



Exploring farmers' knowledge to trace Non-Native Species in aquaculture

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ABSTRACT

Aquaculture is a major vector for the introduction and spread of Non-Native Species (NNS), with negative ecological and economic implications. Costs generated by biofouling, including that of NNS, in bivalve aquaculture, can represent even 20–30 % of production costs. We used Local Ecological Knowledge (LEK) to assess farmers' awareness of NNS and pest species, as well as their observations of changes in fauna associated with cultivated Mediterranean mussels (*Mytilus galloprovincialis*) and Pacific oysters (*Magallana gigas*) in the Adriatic Sea, through questionnaires. While most farmers claimed to understand the concept of NNS and reported their presence in farms, only a few could accurately identify a limited number of these species. Farmers observed increases in flatworms, ascidians, barnacles, hydrozoans and spionid polychaete *Polydora*, which they believe negatively affect mollusks and reduce their marketability. They also reported translocation practices involving seed and adult mollusks between Italy, Greece, France, and Spain, both within and beyond the Mediterranean basin. By combining farmer observations on the abundance of NNS and pests, with their reports of translocation practices, our results suggest that these activities facilitate the introduction and spread of NNS and pests. This highlights that LEK is a valuable tool for identifying challenges related to NNS management in aquaculture. We recommend implementing training programs to improve farmers' capacity to recognize NNS and contribute to their early detection. Furthermore, cross-border collaboration and partnerships among scientists, policymakers, and farmers are crucial for managing the spread of NNS through aquaculture in the Mediterranean.

1. Introduction

With rising global demand for aquatic products, aquaculture is one of the most important and rapidly growing sectors within the fisheries industry and it has increasing importance for several countries (Carvalho and Guillen, 2021). Within the European Union (EU), bivalve mollusks constitute 46.6 % of the total aquaculture production, while in the Mediterranean region they add up to 16 % (Mosca et al., 2021). Among mollusks, the Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) is the main species, with 99,200 tons produced in the Mediterranean in 2019. Italy and Greece are the leading producers, contributing 62 % and 24 % of the regional mussel production,

respectively (Carvalho and Guillen, 2021; Basurco and Lovatelli, 2003). In Italy, mollusks aquaculture is particularly well developed, with production mainly focused on Mediterranean mussels and Manila clams (*Ruditapes philippinarum* Adams and Reeve, 1850), along with limited quantities of common clams (*Ruditapes decussatus* Linnaeus, 1758), Pacific oysters (*Magallana gigas* Thunberg, 1793) and European oysters (*Ostrea edulis* Linnaeus, 1758) (Prioli, 2008; MiPAAF, 2016). The Adriatic Sea, especially its northern part, is one of the main centers for farming of these species (Graham et al., 2020). Production of Pacific oysters is on the rise, driven by growing interest among mollusk farmers, which stems from both successful experiences in various Italian regions and from the oyster production crisis in France caused by health-related

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issues (Tamburini et al., 2020).

Despite its economic benefits, mollusks aquaculture is also a major vector for the introduction of Non-Native Species (NNS) into the Mediterranean Sea (Katsanevakis et al., 2013, 2014). Several aspects of aquaculture practices can facilitate the arrival, settlement, and spread of NNS within farming environments. These include the type and material of rearing structures, which influence the attachment and persistence of fouling organisms; the management of cleaning operations, which can affect the accumulation or dispersal of such species; and the transport of cultured stock between farming areas (Fitridge et al., 2012; Bannister et al., 2019; Cottier-Cook et al., 2019). Among these potential pathways, one of the primary pathways is linked to the movement of cultured stocks between farming areas (Tan et al., 2023). In mollusks aquaculture, these transfers typically involve the exchange of “seed”, juvenile mollusks used to replenish production structures, as well as the movement of adults for re-immersion, a short-term practice in which market-sized mussels or oysters are purchased from other farms and temporarily held in sea in local facilities before sale. Seed can be collected locally or sourced externally, depending on natural recruitment and environmental conditions. During productive years, farms may have surplus seed to sell, whereas in years of low recruitment, they may need to purchase it from other producers or hatcheries. These movements, which can occur both within and across national borders, create opportunities for fouling organisms, parasites, and pathogens to be unintentionally translocated along with the cultured stock (Cottier-Cook et al., 2019; Di Blasio et al., 2023). The term biofouling in aquaculture refers to the unwanted colonization of farming infrastructures and cultured organisms by a wide range of sessile marine species, including both native and non-native taxa. In bivalve farming, fouling communities typically develop on rearing structures such as ropes, nets, and longlines, as well as directly on the shells of the farmed species (Bannister et al., 2019). When established on shells, fouling organisms can interfere with growth, feeding, and overall condition, ultimately reducing the market value of the product. The presence of NNS among fouling taxa can exacerbate these impacts, further compromising product quality and contributing to increased management costs (Bannister et al., 2019). Costs provoked by biofouling have been estimated to represent overall 20–30 % of production costs, but vary according to the commercial species or the geographical location (Lacoste et al., 2015). The extent of impacts provoked by NNS vary depending on the type of farming system used, as different cultivation methods (e.g., long-line, bottom culture, lantern nets) provide distinct substrates that may facilitate the settlement and proliferation of different fouling organisms (Fitridge et al., 2012). Despite these challenges, research on the fauna associated with mussel and oyster farms in the Mediterranean is very limited and outdated (e.g. Agius et al., 1977; Igić, 1984; Perera et al., 1990; Gargouri Ben Abdallah et al., 2012), while most studies focus on wild mussel beds (e.g., Tsuchiya and Bellan-Santini, 1989; Chintiroglou et al., 2004; Çınar et al., 2008, 2020; Curini and Galleni, 1981).

Tracking the presence and spread of NNS in aquaculture facilities is challenging. Traditional monitoring techniques often face limitations such as delayed detection, limited temporal and spatial coverage, and high costs of systematic sampling (Hulme, 2006; Olenin et al., 2011). As a result, alternative approaches are gaining increasing attention. Over the past two decades, an innovative method has emerged, leveraging the knowledge of local communities who maintain a close relationship with nature. This method, known as Local Ecological Knowledge (LEK), has been attracting growing interest from researchers and policymakers, particularly for its role in assessing, monitoring, and managing natural resources (Azzurro, 2018; Cerri et al., 2020; Tiralongo et al., 2019). Compared to other socio-ecological approaches, such as the Knowledge, Attitude and Practices (KAP) framework, LEK focuses more specifically on the ecological dimension of local experience, rather than on behavioral or attitudinal aspects. Accessing the knowledge of local communities includes procedures such as semi-structured interviews, guided by

a set of both closed and open-ended questions that allow flexibility to explore new topics emerging during the conversation, group discussions, participatory mapping, and diagramming techniques (Azzurro and Bariche, M. 2017; Azzurro, 2018; Silvertown, 2009). In the Mediterranean Sea, the ecological knowledge of fisherman has recently been employed to track the geographical expansion of both native and exotic fish species, and its use is considered particularly useful in coastal ecosystems with limited data (Boughedir et al., 2015; Azzurro et al., 2019; Maggio et al., 2022). However, so far, LEK has not yet been used to assess NNS and pest species in mollusks aquaculture.

