Invasion and predation ecology of lionfish (*Pterois miles*)

with a focus on the Mediterranean Sea



Davide Bottacini

Propositions

1. Eradication is an immoral solution to the problem of invasive species.
(this thesis)

2. Every predator is prey and every prey is predator.
(this thesis)

3. Anyone can be a scientist.

4. Academia is no different from any other enterprise.

5. Learning is inefficient.

6. Small talk is no talk.

Propositions belonging to the thesis, entitled

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The world belongs to those who believe

to those who believe that they are part of something bigger than themselves

to those who believe that they can go beyond what's known

beyond what's possible

beyond what's imaginable

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Chapter 1 | General introduction

Angling was my first way of interacting with the fishes in my surroundings. As a young boy, I could catch a bunch of species at every afternoon session after school in my home town in northern Italy. One day, I decided to use a hand net to catch some small fish that I saw along the bank of a ditch. I remember placing them in a plastic bottle, so I could determine whose fry they might be. I was blown away at the first look: the fish were clearly no fry. There were some in there that looked almost exactly the same as female guppies (*Poecilia reticulata*), which I had kept as pets multiple times. Others looked remarkably close to male wild guppies, except that they were colourless. I had heard of some places in Germany where guppies had been released from aquaria and had established wild populations. I thought the same might have happened close to home and that the guppies had lost their colour as an adaptation to the murky waters. Excited, I brought them home to investigate things further. The fish were in fact mosquitofish (*Gambusia* sp.); a species introduced from South America to many places around the world to control mosquito populations. While mosquitofish were a novelty to me, I was already aware that many of the species that I was catching close to home were not native to the area. The more I read about these species and how they had travelled across continents, the more questions I came up with. Why were some of them simply called introduced, allochthonous or exotic while others gained the negative label of 'invasive'? Why were Wels catfish (*Silurus glanis*) hunted down to reduce their number while largemouth bass (*Micropterus nigricans*) were offered protection during their reproductive season when they are both exotic species? How could tench (*Tinca tinca*) be disappearing close to home, but be so abundant that they are considered invasive in northern America? It did not take me long to find out that there is a whole discipline devoted to studying these questions and, when possible, provide answers.

Invasion ecology: the study of invasive species

Invasion ecology is a multidisciplinary branch of ecology dedicated to studying the causes and consequences of biological invasions (Ricciardi 2013). Although the definition of invasive species may vary across disciplines and stakeholders (Ricciardi and Cohen 2007, Haubrock et al. 2025), the general consensus among invasion ecologists is that invasive species are species that establish and spread in a new range with a high colonisation rate (Ricciardi 2013). This definition implies that bio-invasions are always the result of the introduction of organisms in areas different from those that they evolved in. In addition, this definition is agnostic to the impact that the species may have on the invaded area. Organisms can be introduced in a new range through different mechanisms that are often the result of human activities (Hulme 2009). For example, the Asian tiger mosquito (*Aedes albopictus*) was accidentally introduced worldwide through intercontinental trade. The mosquito is now a common nuisance and a major vector of viruses in every continent

except Antarctica (Bonizzoni et al. 2013). The rate of accidental introductions has increased in the last decades as a result of globalisation and worldwide trade (Seebens et al. 2017, 2018).

Humans can also intentionally introduce species to new areas, usually with the intent of gaining valuable services. Natural enemies of agricultural pests used in classical biological control are a prominent example; in areas that are colonised by a new exotic pest, natural enemies from the native range of the pest are released to reduce crop damage (Stenberg et al. 2021). Other examples are the grouper *Cephalopholis argus* and the snappers *Lutjanus fulvus* and *Lutjanus kasmira* introduced in Hawaii with the intent of enhancing fisheries (Randall 1987, Friedlander et al. 2002). Some of these intentional introductions are considered successful because they result in ecological and economic benefits, with limited impact on the local ecosystems. However, some introductions can also originate problematic invasions. For instance, cane toads (*Rhinella marina*) were introduced to Australia to control pests in sugar cane (*Saccharum officinarum*) plantations, but became invasive and required the development of methods to limit their spread (Shanmuganathan et al. 2010, Shine and Baeckens 2023).

Not all introduced species become invasive

Species found outside of their native range are known as introduced, exotic, non-native, non-indigenous or allochthonous and they are only considered invasive when they establish and spread (Ricciardi 2013). The vast majority of species that are reported in a new range never become invasive and the differences between species that do or do not establish is a long-standing area of study in invasion ecology (Van Kleunen et al. 2010, Mathakutha et al. 2019). This is probably because there is great variation among species and ecosystems and even general phenomena may apply only partly to specific situations. For instance, prey naïveté is the lack of an appropriate and effective antipredator response in prey due to a lack of co-evolutionary history between local prey and an exotic predators (Banks and Dickman 2007, Carthey and Blumstein 2018). This is often presented as a potential advantage for any predator colonising a new range. However, if a predator with similar morphology and ecology to the new one is already present in the ecosystem, local prey may be able to adjust to the new predator more promptly than in cases when the new predator has some unique morphological or ecological trait (Rehage and Dunlop-Hayden 2011). This is probably why prey naïveté tends to be more relevant when a predator does not have predators from the same genus in an area (Anton et al. 2020). Enemy release is another phenomenon that can increase the fitness of a species in a new range and can substantially contribute to the success of exotic species (Keane 2002, Mitchell and Power

2003). The enemy release hypothesis posits that species that are new to an area may be favoured by the absence of natural enemies such as predators, parasites and diseases (Sih et al. 2010, Brian and Catford 2023). However, an exotic species could also be susceptible to new enemies, with effects that could be strong to the point that the exotic species never establishes (Colautti et al. 2004). The literature rarely tackles the question of why exotic species do not establish and tends to focus largely on those that do establish (Keane 2002, Mitchell and Power 2003, Torchin et al. 2003, Anton et al. 2020). In biological invasions, multiple phenomena such as prey naïveté and enemy release are at play at the same time, complicating the picture and resulting in no univocal answer to the question of what makes some species more successful at establishing and spreading in a new range.

Measuring the impact of biological invasions

Although only a limited number of introduced species become invasive, invasive species can have a profound impact, either through economic damage or negative effects on the local ecosystems. The economic impact of invasive species can be estimated in monetary terms, since it is directly related to economic losses and costs that arise as the result of implementation of prevention or control efforts (Cuthbert et al. 2021). On the other hand, the ecological impact of invasive species is often underestimated, due to multiple factors that make it more difficult to measure than economic damage (Ricciardi 2013). Examples of ecological problems caused by invasive species are disease spread, habitat alteration, competition with or predation on local species (Fritts and Rodda 1998, Clavero and Garciaberthou 2005, Gozlan et al. 2005, Anderson and Rosemond 2007, Polo-Cavia et al. 2010, Shine 2010). In many invasions, experimental approaches aimed at quantifying the ecological impact of invasive species are limited by the impossibility to manipulate the invasion process, or the density of the invasive species in the wild. As a consequence, studies are often observational and based on comparisons between the state of an ecosystem before and after an invasion. This correlational approach presents a major challenge: changes in state could be due to other stressors such as climate change or changes in density of other local species due to overexploitation (Parker et al. 1999). For example, the spread of invasive Nile perch (*Lates* spp.) in Lake Victoria is often presented as the cause of the decline and extinction of hundreds of species of endemic cichlids. This is based on analyses correlating the spread of Nile perch and fisheries catch rates (Witte et al. 1992). However, recent studies show that declines in the abundance of cichlids began before the introduction of Nile perch, and suggest that other factors such as eutrophication and overfishing were also major contributors to the decline of native cichlids (Van Zwieten et al. 2016, Ngoepe et al. 2024). Overall, the ecological impact of invasive species will probably remain underestimated in comparison with their economic impact due to a scarcity of experimental studies (Katsanevakis et al. 2014, Thomsen et al. 2014, Haubrock et al. 2025).

Why study invasive species?

Invasion ecology is a growing scientific field and part of the increasing interest in invasive species is certainly the result of the large number of bio-invasions in the modern era (Ricciardi 2013). An increasing number of species are introduced into new ranges and are facilitated in their establishment by various factors (e.g., climate change) that make ecosystems more vulnerable to invasions (Seebens et al. 2017, 2018). An important reason to study invasive species is that information on their ecology is essential for devising effective control strategies (Buckley 2008). There are numerous examples of invasive species that had dramatic impacts soon after their introduction but were successfully controlled by leveraging ecological information. Phylloxera (*Daktulosphaira vitifoliae*) is a species of vine aphid native to north America and was first reported in Europe in 1863. Within a few decades, the aphid spread across the continent, where it fed on the roots of *Vitis vinifera*, the only vine species grown for wine production (Ollat et al. 2025). *V. vinifera* has low tolerance to the aphid, whose feeding activity quickly results in the death of a plant. The spread of phylloxera had catastrophic effects on wine production in European countries, with major economic and social consequences (Ollat et al. 2025). Anecdotal observations showed that the aphid can feed on various species of plants of the genus *Vitis*, with levels of tolerance that vary drastically across species. In particular, American vines such as *Vitis riparia*, *Visits rupestris* and *Vitis berlandieri* tolerate the aphid and their growth is not affected by its feeding activity (Granett et al. 2001). The solution to the pest outbreak came from grafting: growers started using hybrids of European and American vines as bottom part of their plants (rootstock), combined with their usual cultivars of European vines as top part of the plant (scion). This allowed grafted vines to tolerate phylloxera, but left the quality and production of grapes from *V. vinifera* unaltered. Grafting on hybrid rootstocks is still at the basis of European viticulture today and resulted in complete and sustainable control of the aphid (Ollat et al. 2025).

Apart from providing the basis for mitigating their negative effects, another prominent reason to study invasive species is that they offer an opportunity to study evolution, adaptations and adjustments to changing conditions (Moran and Alexander 2014). During a bio-invasion, both invader and local species must deal with sudden change; the invasive species is confronted with new environmental conditions and species in the native community must deal with a new set of interactions with the invader. Focussing on how an invasive species adjusts to its new environment can inform us on how we can expect species to adjust to environmental change caused by phenomena such as climate change (Caplat et al. 2013, Moran and Alexander 2014). On the other hand, a focus on interactions between invasive and local species can contribute to answering long-standing questions in

fundamental fields such as predation ecology and evolutionary biology. For instance, what is the relative contribution of innate and learned behavioural responses to a predator? How fast can innate responses to predators evolve? Studies on various species of frogs (*Rana latastei*, *Rana aurora* and *Pelophylax perezi*) confronted by invasive predators in different areas indicate that they have both innate and plastic responses towards predators and that only three to six generations can be enough for frogs to evolve genetic adaptations towards a predator (Nunes et al. 2014, Melotto et al. 2020, Kiesecker and Blaustein 2026).

The Mediterranean Sea as a hotspot of biological invasions

The Mediterranean Sea (hereafter, Mediterranean) is the largest semi-enclosed sea on Earth and it is considered a basin of large ecological value (Coll et al. 2010, Psomadakis et al. 2012). The Mediterranean has a temperate temperature regime in the western part, shifting towards subtropical in the eastern part (Coll et al. 2010). The Mediterranean is also regarded as an ecosystem of key economic importance, offering ecosystem services to 150 million people in the countries on its coasts (Coll et al. 2010). Most of the native Mediterranean fauna is of Atlantic origin (Psomadakis et al. 2012). This is the consequence of re-colonisation of the basin by Atlantic species through the Strait of Gibraltar after the Mediterranean desiccated almost completely 5.6 million years ago, during the Messinian salinity crisis (Duggen et al. 2003, Azzola et al. 2026). In recent years, the Mediterranean has become the most invaded sea in the world, largely due to species influx through the Suez Canal, an artificial canal connecting the Mediterranean and the Red Sea (Edelist et al. 2013). Constructed in 1869 to facilitate global trade, the canal has since been widened and deepened multiple times, to cater for larger vessels and more intense traffic (Galil et al. 2017, Castellanos-Galindo et al. 2020). The Suez Canal represents an opportunity for virtually any species to move in between the Red Sea and the Mediterranean. Species exchange can be the result of active or passive transfer of propagules through the canal or through ship traffic. In the latter case, propagules can travel either on the vessels as part of biofouling or through ballast water (Williams et al. 1988). Which of these routes is the most prominent is not clear (Arndt and Schembri 2015).

Species that enter the Mediterranean from the Red Sea are called Lessepsian species, after Ferdinand de Lesseps, the French engineer who was in charge of the construction of the Suez Canal (Por 1978). Although Lessepsian species have representatives in every taxon, fishes are the organisms that have been monitored most accurately in the Lessepsian invasion. This is because, in comparison with other taxa, their taxonomy is well resolved and they are of high economic interest, which makes it easy to gather data based on fisheries and citizen science initiatives (Golani 1993, Galil et al. 2017, Azzurro et al. 2022a, Bottacini et al. 2024, Azzurro et al. 2025). Lessepsian species represent more than two thirds of the invasive species in the Mediterranean and there are new ones reported every

year (Galil et al. 2017, Fortič et al. 2023, Langeneck et al. 2023, Bariche et al. 2025). Lessepsian species are often regarded as a threat to the Mediterranean ecosystem (Katsanevakis et al. 2014, Kleitou et al. 2021). However, there have been extremely limited efforts aimed at causally linking Lessepsian species with their alleged effects in the Mediterranean and most of the research has focussed on tracking or modelling the spread of invasions or modelling their impacts (D'Amen and Azzurro 2020, Poursanidis et al. 2020). A neat example linking ecosystem-level changes with the spread of invasive species comes from an experimental study on the herbivores *Siganus rivulatus* and *Siganus luridus* (Sala et al. 2011). These Lessepsian rabbitfishes are abundant in the eastern Mediterranean, an area that has faced major reductions in the macroalgal cover in recent years. An exclusion experiment clearly showed that the absence of rabbitfishes leads to macroalgal re-growth and recovery of structural complexity of otherwise barren reefs (Sala et al. 2011). Similar studies are urgently needed to disentangle the effect of more Lessepsian species from that of other stressors on the Mediterranean.

