefore, the study

of the fish mucus microbiome and mycobiome is another promising field for both the discovery of new metabolites and for better comprehension of the mucus itself and its biological and ecological functions. Finally, fish mucus research needs to be approached from an integrative perspective in order to study the entire mucosal system.

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