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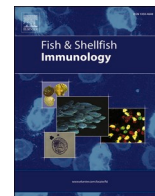
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Haemocyte motility: A marker of inflammation in *Mytilus* sp

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ABSTRACT

Bivalve immunity relies exclusively on innate cellular and humoral mechanisms, during which cells named haemocytes maraud across tissues to survey the organism and cope with invaders through migration towards infected site. Immune response is therefore governed by haemocyte motility. This review focuses on the different types of haemocyte movement in *Mytilus* sp. To address their role in immunity, from random patrolling of organs to directed pathogen elimination. By forming cell clusters or aggregates of different sizes, haemocyte displacements define inflammation *per se* in mussels. Although described for many years, motility can now be quantified by advanced microscopy techniques that give access to cell velocity values, allowing us to quantify inflammation. As various biotic and abiotic factors have been found to modulate haemocyte velocity, this parameter can be considered a marker to assess the inflammation level, paving the way for future developments in determining the immune status of mussels.

1. Introduction

Cell migration is involved in key biological processes in metazoan [1], including morphogenesis, cancer invasion, wound healing and immune response. The ability of cells to move is particularly fundamental for immune cells, as it enables them to reach sites of infection and eliminate invaders in order to maintain the organism integrity and to prevent infectious diseases. Immune cells respond to several stimuli, such as chemotactic and mechanotactic signals, which trigger cell polarization and directed migration to the required sites [2].

In *Mytilus* marine mussels, immune defence is exclusively innate and relies only on haemocytes as cellular effectors [3]. Haemocytes circulate in the body via an open haemolymphatic circulatory system where they can serve different tissues or supply the extrapallial fluid as demonstrated in several bivalves including mussels and clams [4–6]. In addition to their main role in the immune response, these immunocytes have been reported to contribute to various functions such as, nutrient digestion, transport, shell mineralization, excretion and wound repair [7–9]. They are also involved in detoxification system by accumulating metallic and organic xenobiotics in their lysosomes [10,11] using at least 3 classes of ABC transporters [12,13]. The immune response is triggered after the intrusion of a pathogenic agent and requires the recruitment of haemocytes to the sites of tissue damage or infection,

where an inflammatory response is set up [3,14]. Invaders are eliminated mainly by the process of phagocytosis, which involves engulfing pathogens or foreign particles in order to destroy them [3,15]. If the invader is too large to be internalised by haemocytes, the encapsulation mechanism takes effect [3]. During these processes, cascades of reactions are initiated, enabling immunocytes to migrate, recognise, attach the pathogen, and destroy it. Thus, similarly to vertebrates, the movement of immune cells in *Mytilus* appears to be a central process in homeostasis and immune defence.

In this review, we present an overview of current knowledge of haemocyte tissue migration in *Mytilus* species. We provide an historical account of the advances made in this field and point out the remaining gaps.

2. Motile haemocytes are the cellular immune effectors in mussels

In marine mussels, haemocytes form an heterogeneous cell population [16]. The classification of these subpopulations has evolved over time, based on different techniques, and incorporating morphological and functional criteria (Table 1). Initial identification using cytochemical and cytological characteristics combined with microscopy made it possible to distinguish 3 morphological cell types in *M. edulis*, divided

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into 2 categories, basophils and eosinophilic granulocytes [17]. Since then, various studies have suggested other classifications ranging in *M. galloprovincialis* from 2 subpopulations such as hyalinocytes and granulocytes to 4 sub-populations, namely large granulocytes, large semi-granulocytes, small semi-granulocytes and hyalinocytes [18–20]. In general, granular haemocytes are recognized for the many small granules in their cytoplasm, while hyalinocytes have a few rare granules or large phagosome-like vacuoles [19,21–23]. Basophils, on the other hand, have a high nucleus/cytoplasm ratio and contain granules in their cytoplasm [19,21,22].