Invasive lionfish

Common lionfish (*Pterois miles*) entered the Mediterranean Sea as a Lessepsian species in 1991 and were first considered invasive after large aggregations were reported in Cyprus in 2016. Since then, they have colonised the whole eastern Mediterranean, reaching higher densities than in their native range (Bottacini et al. 2024, Azzurro et al. 2025). Lionfish continue to expand in the Mediterranean and have quickly attracted more attention than any other Lessepsian species (Kleitou et al. 2021, Bottacini et al. 2024, Azzurro et al. 2025). This was mostly because before entering the Mediterranean lionfish were already regarded as one of the most invasive marine species after they colonised the tropical western Atlantic Ocean (Côté and Smith 2018). In the western Atlantic, lionfish spread rapidly, reaching densities 10-100 times higher than in their native range and had a dramatic impact on the community of local fishes through direct predation (Albins and Hixon 2008, Green and Côté 2009, Green et al. 2012, Kulbicki et al. 2012). Common lionfish and red lionfish (*Pterois volitans*) were introduced into Atlantic waters from the aquarium trade (Hamner et al. 2007, Côté and Smith 2018). The different origin of this invasion resulted in two species of lionfish in the western Atlantic, but only one in the Mediterranean: both species are present in the aquarium trade, while *Pterois miles* is the only species found in the Red Sea (fig. 1) (Kimball et al. 2004, Kulbicki et al. 2012). The two species are undistinguishable morphologically, can hybridise and were considered one species for a long time (Kulbicki et al. 2012, Bariche et al. 2017). There is no evidence that the two species have any ecological differences, although they are found in different, overlapping,

ranges. For these reasons, they are collectively referred to as ‘lionfish’ or ‘invasive lionfish’ in most ecological studies.

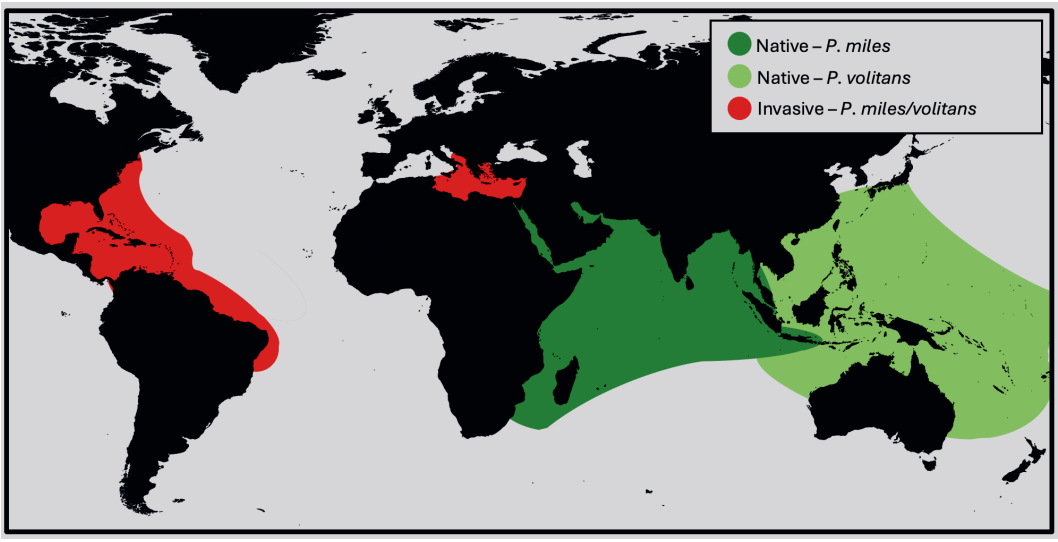

Figure 1. **Global lionfish (*Pterois miles* and *Pterois volitans*) distribution**. The areas in green show the native range of *P. miles* (dark green) and *P. volitans* (light green) and the areas in red shows the invasive range of lionfish in the western Atlantic and eastern Mediterranean. Invasive lionfish in the Mediterranean are *P. miles*, while invasive lionfish in the western Atlantic are mostly *P. volitans*. Source: adapted from Kulbicki et al. 2012, Côté et al. 2013, Azzurro et al. 2025 and Soares et al. 2025.

A large body of knowledge is available on lionfish ecology, mostly composed of information from the invaded ranges (Côté et al. 2013, Côté and Smith 2018, Bottacini et al. 2024). Lionfish are meso-predators that hunt actively with two peaks of activity at sunrise and sunset (Cure et al. 2012, D’Agostino et al. 2020). During the day, they are often found resting in crevices, occasionally in aggregations of multiple individuals and have high site fidelity (Jud and Layman 2012, Akins et al. 2014, Hunt et al. 2019, Phillips et al. 2024). Lionfish feed mostly on fishes and have a stalking hunting strategy: they follow their prey for a long time before striking (Cure et al. 2012, D’Agostino et al. 2020). Stomach content and field observations suggest that lionfish are generalist fish predators, with limited prey preference (Cure et al. 2012, Zannaki et al. 2019, D’Agostino et al. 2020). Ecological information available from the native range of lionfish largely overlaps with what is known from the invaded ranges (Fishelson 1975, Cure et al. 2012, Kulbicki et al. 2012, McTee and Grubich 2014, Gavriel and Belmaker 2021, Gavriel et al. 2021). The primary difference between native range and invaded ranges is density, which is frequently ten or one hundred times higher in invaded ranges (Green and Côté 2009, Kulbicki et al. 2012, Kleitou et al. 2021, 2024, Phillips et al. 2024).

Although the Atlantic invasion had a similar development to the Mediterranean one, there are four important differences between the two invasions. First, they have different origins, which potentially subjected the founder individuals to different initial selection (Bottacini et al. 2024). Second, the Atlantic invasion has developed for a longer time: lionfish have been considered invasive for 25 years there and only for 16 years in the Mediterranean (Côté and Smith 2018, Bottacini et al. 2024). Third, the areas colonised in the western Atlantic are mostly tropical and are dominated by coral reefs, while the Mediterranean is a sea dominated by rocky reefs and has a completely different biodiversity (Bussotti and Guidetti 2011, La Mesa et al. 2011, Kulbicki et al. 2012). Fourth, in the Mediterranean, lionfish do not seem to have reached densities comparable to those reported in the western Atlantic (Green and Côté 2009, Kleitou et al. 2021, 2024, Phillips et al. 2024). Specific information from the Mediterranean is therefore needed to understand the local impact and dynamics of the lionfish invasion. Studying both lionfish invasions also offers the unique opportunity to compare the same invader in markedly different contexts, a rare opportunity in bio-invasions in general and in the marine realm in particular.

Research objectives and thesis outline

While the lionfish invasion in the western Atlantic is the best studied bio-invasion in the marine realm, there are major knowledge gaps remaining on the more recent Mediterranean invasion. The constant spread of lionfish in the basin requires constant updates on their distribution and the impact on the local fish community has never been assessed. In addition, while lionfish effectiveness as predators is often reported as a key aspect of their success as invaders, their stalking predation strategy is largely unstudied. In particular, no studies have investigated whether the complex and colourful morphology of lionfish allows them to camouflage when they hunt on coral reefs and why lionfish blow jets of water while they approach their prey. In this thesis (fig. 2), I tackle these knowledge gaps and contribute to our understanding of the Mediterranean invasion and lionfish predation ecology.

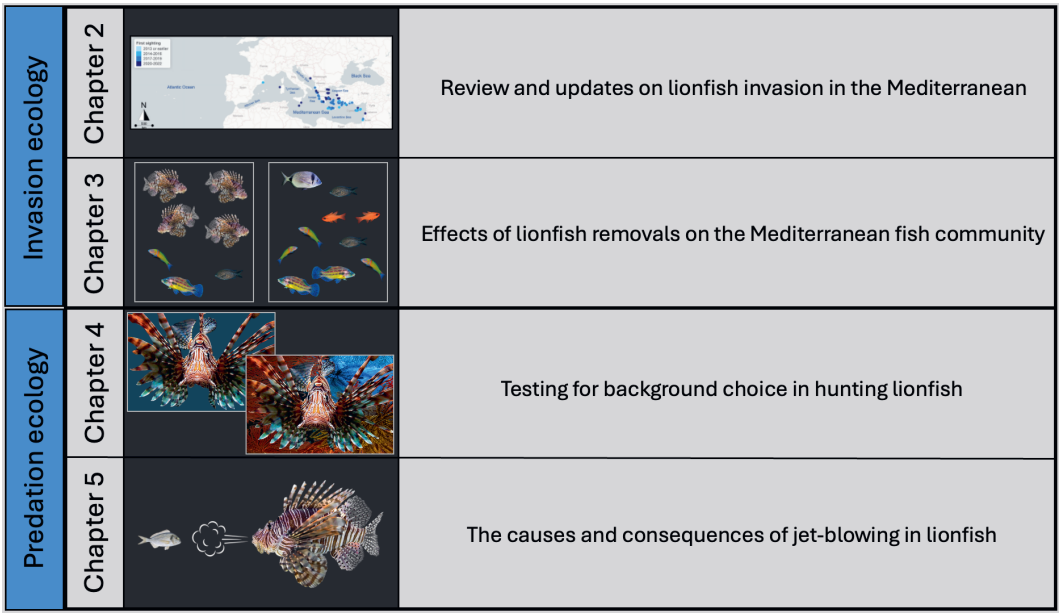

Figure 2. **Thesis outline**. In chapters 2 and 3, I contribute to the field of invasion ecology by providing an update on the spread of invasive lionfish in the Mediterranean Sea and by testing for their impact on the local fish community for the first time. In chapters 4 and 5, I contribute to the field of predation ecology, by investigating the relevance of background choice and the functions of jet-blowing in hunting lionfish.

In **chapter 2**, I provide a review of the scientific knowledge available on the lionfish invasion in the Mediterranean. In addition, the chapter summarises key steps in the colonisation of the Mediterranean by lionfish and provides an update of their spread in recent years. To track the spread of lionfish in the Mediterranean, we asked about 1000 dive centres along the Mediterranean coast whether they see lionfish in their dives in 2023 and compared the results with the ones from the same approach in 2021. The review summarises the information available on the lionfish invasion in the Mediterranean and highlights important knowledge gaps, some of which we closed in the next chapters.

In **chapter 3**, I present the results of a long-term field study aimed at measuring the impact of lionfish on the fish community of the Mediterranean. This is the first experimental study tackling the impact of lionfish in the Mediterranean and one of the few attempts at directly measuring the impact of any Lessepsian species on the local ecosystem. For almost a year, we removed lionfish from six underwater sites from the southern coast of Crete (Greece) and compared the local fish community and biomass with that of six control sites. We did this to test the hypothesis that reducing lionfish densities would result in an increase in the local fish biomass.

In **chapter 4**, I present a study in which we investigated an important aspect in predator-prey interactions: detection. Detection is a key aspect in prey reacting to a predator, because

it is the phase when they realise that there is a potential danger in the environment. If prey cannot detect a predator, they will not react to it, regardless of how naïve or adapted they are to the predator. We tested the hypothesis that the flamboyant appearance of lionfish allows them to camouflage when they hunt on coral reefs. We conducted a behavioural experiment on wild-caught lionfish and observed their approaches towards individual prey. We provided hunting lionfish with a choice of different combinations of backgrounds to test whether they chose backgrounds that match their appearance in colourfulness and structural complexity.

In **chapter 5**, I present an experiment in which we investigated a peculiar behaviour that lionfish show while approaching their prey: they actively blow jets of water at them. Many fishes are known to blow jets of water at their prey, but they all do this to physically dislodge prey in a phase when they cannot escape. On the other hand, lionfish blow water at moving prey, which could react to the blow by escaping. We were the first at testing the causes and consequences of jet-blowing behaviour in lionfish, with the hypothesis that they use jets of water to stimulate prey to turn towards them, so they can more easily catch them head-first. We tested this hypothesis in an experiment in captivity, by analysing trajectories and angles of lionfish and prey during their interactions.

In **chapter 6**, I summarise the results of my thesis and present their implications in the broader context. Studies on lionfish as an invasive species are important, especially in the recently invaded Mediterranean, where they are still expanding and key knowledge is lacking. The predation ecology of lionfish is often presented as key to their success as invaders and has therefore implications for their invasion ecology. Studying lionfish predation ecology is also important in the context of predator-prey interactions, since many aspects of their stalking hunting strategy are both unique among predators and largely understudied. In the highly invaded Mediterranean, lionfish can also be a flagship species that could pave the way for more studies on the other invasive species that are constantly colonising the basin.

In this thesis, I use ‘I’ only when referring to components of the thesis that are the result of my own, individual efforts. I use ‘we’ when referring to the studies that were conducted and written with the contribution of co-authors. See dedicated section (Authorship Statement) for more details on authorship and contributions for each chap

Chapter 2 | Lionfish (*Pterois miles*) in the Mediterranean Sea: a review of the available knowledge with an update on the invasion front

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Abstract
Invasive species often severely impact ecosystems and human activities in the areas that they invade. The lionfishes *Pterois miles* and *P. volitans* are regarded as the most successful invasive fishes in marine ecosystems. In the last 40 years, these Indo-Pacific predators have established in the tropical western Atlantic Ocean, with well-documented detrimental effects on the local fish communities. Around 10 years ago, a second invasion began in the Mediterranean Sea, which is being colonised by *P. miles*. Given the invasive potential of *P. miles* and the fact that the ecology and biodiversity of the temperate/sub-tropical Mediterranean Sea offer a different setting from the tropical western Atlantic, specific knowledge on this second invasion is needed. Here, we (i) review the scientific knowledge available on the ecology of invasive lionfishes, (ii) discuss such knowledge in the context of invasion ecology and (iii) suggest future research avenues on the *P. miles* invasion in the Mediterranean Sea. In addition, we offer an update on the spread of *P. miles* in the Mediterranean Sea. While the history and development of the Mediterranean invasion are resolved and some mitigation plans have been implemented locally, the study of the interactions of *P. miles* with Mediterranean species and their impact on the local biodiversity is in its infancy. Closing this gap will lead to important fundamental insights in invasion ecology and will result in predictions on the impact of *P. miles* on the ecology and ecosystem services of the Mediterranean Sea. Such information will have practical implications for policy makers aiming to devise sound and efficient mitigation plans.