This study intends to evaluate the knowledge of mussel and oyster farmers along the Adriatic coast of Italy about the presence of NNS and pests in mollusks aquaculture. Based on data collected through a semi-structured questionnaire we pursued four main objectives: 1) to assess whether there are trade-related translocations of seed and adult mussels and oysters, that might contribute to the spread of NNS and pests in aquaculture; 2) to evaluate whether farmers' knowledge is sufficiently detailed and reliable to support their involvement in monitoring and management strategies for the containment of these species in aquaculture; 3) to gain information on NNS and pests that have arrived recently, increased their abundance, or caused problems in mollusks farms and 4) to assess the extent to which farmers' observations and perceptions align with current scientific knowledge and documented patterns in the literature. By addressing these aims, this study contributes to exploring the potential role of Local Ecological Knowledge (LEK) in enhancing early detection and management of non-native and pest species, thereby supporting more sustainable aquaculture practices.

2. Materials and methods

2.1. Survey implementation

The survey included one respondent (farmer) per farm, for a total of 35 farmers from 24 Mediterranean mussel (*M. galloprovincialis*) and 11 Pacific oyster (*M. gigas*) farms, of which 5 cultivated both species, located across seven Italian Adriatic coastal regions (Fig. 1). Mussel farms were distributed in Friuli-Venezia Giulia (1), Veneto (4), Emilia-Romagna (4), Marche (4), Abruzzo (6), Molise (1), and Puglia (4). Oyster farms were in Friuli-Venezia Giulia (1), Veneto (2), Emilia-Romagna (5), Marche (1), Abruzzo (1), and Puglia (1). Notably, one oyster facility was not a traditional farm but operated only as a hatchery. Material and human resources available for this study did not permit exhaustive research that would include all mussel and oyster farms along Italian Adriatic coast. Thus, mussel and oyster farms were selected in a way to cover as much as possible the whole study area (Italian Adriatic coast). Farms were selected through a convenience sampling approach, based on the experience and acquaintances of the authors G. Prioli and E. Fossi, affiliated with Cooperativa M.A.R.E., who maintain long-term collaboration with mollusk producers along the Adriatic coast. Each farmer was contacted individually by phone to explain the purpose of the study and invite to participate. None of the contacted farmers declined to participate. All interviews were conducted with farm owners, which are directly involved both in farm management and daily operations, including field work and harvesting. Interviewing farm owners was considered the most appropriate strategy, as they supervise and integrate the experience of all employees, thus providing the most comprehensive and aggregated ecological knowledge available for each farm. To protect farmers' privacy, specific farm locations are not reported.

Data collection took place between April and June 2024, through phone interviews. Considering limited financial resources, this method was chosen to minimize travel costs and time constraints. To reduce interviewer bias and ensure consistency, the same researcher conducted all interviews, strictly following stabilized question order. A semi-structured questionnaire was administered to mussel and oyster farmers (Supplementary material SM1). This type of questionnaire

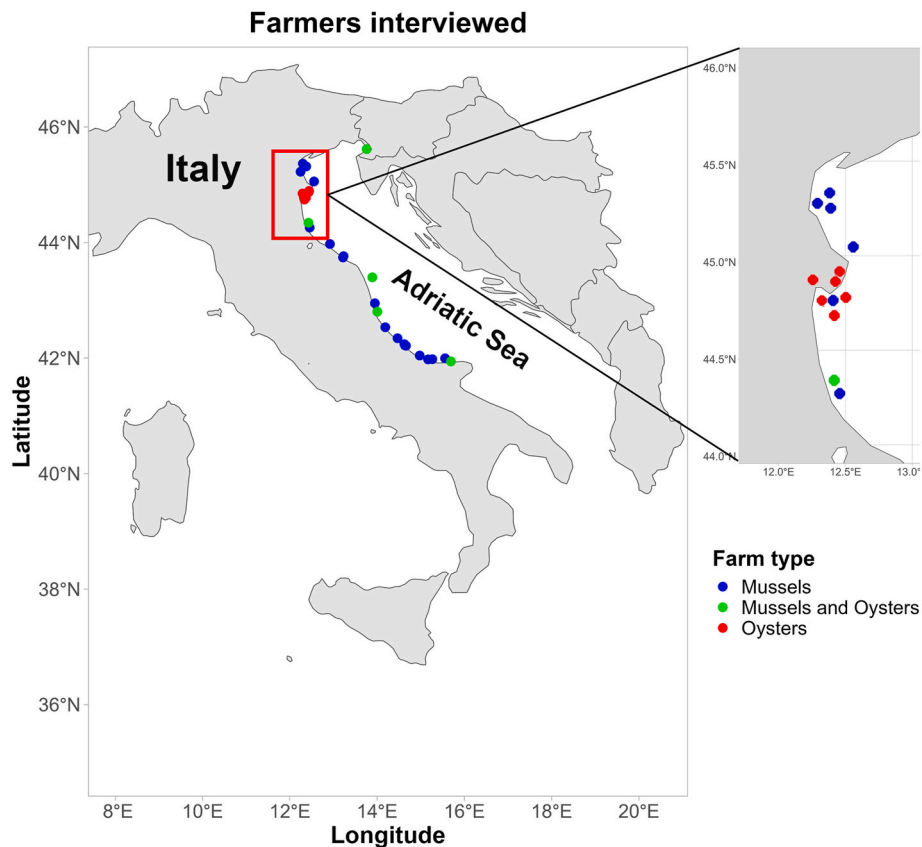


Fig. 1. Geographic locations of oyster and mussel farms in the Adriatic Sea, interviewed between April and June 2024.

includes a combination of closed-ended questions, which allow for quantitative analysis, and open-ended questions, which enable respondents to deepen information provided in closed questions by adding new details based on their experience. Qualitative answers were reviewed and thematically grouped to underline recurring patterns, which were then integrated with quantitative results to support interpretation. Participants were assured of the anonymity of their responses, and any answers that breached privacy were neither analyzed nor disclosed. The questionnaire consisted of 39 questions, divided into two main sections. The first section gathered general information on farms characteristics and farming practices intended as movements of mussel and oyster stocks between farms. Information on farm characteristics were collected to obtain context on farmed species, farming techniques that might influence the presence of NNS and pests and to explore whether farmers' knowledge varies with years of their working experience. Questions on stock movements were specifically designed to address the first objective of the study, that is: are there trade-related translocations of seed and adult mussels and oysters, that may contribute to the spread of NNS and pests in aquaculture?. The second section of the questionnaire was oriented to evaluate whether farmers' knowledge is sufficiently detailed and reliable to support their involvement in monitoring and management strategies and to gain information on NNS and pests that have arrived recently, increased their abundance, or caused problems in mollusks farms. Finally, we confronted questionnaire results with insights on the fauna associated with cultured mussels and oysters in the Mediterranean Sea gathered from the most relevant publications, to assess whether farmers' observations and perceptions align with present knowledge and trends.

One respondent, previously described as operating a hatchery rather than a farm, answered only questions applicable to that context, mainly those included in the first section. Although we did not enquire about farmers' educational level, we know from personal experience that two

respondents were biologists.

To aid species identification, farmers were provided, via text messages or emails, with a document containing images and descriptions of the most frequently known species associated with farmed mollusks, without indicating their Latin or common names (Fig. 2).

During interviews, farmers were free to use their own photographs and short videos to substantiate their answers, without any guidance from the interviewer (Fig. 3). Subsequently, the research team used its taxonomic expertise to identify some of the organisms shown in the photos and videos provided by farmers, based solely on a visual assessment of these materials. These identifications served only to contextualize farmers' observations in the discussion and in the table of impacts; they were not used to modify their original answers.