Despite the interest of these classifications, the morphological criterion alone cannot discriminate between haemocytic populations particularly when microscopic preparations are used. Indeed, haemocytes are motile cells capable of attaching to a substrate and deforming thanks to their remarkable membrane plasticity. In this respect, Le Foll et al. (2010) combined cell motion profiles, hemacolor staining and flow cytometry analysis to classify *M. edulis*, haemocytes according to their ability to move. The authors suggested 3 cell morphotypes, including granular eosinophils, hyalinocytes both marked by transitions between spread and shrunk cell morphologies associated with amoeboid movement, and basophils using small lamellipodia to glide and thick filopodia as traction arms [22]. It is interesting to point out that although the morphological difference between hyalinocytes and granulocytes is significant, the functional characteristics, including phagocytosis and motility, appear to be similar. In this article, the authors highlighted that basophils are involved in cell-cell interaction with granulocytes and also as organization-core of large haemocyte aggregates (Figs. 1 and 3). It has been suggested that the different cell types may result from a single population which undergoes different stages of maturation allowing its granular complexity to change [26]. However, these statements remain hypothetical and require detailed investigation, especially as no specific cellular markers for bivalve haemocytes are available. In this context, a promising study described the selection of oyster granulocytes using an alcohol acyltransferase, named CgAATase, as a specific marker [27]. Authors reported the overexpression of CgAATase transcripts in granulocytes compared to agranulocytes and semi-granulocytes and isolated this subpopulation from total circulating haemocytes by immunomagnetic bead coated with the anti-CgAATase monoclonal antibody [27]. A similar approach could be implemented in mussels to explore the presence of potential cell subpopulation-specific markers.

Besides the complexity of differentiating cell subpopulations, little data is available on the origin, life cycle and lifespan of haemocytes in bivalves and, to our knowledge, no data is available for *Mytilus*. In the Manila clam, a study based on cytochemical analyses reported the formation of mitotic spindles in circulating haemoblasts cells suggesting their ability to act like stem cells [28]. In the Pacific oyster, the hemopoietic transcription factor (Tal1/SCL) has been identified in cells attached to the endothelium of blood vessels [29]. Similarly, Jemaa and al. (2014) demonstrated the presence of haemocytic progenitor cells in oyster gill epithelium using the transcription factor Sox2 as a stem cell marker and the oyster haemocyte-specific Zn/Cu superoxide dismutase (SOD) [30]. More recently, the DNA-binding protein Ikaros, known as a key determinant of haemopoietic lineage, has been characterized in *C. gigas* haemocytes [31].

3. Inflammation in mussels: no *rubor*, *dolor*, *calor*, *tumor* but *cohors*

Activation of the immune system results from the success of a pathogen or foreign element in breaking through the host barriers. In order to protect the organism, various changes are triggered in the internal environment, both in the circulating cells and in the composition of the plasma, the inflammation process [3,32]. Usually, the clinical symptoms of inflammation include *rubor*, *dolor*, *calor* and *tumor* and meaning redness, pain, heat and swelling [33]. If these markers are fundamental to the definition of inflammation in vertebrates, they cannot be relevant to invertebrates, and even less so to mussels. Despite the differences between these two kingdoms, similarities in cellular responses have been observed, particularly about the recruitment of immune cells. As in vertebrates, mollusc immune cells can migrate to the site of inflammation to either destroy the invader or restore tissue lesions by means of specific responses [3]. This widespread cell migration leads in some cases, to cells grouping into aggregates of different sizes (Fig. 1).

In molluscs, haemocyte infiltration has been classified in three morphological histotypes corresponding to different clustering types (*cellula cohors*), i.e. infiltrative/focal-type, nodular-type and encapsulation-type [34]. The cellular infiltration type is characterised by acute or diffuse invasion of the injured tissue by immune cells, but without any signs of clustering [35]. The nodule, on the other hand,

Table 1
Morphological and functional classification of haemocytes sub-populations in *Mytilus* sp. according to the chronology of studies.