1. Introduction

Invasive species are species that establish and spread in a new range at a high rate (Ricciardi 2013), often with detrimental effects on the local ecosystems. Invasive species can cause environmental degradation (Anderson and Rosemond 2007, Ehrenfeld 2010, Villamagna and Murphy 2010), carry and spread parasites (Gozlan et al. 2005, Iglesias et al. 2015) and compete for resources with native species (Bergstrom and Mensinger 2009, Polo-Cavia et al. 2010). Among the most severe ecological problems associated with biological invasions is biodiversity loss through local extinction of native species. This is particularly relevant when there is a direct trophic interaction between invader and local species. For example, invasive mammalian predators caused the extinction of more than 100 species worldwide (Doherty et al. 2016) and the invasion of Lake Victoria by the Nile perch (*Lates niloticus*) drove almost 200 endemic cichlids to extinction (Witte et al. 1992). Because of their dramatic ecological impacts, invasive species are regarded as one of the most serious environmental problems of our time (Ricciardi 2013).

The lionfishes *Pterois miles* and *P. volitans* (hereafter, referred together as 'lionfish') are virtually undistinguishable and show almost identical morphological traits (Kulbicki et al. 2012). They are considered the most invasive fishes in the marine realm; native to the Indo-

Pacific Ocean and Red Sea, lionfish reached the western Atlantic Ocean through intentional or accidental releases by aquarists (Kulbicki et al. 2012, Côté and Smith 2018). Lionfish were first detected in Atlantic waters in 1985 and became a common sight at certain locations in the late 1990s (Whitfield et al. 2002, Schofield 2009). Despite considerable control efforts at the local scale (de León et al. 2013, Dahl et al. 2016, Harris et al. 2019, 2020, Goodbody-Gringley et al. 2023), lionfish have spread through the entire tropical western Atlantic and continue to expand their invasive range along the Brazilian coast (Côté and Smith 2018, Soares et al. 2022, 2023). Lionfish are generalist predators (Green et al. 2011, 2014, Green and Côté 2014, D'Agostino et al. 2020) and are having an impact on the ecosystems of the western Atlantic by preying extensively on various local benthic and demersal fishes, including endemics of high conservation value (Albins and Hixon 2008, Green et al. 2012, 2014, Benkwitt 2015, Rocha et al. 2015, Ingeman 2016). Predation by lionfish can reduce recruitment of juveniles and the biomass of local species by up to 65% (Albins and Hixon 2008, Green et al. 2012). Such marked effects on the local biodiversity have been associated with impacts on the stability of coral reef ecosystems and their degradation (Lesser and Slattery, 2011). More recently, a second lionfish invasion has begun in the Mediterranean Sea (hereafter, Mediterranean), which is being colonised by *P. miles* (Kletou et al. 2016, Bariche et al. 2017, Phillips and Kotrschal 2021). This second invasion raises concerns on possible impacts on the biodiversity and ecosystem services of the Mediterranean (Kletou et al. 2016, Savva et al. 2020).

The Mediterranean is a unique ecosystem: it is the largest enclosed sea on earth and a highly biodiverse basin home to more than 11000 animal species, some of which are found nowhere else in the world (Coll et al. 2010, Psomadakis et al. 2012). For example, of the approximately 540 native species of Mediterranean fishes, around 9% are endemic (Psomadakis et al. 2012). In addition, the sea provides economically valuable services to approximately 150 million people in the numerous countries on its coasts (Coll et al. 2010). At the same time, the Mediterranean biodiversity is suffering from many anthropogenic stressors (Bianchi and Morri 2000, Coll et al. 2010) and it is the most invaded sea in the world. This is largely due to the opening of the Suez Canal (Edelist et al. 2013), which was dug in 1869 to connect the Mediterranean with the Red Sea for commercial purposes (Costello et al. 2021). Initially, there was little scope for invasions due to the small size of the canal and the presence of bitter lakes creating a hypersaline barrier between the two seas. However, the Suez Canal has been widened multiple times in recent years, increasing its capacity to carry propagules and reducing the salinity of the bitter lakes (Edelist et al. 2013, Galil et al. 2017, Castellanos-Galindo et al. 2020). New species ('Lessepsian species') enter the Mediterranean every year and the Suez Canal is now the source of two

thirds of the exotic species present in the basin (Galil et al. 2014, 2015, 2017, Fortič et al. 2023).

There are important differences between the Mediterranean and the tropical western Atlantic. The Mediterranean is a temperate/sub-tropical sea dominated by rocky reefs, seagrass meadows and sandy patches (Bussotti and Guidetti 2011, La Mesa et al. 2011, Kleitou et al. 2021). By contrast, the tropical western Atlantic is dominated by coral reefs, similarly to the native range of lionfish (Kulbicki et al. 2012, Côté and Smith 2018). The species composition and biodiversity of the Mediterranean are also profoundly different from those found in tropical seas (Kallianiotis et al. 2000, Brokovich et al. 2006, Albins and Hixon 2008, La Mesa et al. 2011). Both invasive lionfish populations were founded by individuals that went through selection processes that might favour the survival of the resulting invasive populations in suboptimal conditions. Lionfish establishing in the Atlantic survived through the multiple stressors encountered in the aquarium trade (e.g., catching, transportation) and *P. miles* establishing the Mediterranean survived the suboptimal water parameters encountered either in the Suez Canal or in ballast water. It is unknown how strongly these factors selected on invasive lionfish and to what extent the differences between these stressors are affecting the dynamics of the invasions today. Given the invasive potential of *P. miles*, their different origin from that of lionfish in the Atlantic and the different ecology and biodiversity of the temperate/sub-tropical Mediterranean, specific knowledge on this second invasion is needed. This information will be essential to understand and predict the impact of *P. miles* on the Mediterranean and to design rational and effective mitigation strategies. Here, we review the available information on lionfish ecology, we discuss such knowledge in the context of invasion ecology and highlight major knowledge gaps on the Mediterranean invasion that require future investigation. In addition, we offer an update on the distribution of lionfish in the Mediterranean, where *P. miles* are still spreading.

2. Lionfish in the Mediterranean

2.1 The origin and history of the Mediterranean invasion

The first lionfish ever reported in the Mediterranean was caught by a trawler off the coast of Israel in 1991 and identified as *P. miles* (Golani and Sonin 1992). From that moment, no more lionfish were reported until 2012, when two specimens were captured in Lebanon (Bariche et al. 2013). Soon after, lionfish were reported in Turkey, Cyprus, Greece and Italy (Turan et al. 2014, Crocetta et al. 2015, Iglésias and Frotté 2015, Oray et al. 2015, Turan and Öztürk 2015, Azzurro et al. 2017). Lionfish were first considered invasive in the Mediterranean in 2016, when they were reported in large groups and numbers in Cyprus (Kletou et al. 2016). Lionfish have now established and successfully spread through a large part of the eastern Mediterranean (Gökoğlu et al. 2017, Turan et al. 2017, Dimitriadis et al.

2020, Ulman et al. 2020, Vavasis et al. 2020) and continue to expand their range westwards (Azzurro et al. 2017, Phillips and Kotrschal 2021). Today, invasive lionfish populations are confined to the eastern part of the Mediterranean (Dimitriadis et al. 2020, Phillips and Kotrschal 2021), with only sporadic sightings elsewhere. The northernmost report of lionfish is that of a single individual found near the island of Vis, in Croatia (Dragičević et al. 2021) while the westernmost lionfish was also a single individual sighted in the Alboran Sea, Spain (Fortič et al. 2023). Since no established populations are present at these locations, the individuals in Croatia and Spain may be the result of isolated aquarium releases. The northernmost part of the Aegean Sea has also remained free from lionfish, probably due to the colder waters (Dimitriadis et al. 2020, Phillips and Kotrschal 2021).

Genetic studies revealed that lionfish found in the Mediterranean originate from the Red Sea and that they most likely entered their new range during multiple invasion events through the Suez Canal (Bariche et al. 2017). The origin of Mediterranean lionfish is corroborated by the absence of established populations of *P. volitans* in the basin; while both *P. miles* and *P. volitans* are often sold together in the aquarium trade (Kimball et al. 2004) and, consequently, are found in the invaded western Atlantic, genetic studies showed that only *P. miles* is present in the Red Sea (Hamner et al. 2007, Kulbicki et al. 2012, Wilcox et al. 2018). Thus, the lionfish population of the Mediterranean is considered the result of *P. miles* entering through the Suez Canal and the reports of *P. volitans* in this sea (e.g., Gürlek et al. 2016, Gökoğlu et al. 2017; Ayas et al. 2018) are most likely the result of misidentifications or descriptions of individuals that came from isolated aquarium releases.

The northern Red Sea is inhabited by another lionfish species that is biologically and ecologically similar to *P. miles*; *Pterois radiata*. *P. miles* and *P. radiata* often occur together on the coral reefs of the northern Red Sea and in comparable abundances (Gavriel and Belmaker 2021). Interestingly, *P. radiata* has never established in the Mediterranean (Kulbicki et al. 2012, Gavriel and Belmaker 2021). It was hypothesised that *P. radiata* may be less invasive than *P. miles* due to its smaller size and slightly higher degree of habitat and diet specialisation (Kulbicki et al. 2012, Gavriel and Belmaker 2021). Comparative studies analysing behavioural, physiological, and reproductive traits in a controlled environment may help elucidate what aspects are preventing *P. radiata* from becoming invasive in the Mediterranean Sea.

2.2 Tracking an ongoing invasion

P. miles entered the Mediterranean from one of its easternmost locations and continue to expand westwards and northwards (Bariche et al. 2017, Phillips and Kotrschal 2021), calling for continuous updates to pinpoint the location of their current invasion front.

Citizen science, defined as the involvement of lay people in data collection, is an effective tool to track the expansion of invasive species (López-Gómez et al. 2014, Larson et al. 2020, Hermoso et al. 2021). This is especially true for species such as *P. miles*; they are appreciated by divers for their attractive morphology and colouration, increasing the chances of lay people spotting and recognising them. *P. miles* are also difficult to misidentify, especially in the Mediterranean, where closely related species (i.e., native scorpionfishes) have a markedly different appearance. Finally, the awareness among lay people and stakeholder on the invasiveness of lionfish is high (Kleitou et al. 2021), making them attentive and willing to collaborate with scientists. Citizen science is therefore particularly suited to monitor the invasive range of lionfish in the Mediterranean, a sea where the diving industry is well established and dive centres are numerous (Phillips and Kotrschal 2021).

As a follow-up to Phillips and Kotrschal (2021), we contacted dive centres on the Mediterranean coast to ask whether they see lionfish during their dives and if they remember the first year that they saw them. We used a list of dive centres on the Mediterranean coast compiled in 2021 (Phillips and Kotrschal, 2021). From this list, we contacted all the dive centres that were still open and reachable via email in April 2023. In most countries, we sent emails in two languages: the first language spoken in the country and English. Translations into local languages were provided by native speakers. We sent emails in two languages to make our survey accessible to those who do not speak English fluently and to foreigners running dive centres in countries of which they do not speak the local language. Dive centres in Egypt, Albania, Montenegro, Malta and Israel were contacted only in English. We sent a reminder to every dive centre that did not respond within a week, and we recorded responses for four weeks after the reminder. We used the GPS coordinates of the location of dive centres as an estimation of the point where lionfish are seen as most dives are done in the waters close to a dive centre. Any response that we received in a language different from English were translated through Google translate. When a dive centre reported a range of years as an answer to the date of the first sighting (e.g., 2020-2021) we considered the most recent year in the range as year of first sighting. Data were analysed in R 3.6.2 (R Development Core Team, 2019). Maps were produced with the package 'leaflet' (version 2.0.4.1, Cheng et al., 2021).

Contacting 996 dive centres yielded 326 responses (Fig. 1A). Sightings were reported by 82 dive centres, mostly in the eastern Mediterranean (Fig. 1B). Lionfish were seen by almost every dive centre that responded from Israel, Cyprus, Turkey, Greece, and Albania. The lionfish reported at the furthest locations from the Suez Canal were reported in Croatia (42.6513°N, 18.0608°E), Malta (35.9500°N, 14.4063°E) and the Italian islands of Sicily (36.7330°N, 15.1205°E) and Sardinia (40.5699°N, 8.2430°E). When compared with the results by Phillips and Kotrschal (2021) (Fig. 1C), our data show that, in just two years,

lionfish have expanded their invasive range in the Mediterranean at two fronts: the northern Aegean Sea and the southern Adriatic Sea. While most of the dive centres reported no lionfish in 2021 in the northern part of the Aegean, they almost all did in 2023; the only two dive centres reporting no lionfish in the northern Aegean were also the ones with the northernmost coordinates. A limited expansion can be seen also in the southern Adriatic, where two dive centres reported lionfish sightings in 2023 while none did in 2021.