2.2. Data analysis

The responses were organized using Microsoft Excel program. Data from closed-ended questions were analyzed descriptively by counting and calculating the percentage of responses for each answer category. To explore whether awareness of the knowledge of farmers on what NNS are, varied with their work experience, an exploratory analysis was conducted using Fisher's exact tests with RStudio employing the packages `dplyr`, `tidyr` and `ggplot2`. Respondents were grouped into four experience classes (0–5 years, 6–15 years, 16–25 years, and >25 years). The proportion of farmers reporting positively that they do know what NNS are, was calculated for each class together with 95 % binomial confidence intervals. A Cochran–Armitage trend test was also applied to evaluate potential monotonic patterns. For oyster farmers, experience classes were unequal in size; proportions and 95 % binomial confidence intervals were therefore reported by class, and Fisher's exact test was used to account for small and zero cell counts. To create maps of mollusks movements we used several packages in RStudio, including



Fig. 2. Examples of identification materials provided to farmers to assist with the recognition of common fouling and pest species associated with mussel and oyster farms. (A) *Clavelina oblonga* (Asciacea) (photo found in [Ordóñez et al., 2016](#)); (B) *Styela plicata* (Asciacea); (C) Serpulid polychaetes (Serpulidae); (D) *Amphibalanus eburneus* (Cirripedia) (photo of Peter J. Bryant).



Fig. 3. Visual materials provided by farmers to document fouling and pest species observed in their facilities. These images were used to support taxonomic identification. (A–B) *Clavelina oblonga* (Asciacea); (C) Hydrozoan colonies; (D) Cirripedia (Arthropoda); (E) Polyclad flatworm preying on a mussel.

ggplot2 (Wickham, 2016) for data visualization, sf (Pebesma, 2018) for handling spatial data, dplyr (Wickham et al., 2023) for data manipulation, readxl for reading Excel files (Wickham and Bryan, 2023), rnaturalearth for basemap acquisition (South, 2017), and ggspatial for adding cartographic elements to maps (Dunnington, 2022). Information on movements of mussels and oysters between farms was grouped into Excel spreadsheet. Geographic coordinates of the source and destination areas were approximately retrieved from Google maps, and the number of farmers reporting that specific transfer was noted. These data were used to visualize directional connection patterns among farms. The network representation was purely visual and descriptive.

Qualitative responses from open-ended questions were grouped thematically to identify recurring observations and perceptions among farmers.

To describe changes in the abundance of associated organisms over

the past decade, we relied exclusively on farmers' own knowledge and observations. Respondents indicated which taxa increased in abundance in their farms. The resulting counts were summarized across regions and visualized using stacked bar charts (separately for mussels and oysters) in RStudio using the packages ggplot2 for plotting and dplyr for data manipulation.

Photographs provided by farmers, as well as several reference images shown to farmers by the interviewer, were used only in a later step to confirm the identification of some taxa. These photographs were not used to generate information for the histograms but served to contextualize and interpret certain organisms elaborated in Results and Discussion sections.

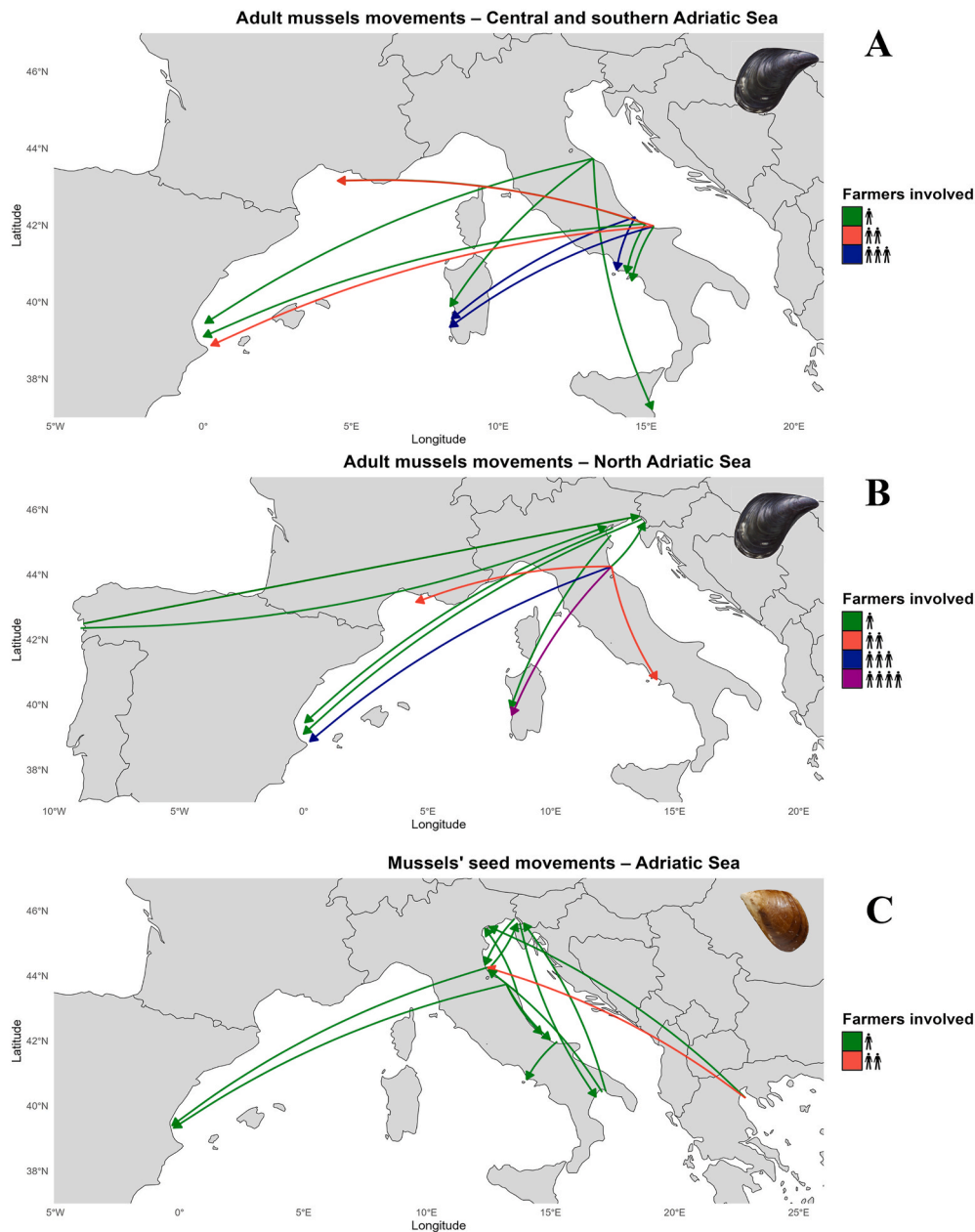


Fig. 4. Network analysis of the movements of mussels (*Mytilus galloprovincialis*) adults and seeds between the Italian farms in the Adriatic Sea and other farms: (A) Movements of adult mussels involving farms located in the northern Adriatic regions; (B) Movements of adult mussels involving farms located in the central and southern Adriatic regions; (C) Movements of mussel seeds involving farms located in the Italian Adriatic regions. Arrow colors represent the number of farmers who reported each movement; in the legend, this number is shown by the corresponding “person symbols” placed next to each color.