Species	Subpopulations	Criteria	Methods	References
<i>M. edulis</i>	Basophils and granular eosinophils	Cell structure (diameter, shape, granularity), phagocytic capacity and enzyme cytochemistry	Light and electron microscopy, staining reactions	Moore and Lowe, 1977 [17]
<i>M. edulis</i>	Hyalinocytes, granulocytes with small granules and granulocytes with large granules	Cell structure (size, granularity) and lectins bindings	Light and electron microscopy	Pipe, 1990 [24]
<i>M. edulis</i>	Basophils, granular eosinophils and agranular cells	Cell structure (size, nucleus/cytoplasm ratio, granularity, etc.), Percoll density gradients, enzyme cytochemistry, lectin bindings, respiratory burst activity and phagocytic capacity	Staining reactions, light and electron microscopy, density gradients, biochemical assays	Pipe et al., 1997 [21]
<i>M. edulis</i>	Basophils, hyalinocytes and granular eosinophils	Cell structure (shape, size, granularity, colour) and motility	Flow cytometry, Hemacolor staining and live cell time-lapse microscopy	Le Foll et al., 2010 [22]
<i>M. galloprovincialis</i>	Granulocytes and hyalinocytes	Cell structure (size, shape, granularity, description of organelles, presence of pseudopodial protrusions, etc.) and phagocytic capacity	Light and electron microscopy	Cajaraville and Pal, 1995 [18]
<i>M. galloprovincialis</i>	Hyalinocytes and granulocytes	Cell structure (shape, cell and nucleus diameter, N/C ratio, granularity, etc.)	Light and electron microscopy	Carballal et al., 1997 [19]
<i>M. galloprovincialis</i>	Large granular cells, large semi-granular cells, small semi-granular cells and small agranular cells	Cell structure (size, granularity), respiratory burst activity and phagocytic capacity	Flow cytometry, Hemacolor staining, light and phase-contrast microscopy	García-García et al., 2008 [20]
<i>M. galloprovincialis</i>	Hyalinocytes, small granulocytes and large granulocytes	Cell structure (size and granularity)	Flow cytometry and light microscopy	Parisi et al., 2008 [25]
<i>M. galloprovincialis</i>	Granulocytes and agranulocytes	Cell structure (size, granularity, shape), Percoll density gradient	Flow cytometry, density gradients centrifugation and light microscopy	Andreyeva et al., 2019 [23]