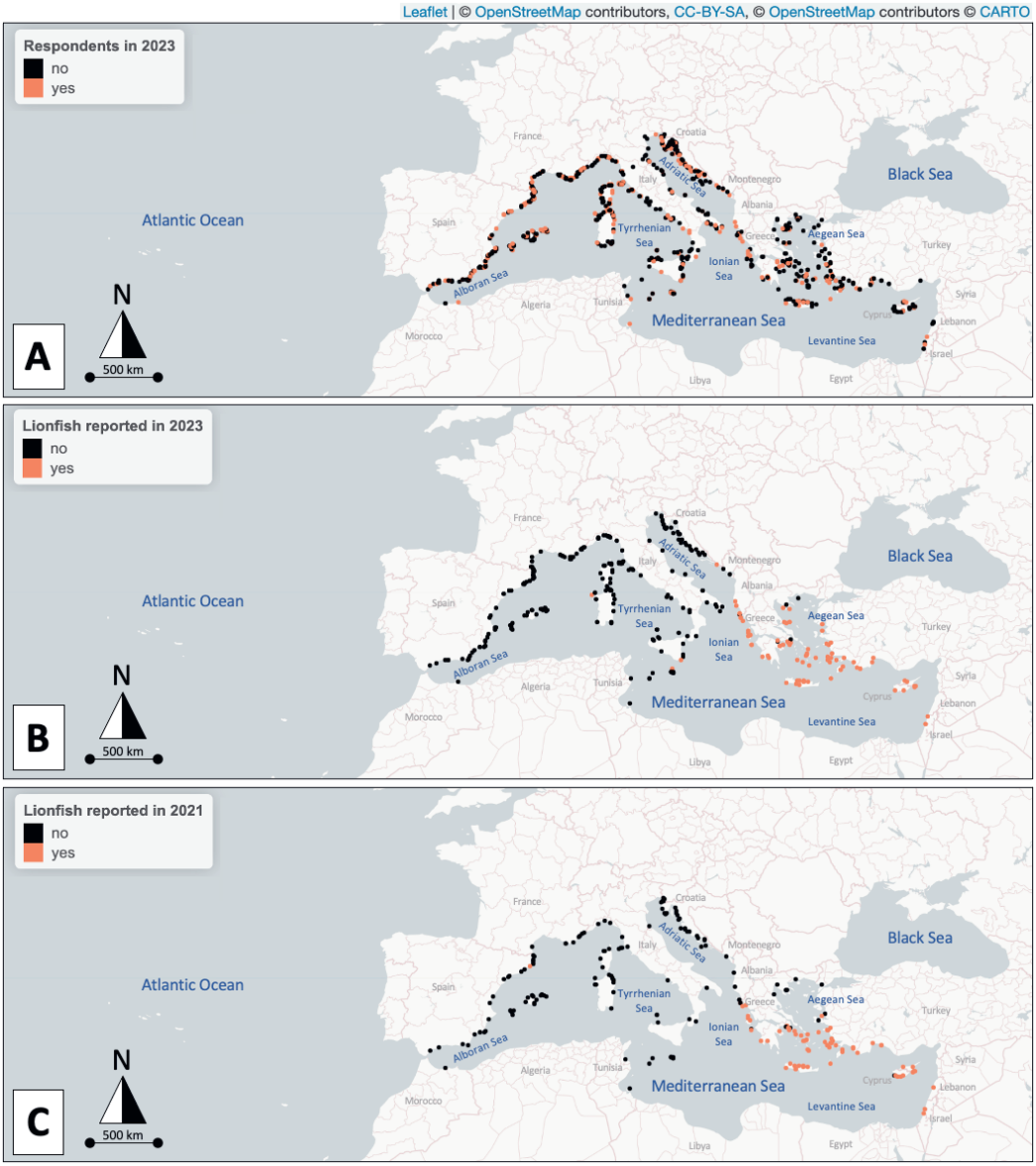

Figure 1. Maps of respondents and lionfish sightings. **A** map of respondents to our survey in 2023. Each dot represents a dive centre that we contacted, with orange dots representing dive centres that responded and black dots representing dive centres that did not. **B** map of responses to our survey in 2023. Each dot represents a dive centre that responded to our survey in 2023 with orange dots representing dive centres that reported lionfish sightings and black dots representing dive centres that reported no sightings. **C** map of responses to the survey in 2021 (Phillips and Kotrschal 2021). Each dot represents a dive centre that responded to the survey in 2021 with orange dots representing dive centres that reported lionfish sightings and black dots representing dive centres that reported no sightings.

The years and locations where lionfish were first seen (Fig. 2) corroborate an expansion of the lionfish invasive range in the Mediterranean. Lionfish were first seen in the northern Aegean, Ionian Sea and southern Adriatic between 2020 and 2022. Individuals in Sicily, Sardinia, Croatia and Malta were also seen only in the most recent year range. This suggests that lionfish found at these locations are probably not just the results of aquarium releases; if that was the case, we could have also expected reports in the past. More likely, these individuals have been transported by strong currents from the eastern Mediterranean, either as larvae or eggs. It is important to note that none of the dive centres reporting lionfish in Malta, Italy and Croatia provided pictures and, therefore, misidentification is still a possibility for these sightings. The dive centres reporting sightings at these locations confirmed that there are no established lionfish populations there.

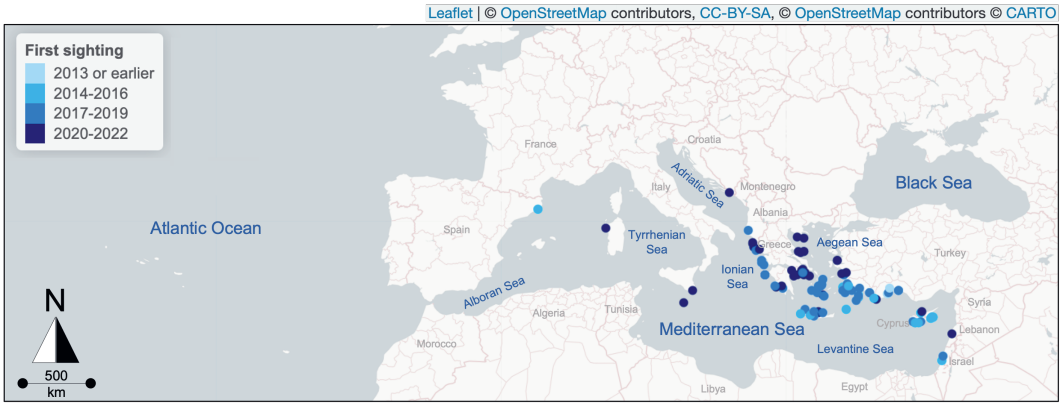

Figure 2. Map of years of first sighting. Each dot represents a dive centre that reported lionfish sightings, either in 2021 or 2023, and included in their response the year when lionfish were first sighted. The darkness of dots shows the year range when lionfish were first sighted.

Our results show that the invasive range of *P. miles* continues to expand rapidly in the Mediterranean. Similarly to most coral reef fishes, lionfish eggs hatch into pelagic larvae (Ahrenholz and Morris 2010, Vásquez-Yeomans et al. 2011). Larvae are the life stage with the highest dispersal potential in coral reef fishes (Shanks 2009) and are arguably the main contributor to the dispersal of lionfish, which are highly site-attached as adults (McCallister

et al. 2018, Gavriel et al. 2021, Phillips et al. 2024). The Mediterranean invasion is following a similar course to that of other Lessepsian species, which typically expand in the Mediterranean starting from the Levantine Sea and gradually spread westwards and northwards towards the Aegean and Ionian Sea (Azzurro et al. 2013).

Many Lessepsian species remain confined to the eastern Mediterranean and are rarely found in high numbers elsewhere (Azzurro et al. 2013, Galil et al. 2017). A modelling study (Johnston and Purkis 2014) predicted that lionfish were unlikely to become invasive in the Mediterranean. However, our and others' (Azzurro et al. 2017, Phillips and Kotrschal 2021) empirical evidence suggests that the lionfish population of the Mediterranean is well-established and keeps expanding westwards. One of the reasons for the unpredicted success of lionfish could be that their invasion is developing under a strong effect of climate change. When climate change is accounted for (Loya-Cancino et al. 2003), lionfish are predicted to find suitable conditions in the Mediterranean. Another reason could be that Johnston and Purkis (2014) considered that lionfish only spread as larvae under the action of natural currents and, because the Mediterranean is less connected by internal currents than the Atlantic, they concluded that lionfish are unlikely to spread across the whole Mediterranean. Although larvae are probably the phase at which lionfish move over long distances, adults can enter new areas as it happened recently in Brazil, where lionfish managed to cross the Amazon-Orinoco plume, most likely by adults moving along deeper mesophotic reefs (Soares et al. 2022, 2023). Additionally, the Mediterranean is highly trafficked, and this could allow lionfish larvae or eggs to cross large stretches of sea independently from currents if they enter the ballast water. It will be challenging to disentangle the effects of the factors contributing to the unpredicted success of lionfish in the Mediterranean, as multiple phenomena are at play without possibilities to manipulate them.

Remarkably, several *P. miles* sightings were reported in areas that were considered to have winter surface temperatures that are too cold for this species (< 15 C) such as the northern Aegean and southern Adriatic (Kimball et al. 2004, Johnston and Purkis 2014, Dimitriadis et al. 2020). Although it is too early to conclude that *P. miles* will establish at these locations, climate change has been predicted to facilitate the expansion of tropical invasive species' ranges in the Mediterranean and other ecosystems (D'Amen and Azzurro 2020). Climate change is resulting in a gradual process of tropicalisation of the Mediterranean; the biological community composition is shifting in favour of Lessepsian species at the cost of native ones (Giorgi 2006, Galil et al. 2017). This has already resulted in Lessepsian fishes outweighing and outnumbering native ones in marine protected areas of the eastern Mediterranean (Giakoumi et al. 2019). Another major difference between the

Mediterranean and the Red Sea is the stronger seasonality of the former. It remains unknown how the seasonality of Mediterranean waters affects the dynamics and distribution of lionfish and other invasive species of tropical origin.

Our study shows that citizen science is a fruitful approach to monitor lionfish populations at the large scale in the Mediterranean, where the dive industry is strong and awareness towards lionfish is high. Different approaches are needed to monitor the state of the invasion on the southern coasts of the Mediterranean, where data is lacking, and the number of dive centres is extremely low (Fig. 1A). When we contacted members of a Libyan spearfishing association through social media, they reported seeing lionfish relatively frequently on the (eastern) Libyan coast. Moreover, lionfish were reported at several locations on the southern coast of the Mediterranean in the past, including Tunisia (Dailianis et al. 2016, Al Mabruk and Rizgalla 2019). This suggests that, as expected, the lionfish invasion and its expansion are not limited to the northern coast of the Mediterranean.

3. The evolutionary ecology of invasive lionfish across ranges

3.1 Lionfish morphology and habitat use

Lionfish have a total of 18 venomous spines; one on each of the first 13 rays of their dorsal fin, one on each of their pelvic fins and three on their anal fin (Aktaş and Mirasoğlu 2017). They show high site fidelity and often return to the same hiding place over the course of several weeks (McCallister et al. 2018, Gavriel et al. 2021, Phillips et al. 2024), although this can vary significantly at the individual level (Tamburello and Côté 2015, Gavriel et al. 2021). Lionfish are often found, either individually or in small groups, hiding in caves and crevices during the day and swim in the open only at dawn and dusk to hunt for prey (Cure et al. 2012, McCallister et al. 2018, D'Agostino et al. 2020, Gavriel et al. 2021). The eastern Mediterranean offers a markedly different habitat from the coral reefs of the Indo-Pacific and the tropical Atlantic (Kulbicki et al. 2012, Côté and Smith 2018). The Mediterranean is a sub-tropical environment dominated by rocky reefs, seagrass meadows and sandy patches (Bussotti and Guidetti 2011, La Mesa et al. 2011, Kleitou et al. 2021). Despite these habitat differences, *P. miles* have established well in the Mediterranean and have already reached higher population densities than in their native range (Kulbicki et al. 2012, Phillips et al. 2024). It is perhaps not surprising that *P. miles* are thriving in the eastern Mediterranean, as in the western Atlantic lionfish have been reported in habitats that are novel for this species, including mangrove forests, river estuaries and seagrass beds (Barbour et al. 2010, Jud et al. 2011, Claydon et al. 2012). Analyses of the population structure and dissections of females indicate that the Mediterranean population of *P. miles* is reproducing and will remain a stable presence (Savva et al. 2020, Mouchlianitis et al. 2022).

3.2 Predation ecology

Fishes make up most of the lionfish diet (Barbour et al. 2010, Harms-Tuohy et al. 2016, Zannaki et al. 2019), although they have been reported to also feed on invertebrates (Valdez-Moreno et al. 2012). Lionfish are stalking, gape-limited predators: they slowly follow their prey, sometimes for several minutes, with flared pectoral fins before striking and swallowing them whole (Green et al. 2011, Green and Côté 2014). They tend to prefer small, shallow-bodied benthic and demersal fishes in the Caribbean (Green and Côté 2014, Ritger et al. 2020) and show a similar prey preference in the Mediterranean, where they also adopt the same hunting strategy (Zannaki et al. 2019, D'Agostino et al. 2020). In their native range and the invaded Atlantic, lionfish are a widespread component of the community of coral reef predators (Lesser and Slattery 2011, Cure et al. 2012, Kulbicki et al. 2012, Côté and Smith 2018). In the Atlantic, *P. volitans* can have strike success rates as high as 85%, the highest reported in animals in the wild (Vermeij 1982, Green et al. 2011).