3. Results

3.1. Duration of aquaculture activity and farming methods

The oldest and the youngest mussel farms included in the survey were established in 1984 and 2019 respectively. For oyster farming, the

oldest farm started in 1985 and the recent most in 2020. As of 2024, one oyster farm has been active for less than five years (2020–2024), eight mussel and four oyster farms have been operating from 6 to 15 years (2010–2019), eight mussel and two oyster farms have been active from 16 to 25 years (2000–2009), while eight mussels and four oysters farms have been operating for more than 25 years (Table SM1).

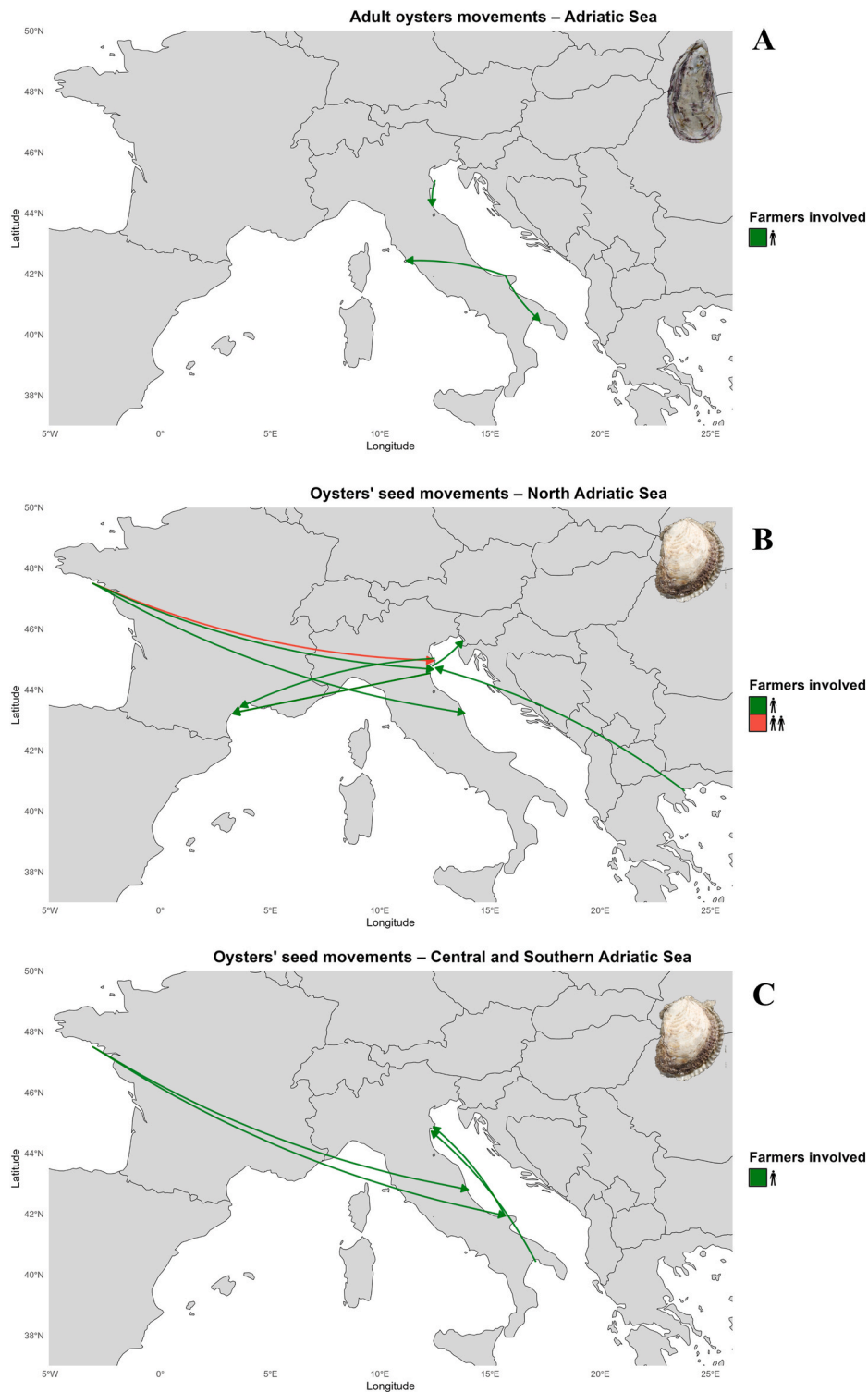


Fig. 5. Network analysis of the movement of oysters (*Magallana gigas*) adults and seeds between the Italian farms in the Adriatic Sea and other farms: (A) Movements of adult oysters involving farms located in the Italian Adriatic regions; (B) Movements of seeds involving farms located in the north Italian Adriatic regions; (C) Movements of seeds involving farms located in the central and south Italian Adriatic regions. Arrows indicate the direction of movement. Arrow colors represent the number of farmers who reported each movement, in the legend, this number is shown by the corresponding “person symbols” placed next to each color.

Three mussel farmers reported that they have been previously cultivating other species, precisely the European flat oyster *O. edulis*. Two oyster farmers had experience with other species in the past, one with *M. galloprovincialis* and one with *O. edulis*. Our careful analyses of responses provided by these farmers, did not indicate that they differed from others in terms of awareness regarding NNS or taxonomic identification capacity (Table SM1).

Concerning farming methods, mussel cultivation predominantly relied on the long-line system (23 farms), while only one farm used fixed poles. Oyster farming was primarily conducted using two types of lantern systems: rigid plastic lanterns (used by 3 farms) and mesh net lanterns (5 farms). Other techniques included the use of bottom substrates (1 farm) and suspended ropes (1 farm) (Table SM1).

3.2. Movements of seeds and adults

Seventeen mussel farms sell adult mussels for re-immersion purposes, primarily to Italy (13 reports, of which only one within the Adriatic Sea), Mediterranean coast of Spain (9), and Mediterranean coast of France (6) (Fig. 4A–B). On the other hand, only two mussel farms purchase adult mussels for re-immersion, and they source them from the northern Atlantic coast of Spain (Fig. 4A–B). Since some farms trade with other farms from multiple areas and countries, the total number of reports exceeds the number of farms. Most mussel farms (17) produce their own seeds, while 7 rely both on external suppliers and self-produced seeds. Purchased mussel seeds originate primarily from Italy (7 reports), followed by Greece (3) (Fig. 4C). On the other hand, 5 farms sell seeds, mostly within Italy (5 reports) and to the Mediterranean coast of Spain (2) (Table SM1).

Only two oyster farms sell adult oysters, exclusively to Italy (3 reports, of which one within the Adriatic Sea), while none of the oyster farms purchases adult oysters (Fig. 5A). Three oyster farms sell seeds to farms at the Mediterranean coast of France (2) and Italy (1) (Fig. 5B–C). On the other hand, oyster farms rely more on the import of seeds: 8 farms exclusively purchase seeds, while 3 combine internal seeds production with external supply. The main suppliers are farms from the Atlantic coast of France (6 farms), Italy (2), and Greece (1) (Fig. 5B–C) (Table SM1).