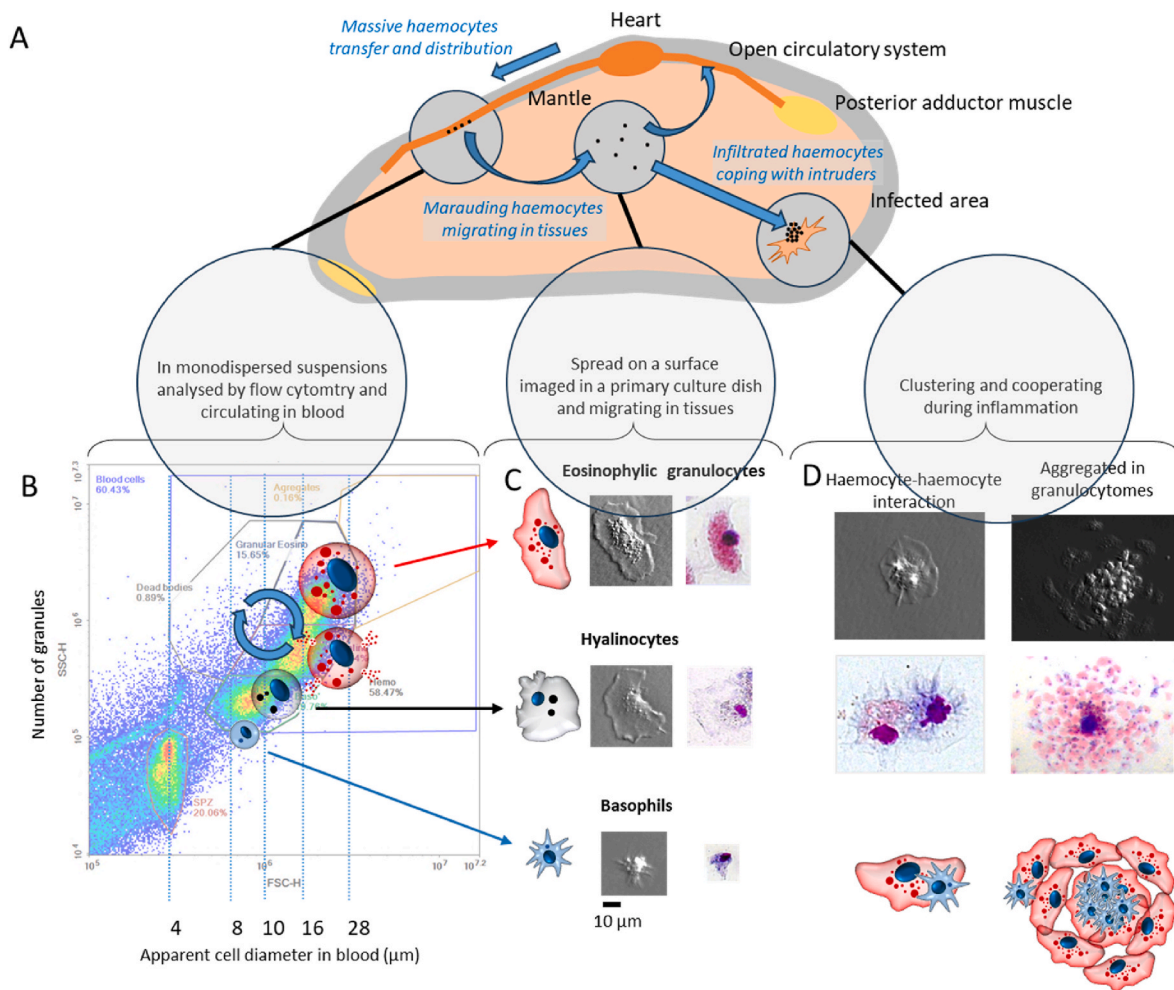


Fig. 1. Haemocyte morphology is linked to tissue interaction and activity. (A) In mussels, haemocytes are found as formed elements in the semi-open circulatory system, as marauding isolated cells in tissues and as infiltrating clusters in infected areas. (B) In suspension in haemolymph -as well as monodispersed cells in a flow cytometry sample-haemocytes are round particles with cell diameters between 8 and 15 μm (see also Le Foll et al., 2010 for electronic volume determination). Since immunophenotyping is not available, subpopulations are distinguished according to size and granule content. Rebelo et al. (2017) suggested that granular cells depopulate and repopulate according to degranulation/granule formation events. (C) Within tissues -as well as on a culture dish surface-most of haemocytes are spread motile cells incorporating large vacuoles similar to phagolysosomes and containing (eosinophilic granulocytes) or not (hyalinocytes) granules according to hemacolor staining. Small basophilic cells display a condensed body with filopodia. (D) A key feature of inflamed tissues is massive infiltration by haemocyte cohorts. While surrounding injured areas haemocytes form cell aggregates of different sizes. (with permission of Rebelo et al., 2017 and Le Foll et al., 2010).

refers to “a lamellated capsule of immunocytes merging into a center of essentially degenerating immunocytes, foreign particles and other debris” [36,37] and is normally associated with the aggregation of haemocytes following bacterial challenge [38]. When particles are too large to be phagocytosed, haemocytes resort to encapsulation. The immunocyte capsule contains the foreign body and cytotoxic products, such as degradation enzymes and free radicals, which are released to eliminate the invader [36,39,40].

Similar patterns of inflammation have been demonstrated in wild and cultivated *Mytilus* sp. (Fig. 2) and in certain cases, have been associated with the presence of pathogens and pollutants. Indeed, a histological study showed inflammatory responses of the infiltrating, nodular and encapsulating type in *M. platensis* and *M. chilensis* mussels linked to the prevalence of metazoans [41]. Similarly, in *M. galloprovincialis*, studies reported haemocytic encapsulation of several parasites such as turbellaria, trematodes and microsporidia [42–44] and haemocyte aggregates surrounding the dinoflagellate *Ostreopsis* cf. *ovata* [45]. On the other hand, *M. edulis* infected with the bacteria *Francisella haliotidica* showed large granulocytomas and haemocytic infiltration [46]. Furthermore, focal and multifocal inflammation were observed in *M. edulis* originating from highly polluted sites [47,48] or after exposure

to microplastics [49] and in *M. galloprovincialis* exposed to pesticides and pharmaceuticals and preservatives [50–53].

The aggregation of haemocytes appears to be reversible, as most of the regrouped cells subsequently disperse and reintegrate the circulatory system during repair of the wound [54]. This mechanism can also be observed *in vitro*, with haemocytes aggregating immediately after extraction, then gradually scattering once spread out on a substrate [22, 55,56] (Fig. 3). Often the use of an anti-aggregate solution such as Alsever’s solution or keeping the cells on ice prevents the formation of aggregates [12,57]. In addition to mechanical action and temperature, haemocytes aggregation can be modulated *in vitro* by chemicals. For example, exposure of *M. californiacus* haemocytes to caffeine or EDTA inhibited cell clumping and the process was reversible [55]. In contrast, the incubation of *M. edulis* haemocytes with the opioid DAMA induced the formation of aggregates of different sizes and the authors reported that larger cell accumulations had a trend towards attracting more wandering cells than smaller ones [58]. According to Le Foll et al. (2010), the centre of haemocyte aggregates is formed by basophils which interact with each other and with the other sub-populations surrounding it (Fig. 3). Notwithstanding these findings, immunocyte agglutination has been the subject of little research and the mechanisms