The high predation effectiveness in their invaded range has been attributed, at least in part, to prey naïveté (Côté and Smith 2018) (but see Cure et al. 2012). The 'naïve prey hypothesis' (or 'prey naïveté hypothesis') posits that prey that are exposed to an exotic predator are not prepared to recognise or effectively react to it due to a lack of coevolutionary history (Sih et al. 2010). Numerous studies support the relevance of prey naïveté in the lionfish invasion in the Atlantic. For instance, several prey species do not react to lionfish with the same readiness as they do with native predators (Anton et al. 2016, McCormick and Allan 2016, Haines and Côté 2019, but see Marsh-Hunkin et al. 2013). In the eastern Mediterranean, exotic prey species from the Red Sea, which coevolved with *P. miles*, are abundant and occur together with Mediterranean prey. Exotic prey show a markedly higher flight initiation distance when a lionfish is approaching them compared to Mediterranean species, supporting the hypothesis that prey naïveté is also relevant in the Mediterranean invasion (D'Agostino et al. 2020).

Experiments on prey naïveté in the context of lionfish invasions raise the question of whether the selection pressure posed by this new predator will result in adaptations in local prey. It follows from the definition of prey naïveté that it can be counteracted by evolutionary adaptation: after several generations of coexistence with a novel predator, prey should evolve innate responses (Anton et al. 2020). But how rapidly can such evolutionary adaptations evolve in prey? This is an unresolved question: some estimates based on data on multiple taxa suggest that hundreds of generations are needed (Anton et al. 2020), while there is evidence showing that 10-30 generations can be enough for predators to drive evolutionary changes in prey (O'Steen et al. 2002, Nunes et al. 2014, Melotto et al. 2020). The great variability in the number of generations needed for local

prey to evolve an innate response to predators is probably explained by factors such as the pressure posed by predators and the genetic variability of prey populations (Nunes et al. 2014). The potential for prey to adapt to a new predator such as lionfish is of high scientific relevance but also has practical implications because it will determine the long-term effects on the local prey communities of the Mediterranean and western Atlantic. In an experiment in the western Atlantic (Kindinger 2015) the antipredator response of damselfish (*Stegastes planifrons*) to *P. volitans* was measured and compared to that displayed against a control, native predator. Damselfish were generally naïve to *P. volitans*, including individuals from populations that had coexisted with lionfish for three and seven years (Kindinger 2015). Local adaptation by prey to *P. miles* has never been tested in the Mediterranean.

Prey naïveté interferes with innate predator recognition in animals (Sih et al. 2010, Anton et al. 2020). However, this is not the only mechanism resulting in prey reacting to a predator. Individual fishes can learn which species can pose a threat to their survival through associative learning (Kelley and Magurran 2003). Predator recognition can be learned either directly, when a fish escapes an attack from a predator, or indirectly when an individual observes predation events or associates the presence of a predator with the presence of danger-related cues (e.g. blood, stress pheromones) from other fishes (Brown 2003). Learned predator recognition is pervasive in fishes (Brown 2003, Kelley and Magurran 2003, Mitchell et al. 2011); prey fishes can learn to associate danger cues with the presence of a predator during a single conditioning event and retain a behavioural response to that predator for extended periods of time (Chivers and Smith 1994, 1995, Mitchell et al. 2011). Could native prey fishes compensate for their lack of innate responses to lionfish through learned predator recognition? This is an open question for both the Mediterranean and the Atlantic invasion. Specific work on how well prey species learn that lionfish pose a threat to their survival is limited to one study on a species from the native range of lionfish. This study suggests that even prey that coevolved with lionfish seem to have difficulties associating them with danger, while other predatory fishes can be learned more readily (McCormick and Allan 2016). This has led to the hypothesis that lionfish circumvent learned predator recognition mechanisms in prey (Côté and Smith 2018). Whether Mediterranean or western Atlantic prey can learn to recognise lionfish as predators is currently unknown and more research is needed to test for the relevance of circumvention of learned predator recognition in lionfish prey.

Predator recognition allows prey to mount an appropriate behavioural response to a predator. Therefore, invasive lionfish are predicted to select on traits that make prey better able to recognise them, either innately or through learning. However, predators can also select on prey behavioural traits that make them less likely to be preyed on due to processes that are not related to predator recognition (Blake and Gabor 2014, Belgrad and Griffen 2016). For example, boldness affects the susceptibility of mud crabs (*Panopeus herbstii*)

to be preyed on, creating the potential for predators to select for boldness in this species. Interestingly, boldness has a different effect on susceptibility to predation depending on the predator: toadfish (*Opsanus tau*) consumed more frequently shy mud crabs, while blue crabs (*Callinectes sapidus*) consumed more frequently bold mud crabs (Belgrad and Griffen 2016). This is due to major differences in the hunting strategies of the two predators. In the context of lionfish invasions, individuals of small, benthic fishes that are bolder or simply more active at dusk or dawn can be predicted to be at a higher risk of predation. This is because lionfish hunt at twilight and show a preference for benthic and demersal prey (Cure et al. 2012, Green and Côté 2014, McCallister et al. 2018, D'Agostino et al. 2020, Ritger et al. 2020). We can therefore expect lionfish to select for prey individuals whose activity peaks do not coincide with peaks in lionfish hunting and that are, overall, less active or hide more in their hiding spots. This could lead to changes in the behaviour of populations of native fishes in the invasive ranges of lionfish. Whether such selection pressure is at play in the context of lionfish invasions and any consequences on prey populations has never been investigated.

3.3 Natural enemies

The 'enemy release hypothesis' posits that exotic organisms benefit from reduced top-down control due to a paucity of natural enemies in their newly invaded ranges (Colautti et al. 2004). The success of lionfish as invaders has been attributed to a lack of natural predators in the areas that they invade (Côté and Smith 2018). However, the natural enemies and source of mortality of lionfish in their native range remain unknown. It seems unlikely that any predator feeds consistently on the venomous and spinous adult lionfish and events of predation remain sporadic and anecdotal, both in their native and invaded ranges (Côté and Smith 2018). The cornetfish *Fistularia commersonii*, and the groupers *Epinephelus striatus* and *Mycteroperca tigris* have been reported to feed on lionfish (Bernadsky and Goulet 1991, Maljković et al. 2008). There is also indication that large groupers may act as biological control agents in the Caribbean (Mumby et al. 2011), although large-scale studies suggest that lionfish density does not correlate with that of groupers (Hackerot et al. 2013). In the Mediterranean, the only convincing example of predation is that of an octopus (*Octopus vulgaris*) filmed while catching and carrying a lionfish in Cyprus (Crocetta et al. 2021). The scarce knowledge on lionfish predators limits any conclusions on the importance of relaxed predation as an explanation for the high invasiveness of lionfish.

There are other factors than reduced predation on adults that could explain the large population sizes that lionfish reach in their invaded ranges. First, parasites, rather than

predators, could be limiting the fitness of adults in their native range (Tuttle et al. 2017). This is supported by data from studies that found relatively low numbers of parasites on invasive lionfish in the Atlantic compared to conspecifics in the native Indo-Pacific (Loerch et al. 2015, Sellers et al. 2015, Tuttle et al. 2017). Such comparisons have not yet included Mediterranean lionfish. Second, a main source of mortality for coral reef fishes is predation at or soon after settlement (Carr and Hixon 1995, Webster 2002, Almany and Webster 2006). Predation on larvae and recruits could therefore be the main source of mortality for lionfish (Phillips and Kotrschal 2021). Lionfish larvae are pelagic and probably less defended than the adults (Kitchens et al. 2017) and could be prey of plankton feeders before settlement on the reef and small demersal predators at settlement (Phillips and Kotrschal 2021). Relaxed predation on larvae and recruits could explain the lionfish population increase in their invaded ranges if there was a lower abundance of plankton feeders and predators than in their native range. However, there are no studies comparing the mortality of lionfish recruits between their invasive and native ranges and hypotheses involving lionfish at these stages are difficult to test since spawning in *P. miles* and *P. volitans* has never been described (Côté and Smith 2018).

3.4 Impact on invaded ecosystems

The high effectiveness of lionfish as predators implies that they are a potential threat to the native fish community of the areas that they are invading. *P. volitans* are indeed having a profound impact on the fish community of the western Atlantic, where they prey heavily on numerous species of very high conservation value (Rocha et al. 2015, Ingeman 2016, Côté and Smith 2018). Invasive *P. volitans* can dramatically reduce the biomass of local species in the Atlantic (Albins and Hixon 2008, Green et al. 2012, 2014), with hypothesised effects on the stability of coral reef ecosystems (Lesser and Slattery 2011). The impact of *P. miles* on the Mediterranean biodiversity has received little consideration. Preliminary assessments suggest that lionfish are reducing the abundance of certain native species and are therefore altering the community composition of the Mediterranean (Turan and Doğdu 2022). However, experiments directly linking *P. miles* density with the densities of Mediterranean species are currently lacking.

4. Interactions with humans and control efforts

The high predation rates shown by lionfish raised concerns on their potential effects on economically valuable species and the fishing industry of the Mediterranean (Kleitou et al. 2019a). It is now well-established that *P. miles* do feed on economically valuable species such as blotched picarels (*Spicara spp.*) and Mediterranean parrotfish (*Sparisoma cretense*) (Zannaki et al. 2019, D'Agostino et al. 2020, Savva et al. 2020). However, specific studies on the impact of lionfish on fisheries are completely lacking, both in the western Atlantic

and Mediterranean. Such studies are difficult to conduct as they necessitate large areas and fish stocks are simultaneously subject to many other stressors such as overfishing, climate change and invasive species other than lionfish (Coll et al. 2010). In addition, the possibility of estimating the impacts of lionfish on Mediterranean fisheries is limited by the lack of knowledge on large-scale effects of *P. miles* on the Mediterranean biodiversity.

Lionfish are venomous and reach large population sizes in their invaded ranges (Kulbicki et al. 2012, Aktaş and Mirasoğlu 2017). Consequently, an additional concern around their invasions is that they could become a danger for bathing tourists and divers (Kosker and Ayas 2022). However, lionfish cannot actively sting; the direction of the spines on the body of a lionfish (bending backwards when the fish is swimming forward) does not allow them to actively attack and sting as wasps or bees do. Therefore, unless they are touched with sufficient pressure, it is unlikely that lionfish spines can penetrate human tissues. Moreover, lionfish tend to move away when a swimmer approaches them closely underwater (Côté et al. 2014). This is probably why only few events of envenomation have been reported in the wild, both in the Mediterranean and western Atlantic. On the other hand, most of the envenomation cases reported in the United States were the result of aquarists manipulating lionfish or doing aquarium maintenance at a close distance from lionfish spines and touched them accidentally (Kosker and Ayas 2022). Similarly, although there is no systematic analysis on lionfish-related accidents in the Mediterranean, most of the cases reported in Cyprus involve people (usually fishermen) directly manipulating lionfish out of the water (Jimenez 2021 personal communication).

Lionfish are highly sedentary and easy to identify by divers due to their conspicuous appearance. This resulted in the involvement of lay people in initiatives aimed at curbing lionfish populations through spearfishing. In so-called ‘culling tournaments’ (or ‘derbies’), divers are encouraged to hunt for lionfish by means of spearguns (often simpler Hawaiian slings) while free or SCUBA diving (Kleitou et al. 2021). Fishing for lionfish has been incentivised by attempts to create a market for lionfish-derived products such as meat or jewellery (Kleitou et al. 2019b, Simnitt et al. 2020). While jewellery will probably remain a niche product, lionfish meat is appreciated for its taste and increasingly served in local restaurants in the invasive ranges of lionfish (Morris et al. 2011, Simnitt et al. 2020). Culling initiatives were shown to be an effective way of limiting lionfish populations at small scales in the western Atlantic and have the potential to become a management tool with beneficial effects on the conservation of local species (de León et al. 2013, Green et al. 2014, Dahl et al. 2016).

Culling tournaments were organised in the Mediterranean soon after the start of the invasion (Kleitou et al. 2021). Although culling can be effective at the local scale,

eradication of lionfish from their invaded ranges is considered impossible for three reasons. First, the effort of having to actively spearfish for lionfish is high and limits the areas that can be covered in culling tournaments (de León et al. 2013, Malpica-Cruz et al. 2016, Kleitou et al. 2021). Second, culling initiatives are restricted to relatively shallow waters (0-40 m), while invasive lionfish can live in much deeper waters, with large aggregations spotted even beyond 300 m of depth (de León et al. 2013, Nuttall et al. 2014, Gress et al. 2017, Rocha et al. 2018). Third, lionfish adjust to the hunting pressure posed by spearfishers by becoming more wary towards approaching divers, decreasing the effectiveness of repeated culling initiatives in the same areas (Côté et al. 2014). Culling should therefore be seen as a containment measure, rather than a definitive solution and should be focussed on areas of high ecological interest.

5. Future research avenues

The history and development of the lionfish invasion in the Mediterranean are well-resolved and can be updated promptly through citizen science initiatives involving the aware and collaborative local dive centres (Phillips and Kotrschal 2021). Our update shows that *P. miles* keep expanding westwards and northwards and are also establishing in waters that were considered too cold for them to live in. Future initiatives should keep monitoring the invasion front as *P. miles* can be expected to continue expanding. Such initiatives should consider approaches that include the southern coast of the Mediterranean. The high awareness of the lay public to the problem of invasive *P. miles* in the Mediterranean resulted in the organisation of successful control initiatives at an early stage of the invasion process (Kleitou et al. 2021). While these initiatives can certainly have beneficial effects at the local scale and contribute to raise awareness towards the major problem of biological invasions, eradication of invasive lionfish is considered impossible.

Studies on the predation ecology of *P. miles* in the Mediterranean remain scant, especially in comparison with the large body of literature available on the Atlantic invasion (Côté and Smith 2018). *P. miles* are thriving in the eastern Mediterranean and are feeding extensively on local fishes of ecological and commercial value (Zannaki et al. 2019, D'Agostino et al. 2020, Savva et al. 2020). However, the community-level impact of *P. miles* on the local biodiversity remains unknown. This is a major knowledge gap for ecologists and policy makers alike. While assessing the effect of invasive lionfish on the productivity of local fisheries is challenging due to the large scales needed and many confounding factors, it is possible to experimentally measure community-level effects of predation by lionfish (Albins and Hixon 2008, Green et al. 2012, 2014). This could be done through a long-term field experiment monitoring the fish community of the Mediterranean and how it varies depending on the lionfish density and time of invasion. Such experiment would benefit from a manipulative component, where the lionfish density is controlled in

experimental patches through culls and the fish community is monitored before and after removals (Albins and Hixon 2008, Green et al. 2012). A control (i.e., unculled patches) can be used to account for natural variation and changes in community composition due to seasonality of the Mediterranean.