3.3. Changes in abundance of associated fauna and knowledge on NNS species

Farmers reported changes in the associated fauna over the last 10 years of their activity, with 14 mussel and 5 oyster farms observing an increase in the abundance of some taxa. The increases showed notable regional variations (Figs. 6 and 7). Among mussel farms, the highest number of these reports came from Puglia (15) and Veneto (13), followed by Abruzzo (9) and Marche (6) (Fig. 6). Organisms that were most frequently observed as increasing their abundance in mussel farms were polyclad flatworms, barnacles (Cirripedia), hydrozoans, and ascidians, all particularly prevalent in Veneto and Puglia (Table SM1). Other groups, such as serpulid annelids, amphipod crustaceans, bivalve *Anomia* sp., and red algae, were also mentioned, though with more regional variability. In oyster farms, increases in associated fauna were primarily reported in Emilia-Romagna, followed by Marche and Puglia (Fig. 7). All oyster farmers who noticed changes mentioned polychaete genus *Polydora*, borers in oyster shells. Other mentioned taxa included the invasive blue crab *Callinectes sapidus* (Rathbun, 1896), barnacles (Cirripedia), ascidians, and the boring bivalve *Rocellaria dubia* (Pennant, 1777).

Awareness of NNS was widespread: 18 out of 24 mussel farmers and 9 out of 11 oyster farmers declared knowing what NNS are (Table SM1). However, their ability to recognize non-native organisms at a species level was limited. Few organisms explicitly named were *Polydora* sp., *C. sapidus*, *Clavellina* sp., *Ciona* sp. and *Pennaria* sp. Except for *Polydora* sp., all other species- or genus-level identifications were only provided by the two farmers who were also biologists, whereas other respondents reported organisms only at higher taxonomic levels.

Regarding the final question on the existence of internal documents, all farmers reported that their companies or cooperatives do not maintain written records or reports concerning the presence of non-native or pest species.

Awareness of the knowledge of mussel farmers on what NNS are, did not show clear differences considering duration of their working experience. Awareness levels were 62.5 % (5/8), 87.5 % (7/8), and 75.0 % (6/8) for farmers with 6–15, 16–25, and >25 years of experience, respectively (Fig. 8). Confidence intervals were wide for all groups, reflecting small sample sizes. Fisher's exact test confirmed the absence

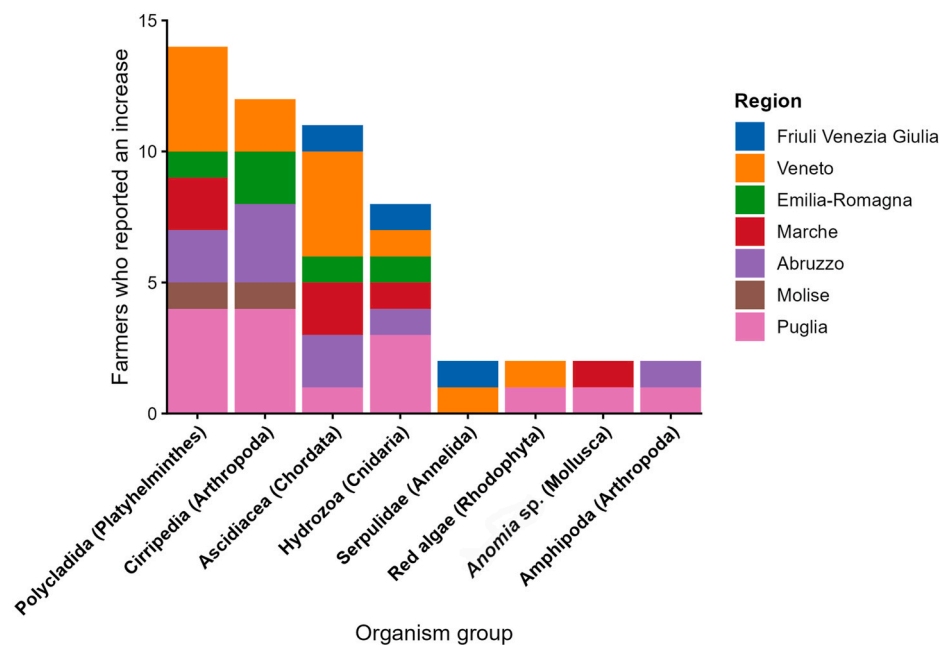


Fig. 6. Number of mussel farmers reporting an increase in the abundance of associated fauna over the last 10 years of their activity, in farms across Italian Adriatic regions.

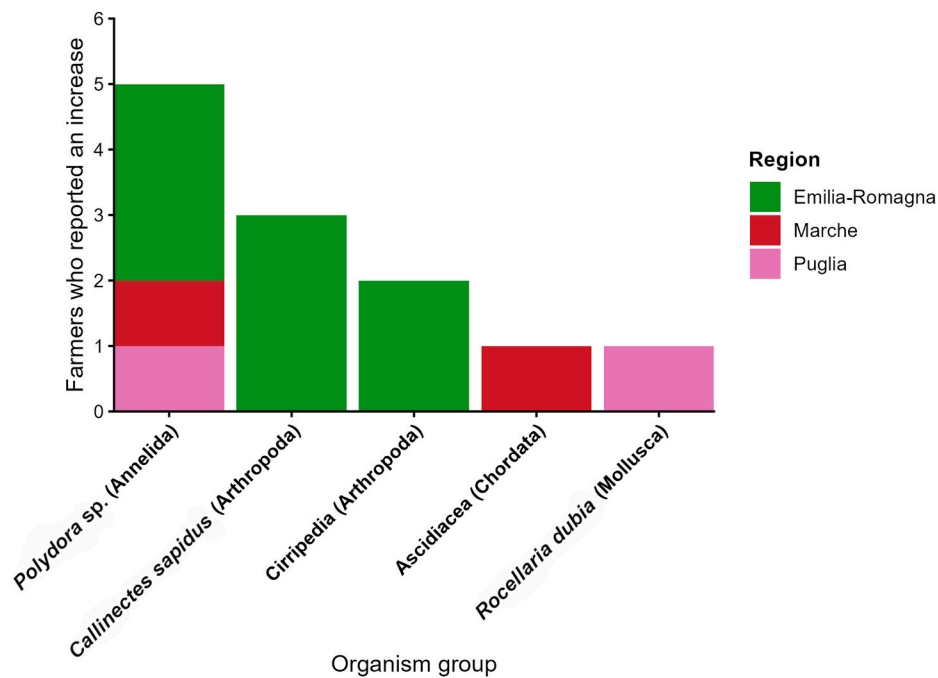


Fig. 7. Number of oyster farmers reporting an increase in the abundance of associated fauna over the last 10 years of their activity, in farms across Italian Adriatic regions.

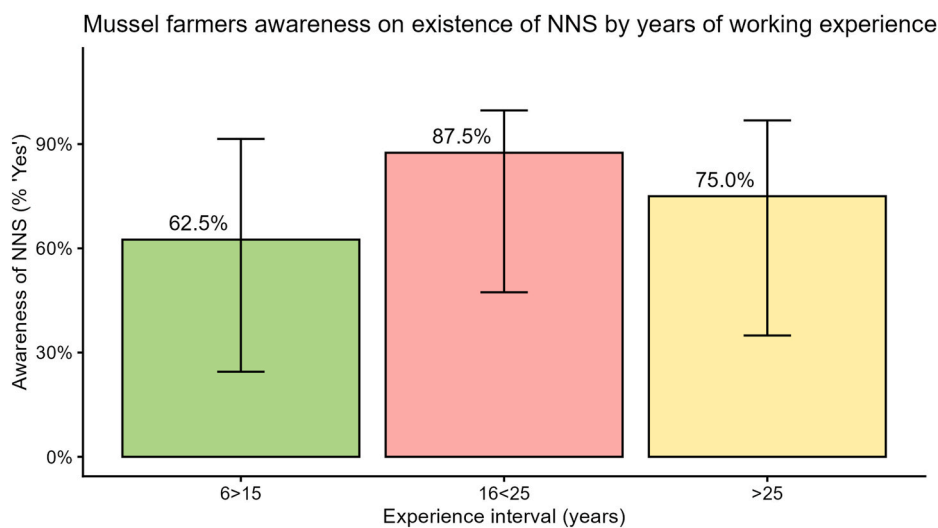


Fig. 8. Awareness of what are non-native species (NNS) among mussel farmers across the classes of length of their working experience (between 6 and 15, between 16 and 25 and >25 years). Bars represent the proportion of farmers responding "yes" to the awareness question; error bars indicate 95 % binomial confidence interval.

of significant differences among classes ($p = 0.837$), and no monotonic trend with increasing experience was detected (Cochran–Armitage $Z = -0.58$, $p = 0.564$). Overall, length of mussel farmers' working experience did not appear to influence their awareness of NNS.