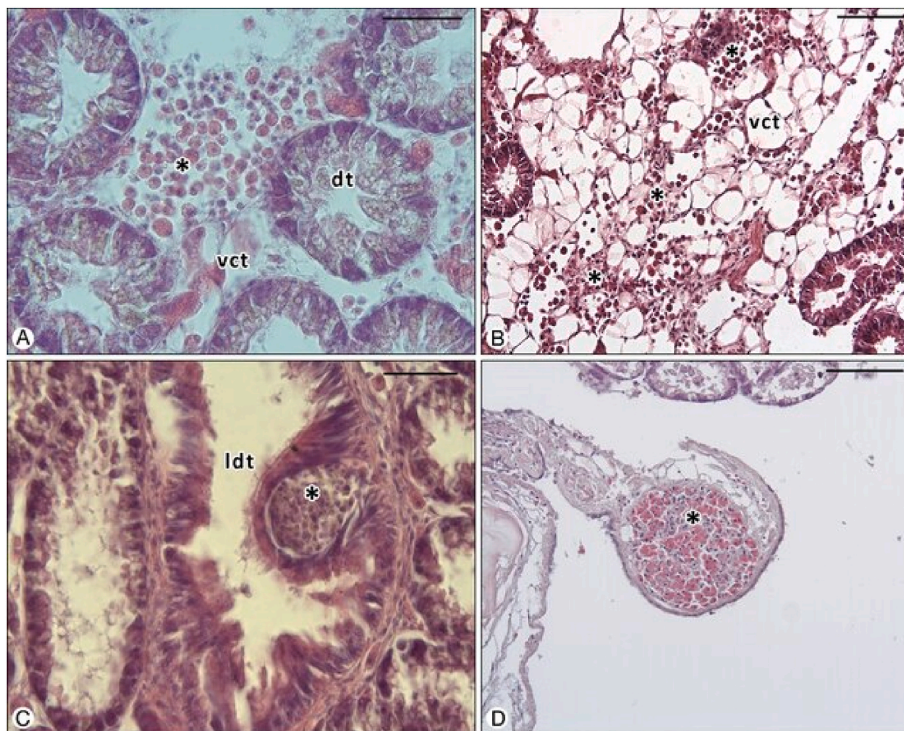


Fig. 2. Histological sections of *Mytilus* spp. showing inflammatory lesions. (A) Focal inflammation (*) in the vesicular connective tissue (vct) of the digestive gland; dt: digestive tubule. (B) Diffuse inflammation throughout the vct of the digestive gland. Note the multiple foci of hemocytic infiltration (*). (C) Intraepithelial 'abscess' (*) in the secondary digestive gland tubule with hemocytic aggregations with melanized appearance; ldt: lumen of digestive tubule. (D) Nodule in the form of a 'granulocytoma' (*) within connective tissue of the mantle. Note both eosinophilic granulocytes and hemocytes with no granules. Scale bars = (A,C) 50 μm , (B,D) 100 μm [41].

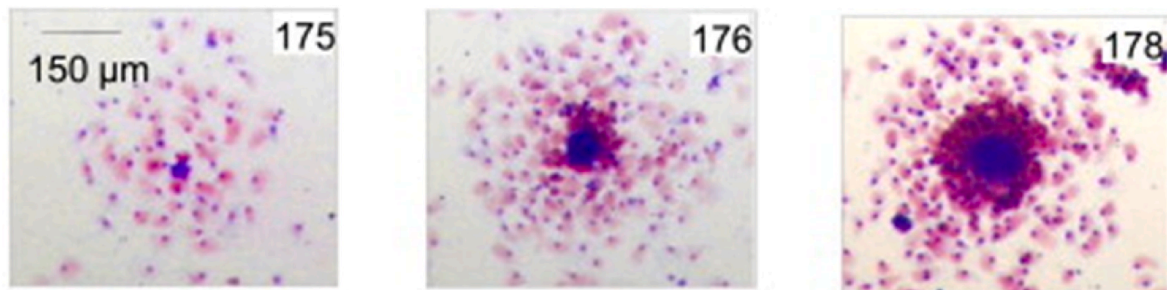


Fig. 3. Haemocyte aggregates in *Mytilus edulis*. Pictures 175, 176 & 178 show three aggregates of different sizes obtained by vigorous pipetting before plating and observed under identical magnification [22].