Prey naïveté is a contributor to the success of lionfish in the Atlantic and Mediterranean, where native prey show virtually no response to this new predator (Anton et al. 2016, D'Agostino et al. 2020). This raises the question of how long it will take local prey to adapt to this new predator through evolutionary change. Invasive lionfish offer an opportunity to test for local adaptations in marine ecosystems, where adaptations to new predators are particularly understudied (Anton et al., 2020). While the high connectivity of marine systems was traditionally thought to limit the possibilities of local adaptations in marine fishes, increasing evidence is suggesting that local adaptation is widespread in marine systems (Anton et al. 2020). The ongoing Mediterranean invasion offers the potential to work with prey populations that have coexisted with lionfish for different lengths of time. This is because many Mediterranean fishes are distributed across the whole basin and lionfish are only present in its eastern part (Phillips and Kotrschal, 2021). Individuals from prey populations that coexisted with lionfish for different lengths of time (from 0 to about 10 years) could be studied in a laboratory setting for their reaction towards an approaching lionfish. Individuals from populations that coexisted with lionfish for longer are expected to show stronger behavioural responses to an approaching lionfish. A major limitation is that, because prey can only be wild-caught, it would be impossible to disentangle the relative effects of innate and learned predator recognition in prey reacting to lionfish.

Individual prey fishes have the potential to learn that lionfish are dangerous through associative learning, even in the absence of coevolutionary history (Brown 2003, Kelley and Magurran 2003). This would give prey an opportunity to rapidly adjust to the presence of new predator. The only study conducted on learned predator responses to lionfish suggests that it is more difficult for prey to learn that lionfish are dangerous compared to other predators (McCormick and Allan 2016). The alleged ability of lionfish to circumvent learned predator recognition in their prey raises the intriguing question of how they do so. We hypothesise that any difficulty in prey learning that lionfish are dangerous might be linked to their unique morphology. According to the hypothesis of 'prepared fear conditioning', fears are acquired more easily and persist for a longer time if the conditioner is an object that had an impact on the survival of the ancestors of a species (Öhman and Mineka, 2001). Because lionfish look remarkably different from other fish predators such as snappers, groupers, and barracudas (Marshall et al., 2019), prey may be less prepared to associate them with danger. The relevance of this hypothesis in the predation ecology of

lionfish could be tested by training naïve (i.e., captive-born) prey to recognise lionfish and other predators as dangerous. This can be done by pairing visual presentations of predators with alarm cues (Brown, 2003) and can be followed by similar experiments based on video presentations of real predators and 3D animated models (Johnson and Basolo, 2003; Fischer et al., 2014; Scherer et al., 2017; Watve and Taborsky, 2019). The use of models shown on screens will allow for changes in the morphology of an approaching lionfish, disentangling which aspects of their morphology and movement contribute the most to their alleged interference with learned predator recognition.

Another major question on the ecology of lionfish, both in their native and invaded ranges, is what their main source of mortality is (Phillips and Kotrschal 2021). This is an important question which could help explain why lionfish reach such high population densities in their invaded ranges. It seems unlikely that any predators feed consistently on adult lionfish because they are well defended by venomous spines and reports of predation events are extremely rare (Côté and Smith 2018). Parasites have been shown to be more abundant on lionfish in their native range compared to the Atlantic, but it is unknown to what extent such parasites exert a control on lionfish population densities (Loerch et al. 2015, Sellers et al. 2015, Tuttle et al. 2017). On the other hand, studies on lionfish parasites in the Mediterranean are entirely lacking. Finally, lionfish could be preyed upon while in their larval or recruit stage but it is challenging to catch lionfish in high numbers before they are juveniles of a few centimetres in length. This is a critical limitation in the possibilities of directly testing the suitability of lionfish to the diet of plankton feeders (Ahrenholz and Morris 2010, Vásquez-Yeomans et al. 2011). To test any hypotheses on the effects of filter feeders it would be necessary to have access to lionfish larvae or eggs, which is currently impossible as they have never been observed spawning (Côté and Smith 2018). However, it is possible to investigate the effects of parasites on lionfish with different approaches. A starting point could be a survey on the parasite load of lionfish fished from the Mediterranean Sea. Such data, combined with the available information from other ranges (Loerch et al. 2015, Sellers et al. 2015, Tuttle et al. 2017), would also ensure that any follow-up manipulative experiments are done with realistic parasite loads. Experimental manipulation of parasite load will reveal how parasites impact lionfish behavioural and physiological traits (Hvas and Bui 2020, Timi and Poulin 2020).

6. Conclusion

While the history and development of the *P. miles* invasion in the Mediterranean are well-resolved and can be easily updated through citizen science initiatives, the study of the predation ecology of invasive *P. miles* is its infancy, especially at high ecological levels. In addition, the ongoing lionfish invasion in the Mediterranean offers the opportunity to test for major fundamental questions on prey naïveté and learned predator responses. We

outlined approaches that could be used to answer these major questions by taking advantage of the ongoing and more recent lionfish invasion in the Mediterranean. Tackling questions such as the community-level impact of lionfish in the Mediterranean and the evolutionary and learned responses in prey will add to the body of knowledge on the best documented invasion in marine ecosystems. This will result in insights into fundamental questions in invasion and predation ecology, but will also be important for policy-makers to estimate the impact of invasive lionfish on human activities.

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Chapter 3 | Regular removals lower invasive lionfish (*Pterois miles*) biomass, but have no effect on the fish community of the Mediterranean Sea

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Abstract

Invasive species are one of the most severe threats to biodiversity worldwide. Invasive predators are particularly harmful as they feed extensively on local species reducing their abundance and causing local extinctions. Lionfish (*Pterois miles/volitans*) are highly effective fish predators and regarded as the most invasive species in the marine realm. Lionfish invaded the western Atlantic Ocean, where they have a negative effect on the abundance and recruitment of coral reef fishes. More recently, lionfish have invaded the Mediterranean Sea through the Suez Canal. The Mediterranean Sea has completely different habitat and fish community from the tropical western Atlantic. Therefore, specific knowledge on the impact of lionfish in the Mediterranean is needed. We assessed the impact of lionfish predation on the Mediterranean fish community in an experimental context for the first time. We manipulated lionfish abundance at six underwater sites (culled sites) on the southern coast of Crete (Greece) by regularly removing lionfish through spearfishing for 11 months. Before every cull, we surveyed the local fish community through underwater visual census. We compared the local fish biomass and community composition of culled sites with that of six unculled sites (control sites). Although culling lowered lionfish biomass, we did not find differences in the local fish biomass and community composition between culled and unculled sites. We interpret these results as the consequence of local fishes moving between our study sites and surrounding areas, with lower lionfish densities. We found a high recolonisation rate at culled sites, indicating that lionfish move for longer distances than previously thought and that culls need to be conducted frequently to lower lionfish biomass in the Mediterranean.

1. Introduction

Biodiversity is decreasing worldwide and humans are the main driver of biodiversity loss (Caro et al. 2022, Jaureguiberry et al. 2022). We threaten biodiversity directly through activities such as lodging and fishing and we are also at the origin of phenomena that have indirect and cascading effects on ecosystems. For example, invasive species are species that establish and spread in a new range at a high rate, often as a result of introductions by humans (Ricciardi 2013). Invasive species severely threaten through various mechanisms linked to lack of coevolutionary history between invader and local species (Jaureguiberry et al. 2022, Haubrock et al. 2025). For instance, the cane toad (*Rhinella marina*) invasion in Australia caused major mortality events of local predators that were naively feeding on the toxic toads and the invasion of African lakes by the Nile perch (*Lates niloticus*) caused the extinction of almost 200 species of endemic cichlids that were naïve to the predator (Witte et al. 1992, Shine 2010). The impact and spread of invasive species can also be exacerbated by climate change (Rahel and Olden 2008, Azzurro et al. 2025, Mitchell and Dominguez Almela 2025).

The Mediterranean Sea (hereafter, Mediterranean) is a sea of high ecological importance and provides ecosystem services to millions of people (Coll et al. 2010, Psomadakis et al. 2012). The Mediterranean biodiversity is threatened by anthropogenic factors such as fishing, pollution, rising temperatures and it is particularly prone to bio-invasions through the Suez Canal (Bianchi and Morri 2000, Galil et al. 2014). The Suez Canal is an artificial canal connecting the Mediterranean with the Red Sea and facilitates movement of organisms between the two basins (Costello et al. 2021). Numerous species from the Red Sea have established in the Mediterranean in recent decades and new ones are reported every year (Galil et al. 2014, 2015, 2017, Fortič et al. 2023, Kleitou et al. 2025). The temperate waters of the land-locked Mediterranean are warming at a faster rate than the global average and it has been suggested that increasing temperatures are favouring the establishment and spread of tropical species from the Red Sea (Hansen et al. 2006, Nykjaer 2009, Pastor et al. 2020, Hassoun et al. 2025). Species that invade the Mediterranean through the Suez Canal are called Lessepsian species and are currently the main contributor to making the Mediterranean the most invaded sea worldwide (Edelist et al. 2013, Galil et al. 2014).

The lionfishes *Pterois miles* and *Pterois volitans* (hereafter, lionfish) are highly invasive (Côté and Smith 2018, Bottacini et al. 2024). Lionfish are native to the Indo-Pacific Ocean and have been invading the tropical western Atlantic Ocean for over four decades. The western Atlantic invasion started from a few individuals released or escaped from aquaria and is still ongoing (Whitfield et al. 2002, Kulbicki et al. 2012, Soares et al. 2025). Since 2016, lionfish have been reported in large numbers in the Mediterranean, where they are now also considered invasive (Kletou et al. 2016, Savva et al. 2020). Genetic studies have shown that the only species of lionfish in the Mediterranean is *P. miles*, which entered the basin through the Suez Canal (Bariche et al. 2017, Bernardi et al. 2024). In the eastern Mediterranean, lionfish have already reached population densities considerably higher than in their native range and are expanding westwards (Kulbicki et al. 2012, Azzurro and Bariche 2017, Phillips et al. 2024, Azzurro et al. 2025). Lionfish are voracious, generalist predators that feed extensively on benthic and demersal fishes (Green et al. 2011, Cure et al. 2012, Green and Côté 2014, Zannaki et al. 2019, D'Agostino et al. 2020, Savva et al. 2020). Studies in the western Atlantic have shown that lionfish cause major biomass declines in local fish communities and have been linked to ecosystem shifts (Albins and Hixon 2008, Lesser and Slattery 2011, Green et al. 2012, 2014).

While the impact of invasive lionfish on the local biodiversity has been studied in the Atlantic, similar knowledge is lacking from the Mediterranean. This information is important, since the Mediterranean is a temperate/subtropical sea, with a completely

different fish community from that of the coral reefs of the tropical western Atlantic (Bottacini et al. 2024). Lionfish are thriving in the Mediterranean, which resulted in some local governments investing in culling to control their populations (Kleitou et al. 2021, 2024, Savva et al. 2024). Culling tournaments are events where free and SCUBA divers are encouraged to remove as many lionfish as possible from an area through spearfishing (Malpica-Cruz et al. 2016). Such events have been shown to reduce lionfish densities, although they have major limitations that prevent them from becoming a large-scale, long-term control method (de León et al. 2013, Kleitou et al. 2021, Goodbody-Gringley et al. 2023, Bottacini et al. 2024, Kleitou et al. 2024). Culling events are organised with the ultimate goal of protecting the local biodiversity (Malpica-Cruz et al. 2016), but no study has assessed whether the Mediterranean fish community indeed responds positively to lionfish removals. We studied the impact of lionfish on the local fish community and the effectiveness of culling as a biodiversity conservation tool for the first time in the Mediterranean. Our study differs from previous attempts aimed at assessing the impact of lionfish in the Mediterranean because of its long duration and fully experimental Before-After, Control-Impact (BACI) design (Turan and Doğdu 2022, Huseyinoglu et al. 2024). We conducted regular lionfish removals at six underwater sites (Impact) in the eastern Mediterranean over 11 months. We surveyed the lionfish density and fish community at four time points (Before-After) and compared them with those at six control sites, where no culls were conducted (Control). Our aims were to assess the speed of recovery of lionfish populations after a cull by repeatedly culling at the same sites and measure the effect of a reduction in lionfish density on the local fish community.

Based on the predatory nature of lionfish and knowledge from the western Atlantic (Albins and Hixon 2008, Lesser and Slattery 2011, Green et al. 2012, 2014), we expected that culling would lead to a gradual increase in the local fish biomass through reduced predation on recruits (Albins and Hixon 2008, Green et al. 2012). These effects should be stronger in the summer, around the peak of recruitment of most coastal fishes in the Mediterranean (García-Rubies and Macpherson 1995). We also expected lionfish to have an impact on the composition of the Mediterranean fish community by feeding more intensely on certain prey species. This could be the result of two mechanisms. First, lionfish prey preference can be influenced by the ecology and morphology of the local species (Green and Côté 2014). Second, a large part of the fish community of the eastern Mediterranean is composed of Lessepsian species (Coll et al. 2010, Galil et al. 2017, Frid et al. 2021). These species coevolved with lionfish in the Red Sea and have been suggested to be less naïve to the predator compared to Mediterranean prey (D'Agostino et al. 2020). Monitoring recolonisation of culled sites is essential to determine how often an area needs to be culled to keep the lionfish density below thresholds that are considered ecologically meaningful.