A similar pattern was observed among oyster farmers: awareness was high independently of the length of working experience (100 % in <5 years, 75 % in 6–15 years, and 100 % in both 16–25 and > 25 years), with wide confidence intervals due to small group sizes (Fig. 9). Fisher's exact test detected no significant differences among classes ($p = 1.000$), and no linear trend with increasing experience was observed (Cochran–Armitage $Z = -0.83$, $p = 0.404$). As with mussel farmers, length of working experience did not appear to be a determinant for awareness on what NNS are.

3.4. Presence and impacts of pest species and mitigation strategies

Pest species were reported in all farms, with interannual fluctuations in abundance. For some of the most impactful pest taxa, we succeeded posteriorly to discern to which species they belong, by reviewing together with farmers the photos, videos, and descriptions they provided. Among mussel farms, polyclad flatworms (later identified as *Stylochus mediterraneus*), were considered the most problematic (16 farmers), due to their predation on mussels (Table 1; Table SM1). Ascidians were also frequently cited as pests (12 farmers). They were later identified, through photos provided by farmers, mostly as *Clavelina oblonga* (Herdman, 1880). Farmers reported that these organisms smother ("suffocate") mussels by overgrowing them, ultimately leading to mortality. Barnacles (Cirripedia) were reported by 6 mussel and 2

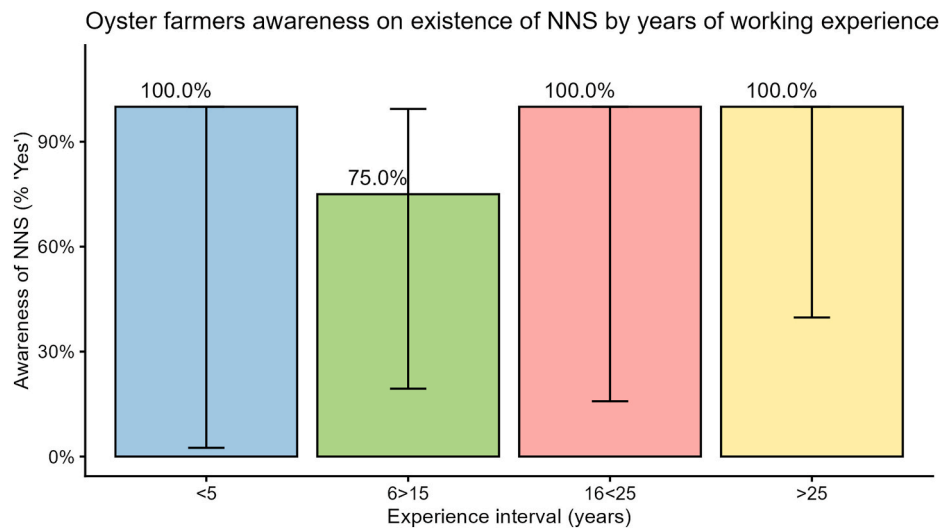


Fig. 9. Awareness of what are non-native species (NNS) among oyster farmers across the classes of length of their working experience (<5, between 6 and 15, between 16 and 25 and >25 years). Bars represent the proportion of farmers reporting “yes” to the awareness question; error bars indicate 95 % binomial confidence interval.

Table 1

Pest organisms in mussel and oyster farms and their impacts reported by farmers. Values represent the number of mussel and oyster farmers who report impacts.

ORGANISMS	NUMBER (%) OF MUSSEL FARMERS	NUMBER (%) OF OYSTER FARMERS	IMPACTS					
			Predation	Suffocating mollusks	Reducing shell quality	Cleaning problem	Reducing marketability	Harmful to operators
<i>Stylochus mediterraneus</i>	16 (66 %)	0 (%)	16					
Ascidians	11 (46 %)	1 (9 %)		12				
<i>Polydora</i> sp.	0 (%)	9 (82 %)			4			9
Serpulidae	1 (4 %)	0				1		1
Barnacles	6 (25 %)	2 (18 %)				2		6
<i>Callinectes sapidus</i>	0	1 (9 %)	1					
Hydrozoa	4 (17 %)	0		2				4
<i>Anomia</i> sp.	1 (4 %)	0				1		
Turtle	1 (4 %)	1 (9 %)	2					

oyster farmers. While they reduce product marketability, farmers also emphasized the significant amount of time and labor required to remove them from the shells. Hydrozoans were mentioned by 4 farmers and were considered troublesome both for their potential to “suffocate” mussels and for their urticant properties, which can cause health issues for farm workers during handling. Other fouling organisms included the bivalve *Anomia*, and the serpulid polychaetes (Serpulidae), both reported only once, with posing problems for cleaning and sales. Moreover, the invasive blue crab *C. sapidus* was mentioned by one mussel farmer as occasionally feeding on mussels’ and oysters’ seeds. Additionally, 2 farmers reported predation of mussels by marine turtles, which, although less frequent, were considered a source of direct stock loss (Table 1).

In oyster farming, *Polydora* sp. was the most frequently cited pest (9 farmers), reported to bore into shells and cause mud blisters that compromise shell integrity and reduce commercial value (Table 1). Barnacles were also mentioned, mainly for causing cleaning problems and reducing marketability, while ascidians were reported for their ability to “suffocate” oysters.

Despite these negative impacts, none of the mussel farms are implementing control measures against the attachment and proliferation of pest species. On the other hand, 4 oyster farms had adopted mitigation strategies, including frequent cleaning of oysters using pressure washer, exposing them to the air for a few hours (3 farms) and using a mechanized immersion/emersion system (1 farm) (Table SM1).

4. Discussion

Farmers interviewed along the Italian Adriatic coast provided first-hand information on the presence and spread of non-native and pest species affecting mollusks aquaculture, as well as on farming practices that may facilitate biological invasions, in particular movements of seed and adult stocks. Most farmers had decades of experience in aquaculture, offering a long-term perspective that may allow them to notice gradual ecological changes in farm-associated fauna. Some farmers also reported previous experience cultivating other bivalve species, which could in principle contribute to a broader taxonomic familiarity. However, our analyses showed that neither years of working experience nor prior cultivation of other species appeared to influence farmers’ awareness or their ability to recognize non-native or pest organisms. In the Mediterranean Sea the problem of biological invasions gained high attention and visibility across different sectors including aquaculture, in the last years. As a result, it is possible that farmers with long careers and those more recently involved in aquaculture may now share a similarly updated understanding of NNS, independently of differences in their professional experience. Results of our survey suggested that educational background influences taxonomic resolution to which farmers can recognize associated organisms. Although education level was not formally recorded in the questionnaire, it was known that two respondents were biologists. These were also the only farmers who consistently identified organisms to a genus or species level. This suggests that formal biological education, rather than working experience

or job position, is the primary driver of farmers' ability to distinguish non-native and pest species.