governing cell-cell interactions are unclear. Some studies have correlated nodule formation in insects with Toll signalling systems and eicosanoid biosynthesis [59]. Other investigations have suggested that in the snail *Pomacea canaliculata*, calmodulin and protein kinase-C might contribute to calcium-mediated haemocyte aggregation [60].

4. Haemocyte movement can be evaluated by cell velocity

As in vertebrates, different categories of cell movement can be distinguished in invertebrates: directional haemocyte migration and non-directional haemocyte motility. Cell migration refers to the general processes that involve the movement of a cell in response to an attractor or repellent whereas cell motility is the ability of a cell to move autonomously and randomly, which occurs in cell cultures [61]. In a series of pioneering works published between 1972 and 1976, the movement of haemocytes was studied for the first time in a marine mollusc through a description of the morphology, spread, motion and aggregation capacity of haemocytes in the limpet *Patella vulgata* [62–65]. Using time-lapse

cinemicrography and photomicrography techniques, the authors observed cell movements and measured the speed of different parts of haemocytes (nucleoli, spikes and lamella margin). Haemocyte displacement was then reported in several bivalves including oysters, clams and mussels in the presence of several compounds including live bacteria, their extracts or synthetic peptides [66–68]. When deposited on a substrate, the mussel haemocytes flattened out, formed pseudopodia and then moves in amoeboid movements [58]. The opioid DAMA stimulated directed migration and clustering of immunocytes to the injection site and enhanced the locomotory activity when in direct contact with the cells [58,69]. Similarly, changes in haemocyte shape and chemokinetic responses have been described in contact with lipopolysaccharides [70,71].

Most of these studies were based on microscopic observations demonstrating the movement of haemocytes and lacked quantitative data. Decades later, the approach to measuring haemocyte velocity was taken up by Rioult et al. (2014) with several technical improvements. Using an automated system for tracking nuclei by videomicroscopy, the

authors followed the trajectories of haemocyte nuclei and measured their travel speeds by using an automated nuclei tracking via video-microscopy (Fig. 4) [13]. In addition to the ease of use and practicality of automation, this method overcomes the difficulty of tracking haemocytes in the open field. Indeed, haemocytes deform to move and interact with each other, making them difficult to track. Despite its advantages, the technique is limited to the determination of an average velocity of the haemocyte population without taking into account the different sub-populations. In addition, it is restricted to use exclusively *in vitro* and not *in vivo*.

5. Haemocyte velocity is modulated by abiotic and biotic factors

As filter-feeders, mussels are exposed to a variety of microorganisms and pollutants present in the surrounding water, which can have a deleterious impact on their physiology and in particular their immune defences. In the context of ecotoxicological or pathophysiological studies, some researchers have been concerned with the modulation of haemocyte velocity by various biotic and abiotic factors (Table 2). Cell motility can be enhanced or inhibited by bacteria in accordance with their degree of virulence. Incubation of *M. edulis* haemocytes with a non-virulent strain of *Vibrio splendidus* accelerated their displacement contrary to the pathogenic *V. splendidus* strain, which induced a slowing down of the cells [72]. Immobilisation of haemocytes in the extrapallial fluid has been reported in clams infected with *V. tapetis*, reducing immunocyte adhesion and transfer to haemolymph or tissues [73,74]. Similar changes in haemocyte speed were observed in response to different categories of pollutants. In the Mediterranean mussel, paracetamol stimulated haemocyte movement in a dose-dependent manner, while nanoplastics modulated the speed according to their size [75,76]. In contrast, in the blue mussel, the plasticizer Bisphenol A, the steroid oestradiol, the heavy metal cation Cu^{2+} and the drug Caffeine had a decelerating effect on haemocyte motility, and the inhibition was even more accentuated in combination with the non-virulent bacteria *V. splendidus* [77]. Likewise, Rioult et al. (2014) reported the acceleration of haemocyte speed by the rise in temperature and a lower haemocyte velocity in mussels sampled from polluted site compared to animals sampled from the control site [78].