2. Materials and Methods

2.1 Underwater sites

We selected 12 underwater sites on the southern coast of Crete (fig. 1A), where lionfish are abundant and had been present for almost a decade at the start of our study (Phillips and Kotrschal 2021, Bottacini et al. 2024, Azzurro et al. 2025). The sites were selected after scouting various remote areas through SCUBA diving. We prioritised remote sites to minimise the impact of tourism and recreational fishing on our study. We restricted scouting to areas dominated by rocky reefs, which are the only habitat of lionfish in the Mediterranean (Phillips et al. 2024). We only selected sites in areas in which a diver could count at least 20 lionfish during a dive of one hour. All the sites consisted of a portion of rocky reef, delimited by sand on the deep part and either the water surface or a flat reef edge on the shallow part. The boundaries of a site were chosen arbitrarily and kept constant throughout the study using natural landmarks. The size of the sites was chosen based on the available underwater topography and was limited to an area that could be surveyed during one dive of approximately one hour. The projection of the sites to the water surface varied between 1390 m^2 and 3147 m^2 (tab. S1). The average depth of the sites ranged from 4 to 30 m (tab. S1). We grouped the sites into four clusters (fig. 1B), based on accessibility from land: sites in a same cluster could be accessed from land from the same bay through a short boat or DPV (diver propulsion vehicle) ride from the shore. Because of the considerable distance among clusters, only sites within the same cluster could be visited on the same day. Within each cluster, we randomised the assignment of two treatments: unculled and culled (fig. C-F).

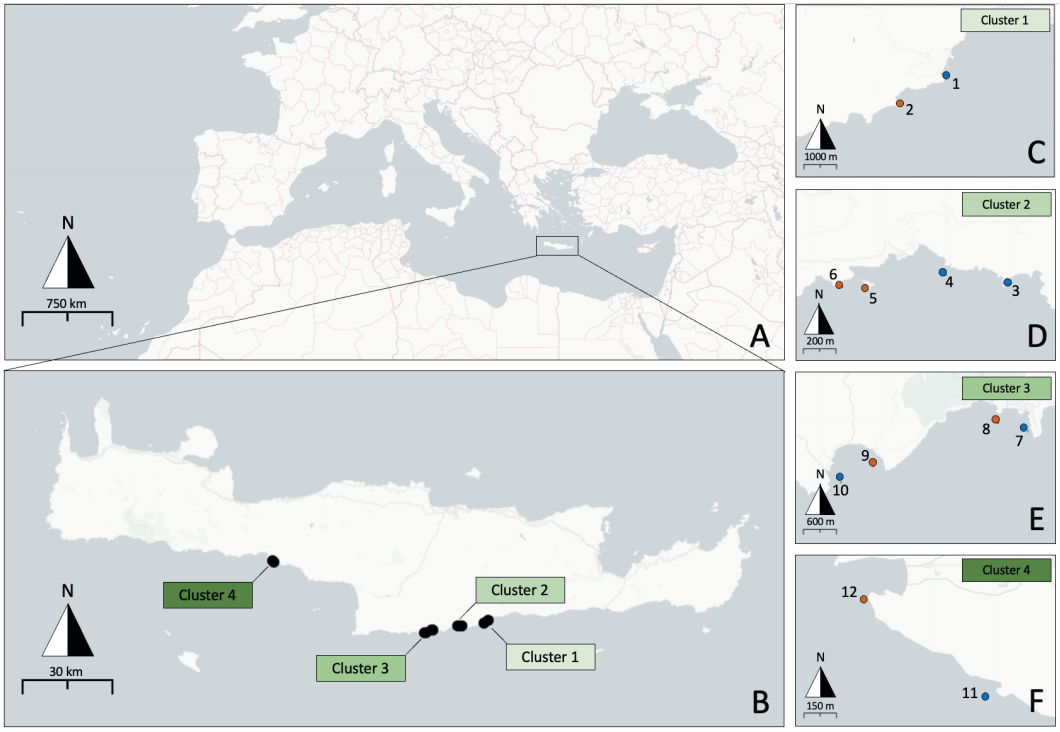

Figure 1. Location of Crete in the Mediterranean Sea (**A**), details on the location of clusters of experimental sites on the southern coast of Crete (**B**) and details on the location of experimental sites within each cluster (**C-F**). Panels C-F show the treatment assigned to each site, with blue dots indicating control (unculled) sites and red dots indicating culled sites.

2.2 Lionfish removals and fish surveys

Field observations encompassed 11 months, with field activities at five time points: November 2023, December 2023, April 2024, July 2024 and October 2024. At every time point, we removed all the lionfish at six sites (culled sites). The names of the time points refer to the beginning of the culling activities. Culling took between one and three weeks to be completed at all sites, depending on the weather conditions and sites accessibility. A culling event consisted of two dives at the same culled site, where a team of SCUBA divers removed every lionfish encountered with Hawaiian slings (Kleitou et al. 2021). There was one occasion when culling took longer to complete: at the second time point, culling started on 30/11/2023 and took until 05/02/2024, due to inclement weather in the winter months. For the sake of simplicity, we refer to this time point as December 2023. During a cull, we removed lionfish from a site and from additional 50 m on either end of the site (buffer zone), to limit immediate recolonisation from neighbouring areas. We weighed every lionfish with a scale and measured their total length with measuring tape. In less than five percent of the cases, lionfish had to be caught alive because they were too small to spear or had to be left dead on the spot due to logistical constraints. In these cases, the length was

estimated visually. At the end of the study, we also culled at all the control sites. To estimate the proportion of lionfish that were not caught during a cull, we re-culled three control and three culled sites 5-13 days after the last cull (additional culls). We assumed that any lionfish present at the sites after such a short time from a cull were already present during the culling event, but were either missed or not detected.

In November 2023, April 2024, July 2024 and October 2024 (survey times), we surveyed the fish community (including lionfish) at every culled and control (unculled) site with underwater visual censuses conducted by SCUBA divers. We restricted our surveys to fishes, since these are the preferred prey of invasive lionfish (Côté and Smith 2018, Bottacini et al. 2024). We conducted the fish surveys in the three weeks preceding the beginning of culling activities to minimise the impact of a culling event on the surveyed biodiversity. In addition, this allowed us to have a measure of the recolonisation rate of lionfish at our culled sites from transect data. This is important since this data is directly comparable to transect data from the control sites. Transects were conducted by a team of two SCUBA divers trained to recognise the local species and the size of objects underwater. Transects were 25 m x 4 m (100 m^2) and were limited to 3 m above the substrate on the third dimension. We visited every dive site at least twice per time point; once in the morning and once in the afternoon. Depending on the depth of a site and logistical constraints, each diver could do between two to six transect per dive. When two divers were surveying, we kept a minimum distance of 5 m between them to minimise disturbance. On every transect, we counted every fish species encountered and estimated their total length in the following size classes: < 2 cm, 2-5 cm, 5-10 cm, 10-15 cm, 15-20 cm, 20-25 cm, > 25 cm. Fishes were identified to the species level, with the exception of gobies (Gobiidae) and blennies (Blenniidae), which were identified at the family level. School and shoal sizes were counted from one to ten and estimated in steps of five after ten. On survey days, visibility was always at least 15 m and current was either absent or low. Transects within the same dive were laid at different depths, moving from deep to shallow within the same dive with the aim of covering the entire depth range of a site.

2.3 Lionfish density, local fish biomass and community composition analyses

To assess the impact of a culling event and recolonisation rates, we analysed the lionfish biomass that was caught during a cull (culling data) with a paired t-test at every time point. This tested the difference at each site between biomass of caught lionfish at each time point and biomass of caught lionfish in the first cull. For lionfish that were not weighed, we calculated weight based on their estimated total length using the formula: weight = e ^ (-0.2996393 + 0.3157668 * length - 0.0037164 * length ^ 2). The coefficients were extracted

from a model that we fit on lionfish of which we measured weight and total length. Because there were no culls at control sites, we used lionfish biomass from the fish surveys (transect data) to compare the density of lionfish in control and culled sites throughout our study. We used the same formula and coefficients to calculate biomass of lionfish at control and culled sites based on count data and size classes, using the mid-point of each size class as an estimated total length. We analysed lionfish biomass with a generalised linear mixed model (GLMM) with treatment (culled or unculled), time point and their interaction as fixed factors and site as a random factor.

From the transect data, we calculated biomass based on length-weight relationships specific for the species, with coefficients from FishBase (Froese and Pauly, 2026). When coefficients were not available for a species, we used coefficients from morphologically similar species. We assigned every species to the categories demersal, pelagic or benthic, depending on their ecology. Most of the biomass on our transects was composed of demersal species between size classes 2-5 cm and 20-25 cm (fig. S1), which correspond to what lionfish eat in the Mediterranean (Zannaki et al. 2019, D'Agostino et al. 2020, Savva et al. 2020). Therefore, all our analyses on biomass and fish community were conducted on demersal fishes of size classes 2-5 cm and 20-25 cm. We used the mid-point of a size class as an estimate for the total length of every fish counted in the size class. For all the analyses on the fish biomass and community composition, we excluded lionfish from the data set. We modelled the fish biomass with a GLMM that included time point, treatment, size class and their interactions as fixed factors and site as a random factor. Biomass was averaged across transects for every species, size class, time point and site prior to analysis. We are not aware of similar long-term experimental studies on the fish community in the Mediterranean. Therefore, we also performed power analysis to assess the minimum difference in biomass that could have been detected in our study design between treatments at each time point. We did power analysis based on a separate Montecarlo simulation for each time point, based on the most similar model to our final one.

We analysed for potential shifts or changes in the community composition through principal component analysis (PCA). We computed principal components for the biomass of every fish species at every site and time point. If the biomass at a specific combination of these three factors was 0, we removed it from the PCA for computational reasons. We did cluster analysis on the scores along principal components, under the prediction that sites in the same treatment should progressively cluster together after the first culls (i.e., after November 2023). We also conducted non-metric multidimensional scaling (NMDS) on the same data used for PCA, with a focus on the first and last survey point (November 2023 and October 2024). We computed the direction of the vectors connecting the scores along NMDS1 and NMDS2 at the first and last survey time of the same site. We used

circular statistics (Watson-Williams test for homogeneity of means) to test the prediction that the direction of vectors was different between sites of the two treatments.

We used R 4.4.1 (R Core Team 2024) for all statistical analyses and visualisations. We fit GLMMs with the 'glmmTMB' (Brooks et al. 2017) and the 'lme4' packages (Bates et al. 2015). We included interaction terms when they were biologically meaningful and checked for violations of the model assumptions using the package and test of assumptions were done with the package 'DHARMa' (Hartig 2022). We used the package 'simr' (Green and MacLeod 2016) for power analysis, 'vegan' (Oksanen et al. 2001) for multivariate analyses on the community data and 'circular' (Lund and Agostinelli 2004) for circular statistics. We used 'tidyverse' (Wickham et al. 2019) for visualisations of plots and maps.

3. Results

3.1 Lionfish abundance

During the first culling event (November 2023), we removed a mean lionfish biomass of 16.5 kg/site (fig. 2). During subsequent culls, the biomass removed was significantly lower than at the first time point: 1.8 kg/site in December 2023 (paired t-test, $p < 0.001$), 1.9 kg/site in April 2024 (paired t-test, $p < 0.001p < 0.001$), 11.7 kg/site in July 2024 (paired t-test, $p < 0.001p = 0.002$), 9.4 kg/site in October 2024 (paired t-test, $p < 0.001p = 0.009$) (fig. 2). This trend was consistent across sites (fig. S2) and did not change when the area of a site was accounted for (fig. S3). Recolonisation rates varied between 11 and 72% (tab. 1) and the additional culls conducted at the end of the experiment indicate that approximately 18% of the lionfish present at a site was not caught during a cull (tab. 2). Before culling, we had comparable lionfish biomass between culled (mean density = 23.0 kg/ha) and control sites (mean density = 18.3 kg/ha) (GLMM, $p = 0.737$) (fig. 3). After the first cull, control sites had a higher lionfish biomass than culled ones: in April 2024 difference = 16.1 kg/ha (GLMM, $p = 0.015$), in July 2024 difference = 16.3 kg/ha (GLMM, $p < 0.001$), in October 2024 difference = 22.9 kg/ha (GLMM, $p = 0.109$). Lionfish counts show a very similar trend (fig. S4). Lionfish biomass made up between 0.9% and 20% of the total fish biomass during the study (tab. S2). In the control sites, the mean lionfish biomass increased throughout the study, although not always significantly (fig. 3). This was probably caused by high and inconsistent variation in lionfish biomass across control sites throughout the study (fig. S5).