It is important to acknowledge that interview format used in this survey might have shaped the type of information farmers were able to provide. Conducting interviews by phone may introduce uncertainty in species identification, as farmers can describe organisms in general or imprecise terms. However, this limitation did not alter our objective: assessing the level of taxonomic detail that farmers can provide. Photographs and videos exchanged during interviews were used only to clarify ambiguous cases, allowing us to evaluate both the accuracy and the limits of farmers' recognition abilities. During the interviews, respondents shared images from their farms, and researchers provided reference material when needed, making it possible to clarify ambiguous descriptions and document both correct and incorrect identifications. This process showed that farmers could reliably distinguish organisms at broad taxonomic levels without error (e.g., ascidians, hydrozoans, barnacles, polychaetes), even when they were unable to identify them at finer levels such as genus or species.

Our findings confirm that movements of adult mussels and oysters for re-immersion, as well as exchange of mollusks seed, occur in both directions, to and from the Adriatic Sea. International trade of seed and adult stocks to and from Italy, is established with France, Spain, and Greece. Importations of adult mussels from Spanish Atlantic farms and oyster seed from French Atlantic hatcheries, create direct pathways for the transfer of fauna between the Atlantic Ocean and the Adriatic Sea. All these movements facilitate unintentional spread of fouling organisms, pests, and pathogens alongside the commercial stock, contributing to the introduction of NNS. Accordingly, Italy and France have been identified as leading contributors to aquaculture-mediated NNS introductions (Nunes et al., 2014). Di Blasio et al. (2023) documented, based on literature research, the introduction and spread of several non-native taxa associated with movements of commercial bivalves, including mussels, between Italian marine waters and the Atlantic Ocean. Recently, Martinelli et al. (2025) document that spionid polychaete pests *Polydora* spp. can be transported through hatchery-produced oyster' seed, confirming that such exchanges may spread pest polychaetes even at early life stages of farmed species.

Important factor influencing the potential spread of non-native species is the way in which mollusks are transported. According to additional information provided by some farmers, oyster' seeds are typically sold as loose, individual organisms packed in mesh bags, which allow for better handling and possible cleaning prior to deployment. In contrast, adult mussels are moved inside the polypropylene ropes in which they were grown, with individuals naturally clustered together by byssus threads, which support the rich associated fauna. Clearly, the two types of transport are used for different life stages and carry distinct assemblages of associated organisms in terms of their identity and abundance. This aspect, although not directly assessed in our study, represents a relevant variable to consider in future evaluations of aquaculture-mediated species movements.

Although exchange of cultured species with France and Spain fall under the EU TRAcability and veterinary CErtification System (TRACES), implemented in Italy through Law 428/1990, the reported movement are not publicly accessible. Moreover, TRACES focuses on monitoring of several disease provoking pathogens and parasites and does not document the wider assemblage of fouling organisms, pests, or non-native species that may accompany traded stock. As a result, it is not possible to directly compare observations reported by farmers with official records, nor to evaluate how effectively current biosecurity procedures capture the full spectrum of organisms transported through aquaculture. Our findings therefore highlight the importance of closer collaboration between farmers, researchers, and competent authorities, and emphasize the need for greater transparency and accessibility of movements data to support monitoring of species introductions and trace changes in associated fauna.

Most of the taxa reported by farmers as increasing their abundance in

farms, are documented expanding fouling organisms or new emerging invaders in the Mediterranean aquaculture, as highlighted by Mikac et al. in the extensive review of fauna associated with mussel and oyster habitats in the Mediterranean Sea (in preparation). In our study, ascidians, barnacles and flatworms were frequently mentioned in mussel farms, while *Polydora* sp. was highlighted in oyster farms. Several non-native ascidian species have been newly reported in mollusks farms in the Mediterranean Sea in recent years, some of which have been associated with negative impacts (Papadopoulos et al., 2023; Alvanou et al., 2024). Ascidians can outcompete bivalves and interfere with culture by dominating settlement surfaces (Dupont et al., 2010; Majnarić et al., 2022). Our results suggest that the importation of seeds from other areas into the Adriatic farms may have contributed to the introduction of some invasive ascidians. In fact, farmers from Veneto described increasing infestations and problems caused by these organisms, subsequently identified herein through the photos provided by the farmers, as *C. oblonga*. The same species has been reported as a problematic fouling organism in mussel farms in Greece (Tsotsios et al., 2023; Alvanou et al., 2024). Farmers in Veneto primarily source mussel' seeds from Greece, which suggests that seeds importation may have facilitated the introduction of *C. oblonga*.

Barnacles were also frequently reported by farmers as problematic fouling organisms, due to their persistence on mussels' and oysters' shells and difficulty of removal. Farmers noted that barnacles' encrustations lower aesthetic and commercial quality of mollusks and reduce their marketability. Previous research shown that barnacle fouling may alter shell morphology and disrupt feeding efficiency in mussels (Fernández-Rodríguez et al., 2022). While several species of barnacles are invasive in the Mediterranean and some have been reported in association with farmed bivalves (Igić, 1984; Osca and Crocetta, 2020), the ecological and economic impacts of such colonization remain largely underexplored and call for further research. Farmers also reported the blue crab, *C. sapidus*, invasive species that was already widely recognized by scientists, farmers, and managers as preying on farmed bivalves provoking economic losses in aquaculture (Chiesa et al., 2025; Izquierdo-Gómez, 2022).

Several mussel farmers reported increased abundance of pest flatworms in the last 10 years. Although they did not support precise identity of the species, photographic material they provided allowed us to recognize *Stylochus mediterraneus* Galleni, 1976, a native Mediterranean polyclad flatworm well known for its negative impacts on mussel aquaculture (Privileggio et al., 2024; Mandić et al., 2024). Results of our study support the growing presence of *S. mediterraneus* in the northern Adriatic, particularly in areas already heavily affected by ascidian fouling (Privileggio et al., 2024). In fact, the increasing pressure by ascidians (in particular the invasive *C. oblonga*), noted by farmers, might have weakened mussels, and could explain the reported increased infestation of *S. mediterraneus*. These effects are likely to be exacerbated by climate change since higher temperatures, besides having direct negative impacts on mussel physiology, weaken the mussels making them more vulnerable and susceptible to flatworm pest predation. The combination of these synergistic stressors could be the reason for the mass mortality of mussels noted in the Adriatic Sea (Mandić et al., 2024). Moreover, climate change will probably contribute to the expansion of more thermophilic non-native pests. All these combined effects could have deleterious effects on mollusk aquaculture in the Mediterranean. It is noteworthy that flatworms were reported exclusively by mussel farmers and never by oyster farmers. Although flatworms of the genus *Stylochus* are well known oyster pests, commonly known as "oyster leeches", *S. mediterraneus* is a predator that primarily targets mussels (Gammoudi et al., 2017; Teng et al., 2022).