The study of the modulation of haemocyte velocity in *Mytilus* sp. has so far been restricted to non-directed cell movements. Furthermore, the mechanisms involved in the responses observed remain unknown, although some authors have associated the speed modulations with impairment of other immune parameters such as ROS production [72, 75].

6. Future challenges in the study of haemocyte movement

Studies of directional or non-directional haemocyte movement in mussels have focused mainly on *M. edulis* and *M. galloprovincialis*, with little or no research on other *Mytilus* sp. such as *M. californiacus*, *M. coruscus* or *M. chilensis*. Measuring cell speed via nuclei tracking have expanded the knowledge of haemocyte motility by showing the effect of different stresses on their locomotion. While useful, these studies are limited to haemocyte motility *in vitro* and do not reflect cell migration in tissues. *In vivo*, immunocytes encounter a variety of environments with different physical and chemical characteristics that can influence their migration. The protein composition of the extracellular matrix (ECM), the stiffness, porosity and geometry of the tissue and the presence of chemotactic cytokines can shape the direction and speed of cell movement [2,80,81]. Thus, to fully understand the behaviour of haemocytes in mussel tissues, we need to take in account their natural environment. In this respect, experiments have been carried out in vertebrates to visualise the migration of immune cells in a living animal or in an externalised tissue or organ using intravital microscopy [82–85]. In most of cases, these trials required the sacrifice of animals and the results are still difficult to interpret [2]. To overcome these issues, various microfabrication techniques have been developed to design *in vitro* experiments that recreate the ECM with which cells may be confronted in their native environment [2]. Combining these new tools with the measurement of movement speed could provide an opportunity to study the impact of different stresses on haemocyte migration under realistic conditions. In addition, it is important to consider the role of haemocyte sub-populations in grouped cell migration and cell-cell interactions.

The mechanisms governing the migration and motility of haemocytes in mussels are another major challenge. Although the earliest works describing cell migration in mussels and other bivalves date far back, no studies have been carried out on the signalling effectors controlling this phenomenon. Some data have been obtained in a few invertebrate models concerning, among other things, the involvement of the calcium signal. Calcium signalling appears to be essential in the migration of neutrophils and eosinophils by mediating the structural and functional modifications required for cell movement [86,87]. In *Drosophila* haemocytes, calcium bursts allow a rapid reorganization of the actin cytoskeleton through the calcium (Ca^{2+})-regulated cross-linking protein EFhD2/Swip-1 [88]. Authors showed that this protein is required for epithelial wound closure *in vivo*. Likewise, in the gastropod *Biomphalaria glabrata*, calcium dynamics were associated to haemocyte morphological changes [89]. Given the similarities between the mechanisms of action in the different species, the calcium signal may be one of the pathways to be considered in understanding the regulation of haemocyte migration in *Mytilus* sp.

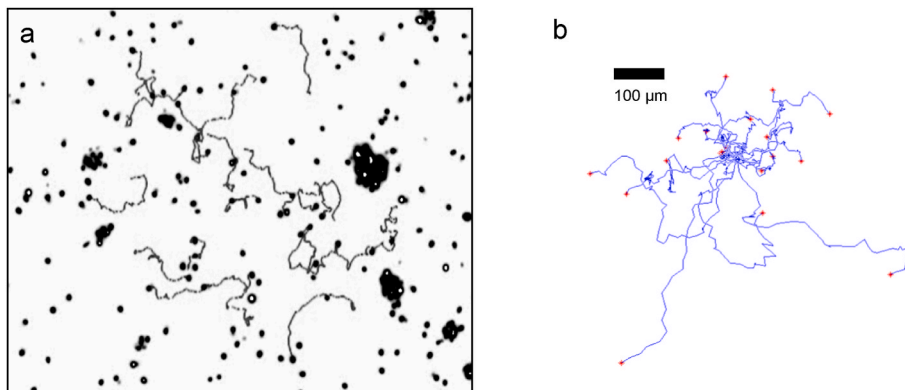


Fig. 4. *M. edulis* haemocytes tracking by time lapse imaging of Hoechst 3342-stained nuclei. a) twenty haemocytes tracks computed from the time-lapse sequence, b) overlay of twenty haemocytes trajectories displayed from a common origin. The point that each cell reached after 30 min of recording is indicated by a small cross [13].