Reported rising infestations of the polychaete *Polydora* in Italian oyster farms might have been exacerbated by their translocation through oyster' seeds. In fact, most Italian oyster farms obtain seeds from northern France, a region where the presence of invasive polydorid worms, including *Polydora*, is well documented in association with

farmed *M. gigas* (Sato-Okoshi et al., 2023). Worldwide distribution and ecological plasticity of many boring *Polydora* species, including their tolerance to a wide range of environmental conditions, make them particularly successful invaders in aquaculture (Malan et al., 2020; Martinelli et al. 2020, 2024; Rodewald et al., 2021). In our survey, *Polydora* was reported exclusively by oyster and not by mussel farmers. This is expected because shell-boring polydorids, although found to bore also in thinner mussel shells, preferentially settle and excavate into thick oyster shells (Goedknecht et al., 2019; Waser et al., 2020; Stadnichenko et al., 2024). Several farmers in our survey reported *Polydora* infestations as a direct cause of economic loss. These worms produce mud blisters and shell deformities that can downgrade oysters from the high-value half-shell market, increase labor requirements for cleaning, and in severe cases lead to rejection of entire batches (Malan et al., 2020; Rodewald et al., 2021; Martinelli et al., 2024). The problem is particularly relevant in the Adriatic Sea, where non-native *Polydora websteri* Hartman in Loosanoff and Engle, 1943 and *P. caeca* Webster, 1879 were recently reported for the first time in the Mediterranean (Mikac et al., 2025). Given their economic and ecological significance, integrating *Polydora* monitoring into routine health programs and developing effective management practices should be considered a priority, especially for small-scale producers where control measures can be labor-intensive and only partially effective (Martinelli et al., 2022).

Some mussel farmers described turtles foraging near aquaculture installations and believed they cause measurable losses in production. In fact, *M. galloprovincialis* has already been confirmed as part of the natural diet of *Caretta caretta* Linnaeus, 1758, the most common turtle species in the Adriatic Sea (Hochscheid et al., 2013). This interaction may become increasingly relevant in the northern Adriatic, where recent studies have documented a rise in loggerhead turtle presence and aggregation in coastal waters, connected with protection measures for this species, which probably leads to greater predation pressure on farmed mussels (Lucchetti et al., 2021; Di Renzo et al., 2025).

In addition to well-documented biofouling and pest species, farmers reported several other harmful organisms whose interactions within farmed mollusks are less explored. One farmer reported increased abundance of the native Mediterranean bivalve *R. dubia* which lives as endolith in oysters' shells. This species was only recently reported as a parasite which severely alters shell morphology and meat quality in Pacific oyster farms in the northern Adriatic Sea (Mikac et al., 2021). Our results confirm that infestation of *R. dubia* could represent an emerging problem for oyster aquaculture and warrant further assessment of its presence in farms. Physiological characteristics and environmental features of this bivalve that may contribute to its proliferation in aquaculture, should also be explored. Hydrozoans were reported by farmers as provoking skin irritation while handling and being potentially harmful to mussels. Several interviewees suggested that dense hydrozoan colonies may interfere with bivalve respiration and feeding by physically obstructing the shell aperture, potentially leading to stress or suffocation. However, no studies to date have explicitly documented hydrozoans as harmful fouling organisms in the Mediterranean bivalve aquaculture. Farmers also reported problems associated with the presence of serpulid polychaetes and bivalve *Anomia* sp., (presumably *Anomia ephippium* Linnaeus, 1758, the most common reported species of that genus, associated with bivalves). These organisms were frequently observed by encrusting farmed mollusks, lowering their aesthetic value and being very hard to remove. Again, no studies were found which directly link these taxa to negative impacts in mollusks aquaculture in the Mediterranean. All these results emphasize the importance of considering farmers local ecological knowledge in assessment of non-indigenous and pest species in aquaculture, especially for data deficient issues and organisms.

Understanding of seed and adult mollusks translocations as major pathways for the transport of non-native and pest species in the Adriatic Sea, from our study, underscores the need to strengthen monitoring, certification protocols, traceability systems, and coordinated control of

transfers across the trade networks. Despite the evident impacts of biofouling and pest species, mitigation strategies were largely absent in mussel farms, while some oyster farms adopted cleaning regimes and mechanized immersion/emersion systems consistent with literature protocols (Martinelli et al., 2022). Although a variety of physical and chemical treatments, such as acetic acid dips for invasive tunicates (Carman et al., 2016), are documented globally, their feasibility largely depends on the farming system and local environmental conditions (Cahill et al., 2022). In this light, the proactive measures of prevention of unintentional species introduction and spread is the most viable strategy.

In relation to non-native and pest species, the information provided by farmers proved to be useful mainly for identifying which organism groups were becoming more abundant and where such changes were occurring. However, our results also show clear boundaries in what LEK can offer. Farmers were generally unable to distinguish species within problematic groups, and only two respondents (both trained biologists) identified taxa at genus or species level. Although educational background was not included among the survey questions, our study indirectly indicated that this factor can strongly influence farmers' ability to identify non-native and pest species. Future studies should explore how education level shapes farmers' taxonomic skills. While highlighting some known limitations of LEK, such as shortage and variability in species identification skills (Azzurro et al., 2019; Kousteni et al., 2022), our research confirmed that it could be a useful tool for monitoring and management of non-native species in aquaculture. Unlocking its full potential will require a collaborative framework that links farmers, scientists, and decision-makers. There is a need for targeted educational programs to improve farmers' recognition and reporting of invasive species and to promote a culture of active documentation, for example through photographic records, to facilitate their early detection.

Finally, our findings also point to several practical actions that could strengthen biosecurity in the Adriatic bivalve aquaculture. Farmers would benefit from adopting systematic tools for documentation of their environmental observations during routine operations. Such tools should ideally be developed collaboratively by researchers and farmers. Examples might include the routine use of photographs, which would allow verification of observations, the use of simple identification sheets co-designed with researchers to support recognition of the main fouling organisms, and easy to use tool for reporting of observed species and changes. At a regional scale, developing shared cleaning protocols and harmonized monitoring practices, differentiated for mussel and oyster farms, which face distinct pest assemblages, would help reduce the risk of NNS spread. Because seed exchanges between Italy, France, Spain, and Greece represent the main routes for species introduction, stronger cross-border cooperation is essential. Collaboration should involve farmers, who manage and exchange seeds, researchers and competent authorities, who can provide monitoring tools and harmonized criteria to assess the organisms associated with traded stock. Implementing joint monitoring of pest and non-native species associated with traded stock, and improving fair access to movement data would substantially enhance the ability to detect and manage introductions across the Mediterranean aquaculture network.

Statement and declaration

This research was conducted in the framework of the PhD project of the author EF (supervisor BM, co-supervisors FC and GP) in collaboration between the University of Bologna (Department of Cultural Heritage, Ravenna, Italy) and M.A.R.E. Soc. coop. a r. l. (Cattolica, Italy), in accordance with D.M. 352 of April 9, 2022, under the National Recovery and Resilience Plan (P.N.R.R.). The research was also part of the case study "Alien *Polydora* (Annelida: Spionidae) oysters' pests in the Adriatic Sea (Mediterranean)" led by the University of Bologna in the frame of the project Biodiversity Genomics Europe (Grant agreement ID: 101059492) funded by Horizon Europe under the Biodiversity, Circular

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CRedit authorship contribution statement

Eugenio Fossi: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Federica Costantini:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Marina Antonia Colangelo:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Lucia Palazzi Rossi:** Writing – review & editing, Formal analysis. **Giuseppe Prioli:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Barbara Mikac:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ocecoaman.2025.108058>.

Data availability

Data will be made available on request.

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