Table 2
Modulation of *Mytilus* sp. haemocyte velocity by culture conditions, pollutants and bacteria.

Species	Stress and exposure conditions	Basal velocity	Velocity after stimulation	References
<i>M. edulis</i>	Increase in haemocytes culture time, 30 min of recording at 15 °C	2.78 ± 0.12 µm/min	4.56 ± 0.17 µm/min after 24h Fastest cells reached 7 µm/min	[79]
<i>M. edulis</i>	Increase in haemocytes culture temperature, 30 min of recording	1.5 µm/min at 14 °C	2.4 µm/min at 18 °C 3 µm/min at 22 °C	[13]
<i>M. edulis</i>	Mussels from 2 sites with contrasting pollution, 30 min of recording at 14 °C	2.72 µm/min in the control site (Yport)	1.89 µm/min in the polluted site (Le Havre)	[13]
<i>M. edulis</i>	Exposure of haemocytes to 2 strains of <i>V. splendidus</i> with contrasted virulence for 2h at 15 °C	Stable around 1 µm/min	Virulent bacteria: transient acceleration and decrease to around 1.5 µm/min after 2h, Avirulent bacteria: around 5 µm/min after 2h	[72]
<i>M. edulis</i>	Exposure of haemocytes to bisphenol BPA (50 mg/L), β-oestradiol E2 (10 mg/L), CuCl ₂ (100 mg/L), caffeine (20 mg/L) for 2h at 15 °C	2.32 ± 1.57 µm/min	1.86 ± 0.2 µm/min with BPA 1.78 ± 0.2 µm/min with E2 1.68 ± 0.2 µm/min with CuCl ₂ 2.08 ± 0.42 µm/min with caffeine	[77]
<i>M. edulis</i>	Exposure of haemocytes to an innocuous strain of <i>V. splendidus</i> for 2h at 15 °C	2.32 ± 1.57 µm/min	Significant increase the first hour, with a maximum velocity of 2.38 ± 0.15 µm/min	[77]
<i>M. edulis</i>	Combined exposure of haemocytes to chemicals (BPA 50 mg/L, E2 10 mg/L, CuCl ₂ 100 mg/L, caffeine 20 mg/L) and <i>V. splendidus</i> for 2h at 15 °C	2.32 ± 1.57 µm/min	BPA + bacteria exposure: 1.53 ± 0.16 µm/min E2+ bacteria: 2.08 ± 0.3 µm/min CuCl ₂ + bacteria: 2.09 ± 0.19 µm/min Caffeine + bacteria: 1.43 ± 0.16 µm/min	[77]
<i>M. galloprovincialis</i>	Exposure of haemocytes to polystyrene nanoplastics for 3h	2.3 ± 1.5 µm/min	5.6 ± 1.5 µm/min with 50 nm nanoplastics 1.82 ± 1.5 µm/min with 100 nm nanoplastics 0.4 ± 0.2 µm/min with 1 µm nanoplastics	[75]
<i>M. galloprovincialis</i>	Exposure of haemocytes to paracetamol	After 1h: 5.7 µm/min	Increase in velocity for 0.2 and 0.02 mg/L	[76]

Table 2 (continued)

Species	Stress and exposure conditions	Basal velocity	Velocity after stimulation	References
	for 1h or 24h incubation, 10 min of recording	After 24h: 8.2 µm/min	after 1h Slight reduction in speed for all concentrations after 24h	

7. Conclusion

Motility of haemocytes is central to immune defence of mussels. For and by moving across tissues, these cells use paracrine (and maybe juxtacrine) communication, migrate towards sites of infection, set up the inflammatory response and eliminate pathogens. If any of these stages fail, survival of individuals is jeopardised. Mussels live in environments exposed to various types of biotic and abiotic stressors, some of them being able to affect physiological functions, including haemocyte motility. The study of haemocyte movements in *Mytilus* sp. has evolved recently with the development of automated tracking tools. These advances have highlighted the effect of chemical, physical and biological factors on haemocyte velocity, particularly in *M. edulis* and *M. galloprovincialis*. Taken together, these findings point to the potential of using haemocyte migration quantification as an indicator of the state of inflammation of organisms under stress. Future better characterisation of basal or stimulated levels of haemocyte velocity, reflecting normal or inflammatory state respectively, may enable us to follow disease processes and to determine immune status of mussels.

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Data availability

No data was used for the research described in the article.

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