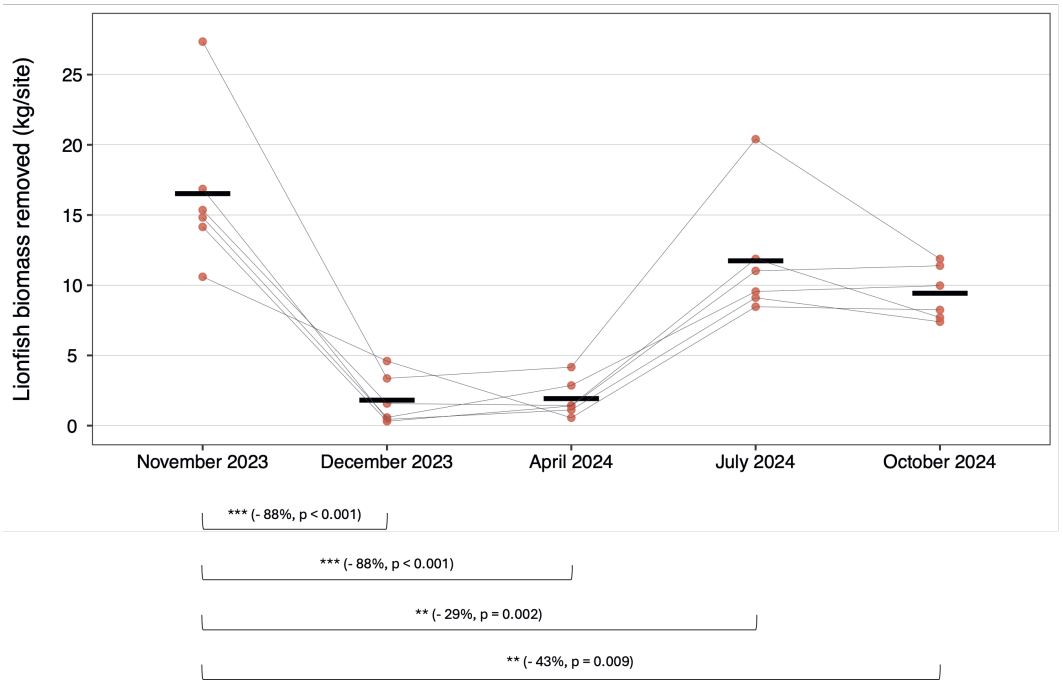

Figure 2. Lionfish biomass (kg/site) removed from culled sites throughout the study. Red dots represent the biomass of lionfish removed at each of the six culled sites and horizontal lines represent means for each time point. The thin grey lines connect data points from the same site. P-values are two-sided and based on paired t-tests on the differences between biomass removed from a site at every time point and biomass removed at the same site at the first time point.

Table 1. Lionfish catch (number of lionfish removed) at every culled site throughout the study. Absolute numbers show the counts of lionfish removed at each culling event and relative numbers show the ratios between the absolute numbers at each time point and the absolute numbers in November 2023. The 'mean catch' row shows the mean for every column.

Site n.	Lionfish catch (n. lionfish)								
	November 2023	December 2023		April 2024		July 2024		October 2024	
	absolute	absolute	relative	absolute	relative	absolute	relative	absolute	relative
2	79	9	0.11	5	0.06	51	0.65	43	0.54
5	79	5	0.06	14	0.18	46	0.58	62	0.78
6	65	3	0.05	4	0.06	33	0.51	35	0.54
8	53	13	0.25	3	0.06	43	0.81	41	0.77
9	108	14	0.13	16	0.15	93	0.86	61	0.56
12	52	2	0.04	6	0.12	48	0.92	47	0.90
Mean catch	**73**	**8**	**0.11**	**8**	**0.10**	**52**	**0.72**	**48**	**0.68**

Table 2. Lionfish catch (number of lionfish removed) at the end of the study at three culled and three unculled sites. Absolute numbers show the count of lionfish removed at each site in the last culling event and the number of lionfish removed during an additional cull conducted a few days later. Relative numbers show the ratios between the absolute numbers at the additional cull and the numbers at the last cull. The 'time' column indicates how many days after the last cull the additional cull was conducted. The 'mean catch' row shows the mean for every column.

Site n.	Treatment	Lionfish catch (n. lionfish)			Time (days)
		Last cull	Additional cull		
		absolute	absolute	relative	
3	Unculled	134	13	0.10	10
4	Unculled	50	9	0.18	11
5	Culled	62	18	0.29	5
6	Culled	35	3	0.09	13
7	Unculled	39	10	0.26	13
8	Culled	43	12	0.28	9
Mean catch		**61**	**11**	**0.18**	**10**

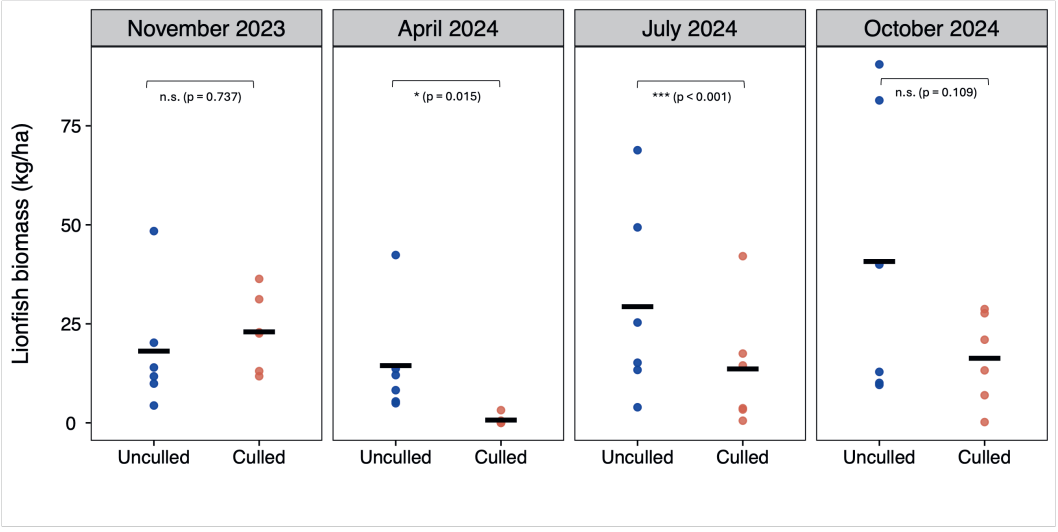

Figure 3. Difference in lionfish biomass (kg/ha) between culled and control sites during the study. Dots represent the mean lionfish biomass calculated from transect data at each unculled (blue) and culled (red) site at each time point. Horizontal lines represent means for each time point and treatment. P-values are two-sided and based on a models of log-transformed lionfish biomass.

3.2 Fish biomass and community composition

Fish biomass did not differ systematically between culled and control sites throughout the study (GLMM, p = 0.43, p = 0.08, p = 0.39, p = 0.34 at the four time points) (fig. 4). This was also true for the three most abundant species on the transects taken individually: *Chromis chromis*, *Siganus rivulatus* and *Thalassoma pavo* (fig. S6). The power to detect differences in biomass between culled and control sites was 20%, 60%, 90% and 100% for biomass differences of 25%, 50%, 75% and 100% (tab. 3). Lionfish removals did not affect the composition of the fish community throughout the study: cluster analysis revealed clustering patterns that did not match the distinction between culled and unculled sites (fig. 5 and S7) and the mean orientation of the vectors from NMDS analysis was not different between the treatments (Watson-Williams test, F = 0.12, d.f.1 = 1, d.f.2 = 10, p = 0.74) (fig. 6).

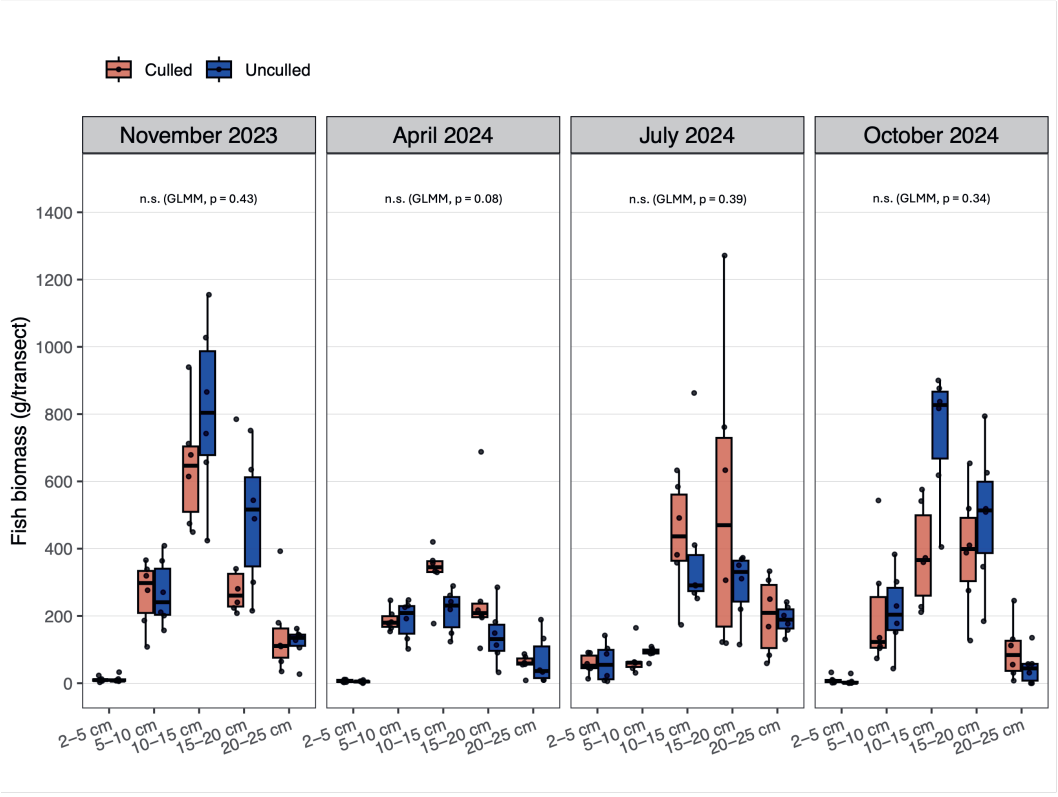

Figure 4. Fish biomass (g/transect) throughout the study, calculated from transect data. The surface of a transect is 100 m^2. Dots represent the mean biomass for each site at each time point and boxes show summary statistics for culled (red) and unculled (blue) sites at each time point.

Table 3. Outcome of power analysis. **Effect size** indicates a difference (in g/transect) that would be expected at each trip after the first cull if culling resulted in a 25%, 50%, 75% or 100% biomass increase. The differences were calculated as proportions of the mean biomass at each time point. **Power** indicates the probability of each difference to be detected as significant in the GLMM at alpha = 0.05. The actual differences extracted from the model were: 105, 112 and -26 g/transect for April 2024, July 2024 and October 2024 respectively.

Effect size	Survey time	Power	Effect size	Survey time	Power
25% (58 g/transect)	April 2024	22%	75% (175 g/transect)	April 2024	92%
	July 2024	20%		July 2024	91%
	October 2024	20%		October 2024	92%
50% (117 g/transect)	April 2024	61%	100% (233 g/transect)	April 2024	100%
	July 2024	60%		July 2024	99%
	October 2024	63%		October 2024	100%

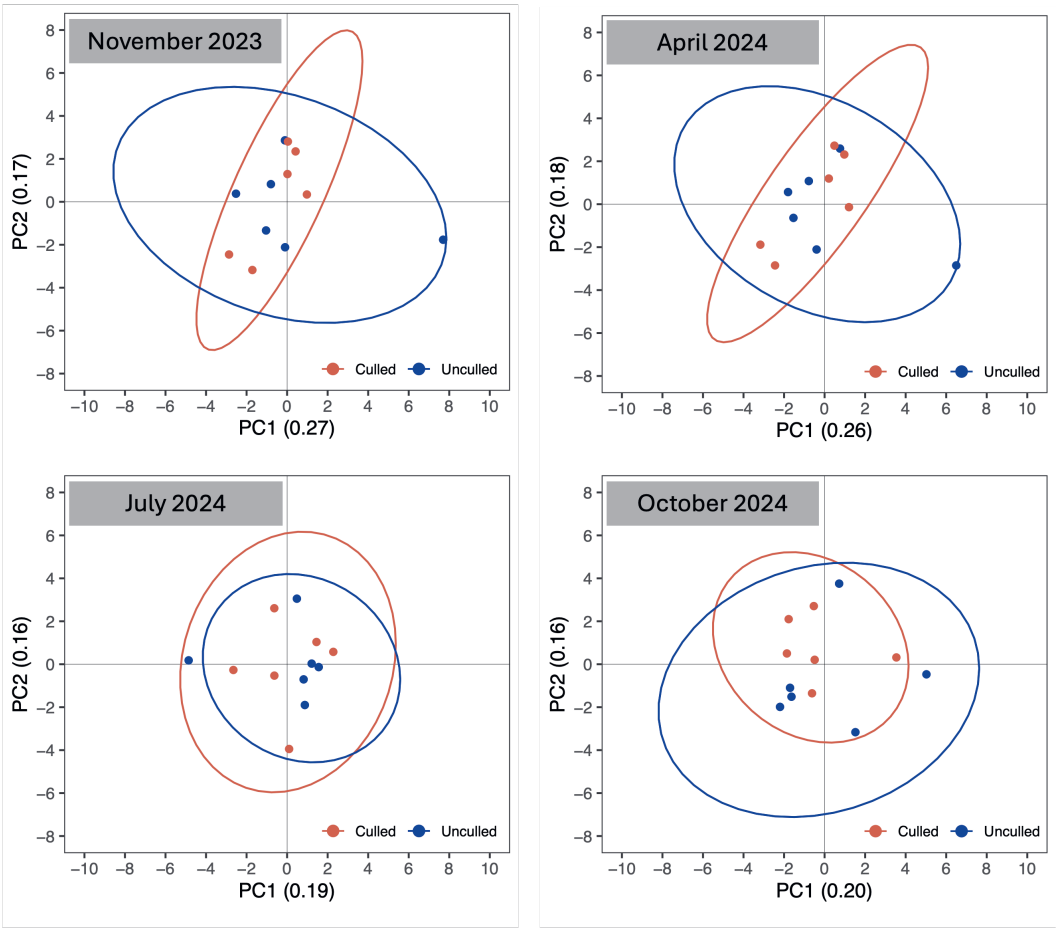

Figure 5. Effect of culling on the composition of the fish community from cluster analysis on principal components (PC). The plots show scores along PC1 and PC2 of fish biomass at each of the 12 sites at the four survey times (culled sites in red, unculled sites in blue). Numbers in brackets show the proportion of the variance explained by a PC. PCs were computed for the biomass of every fish species at every site and time point.

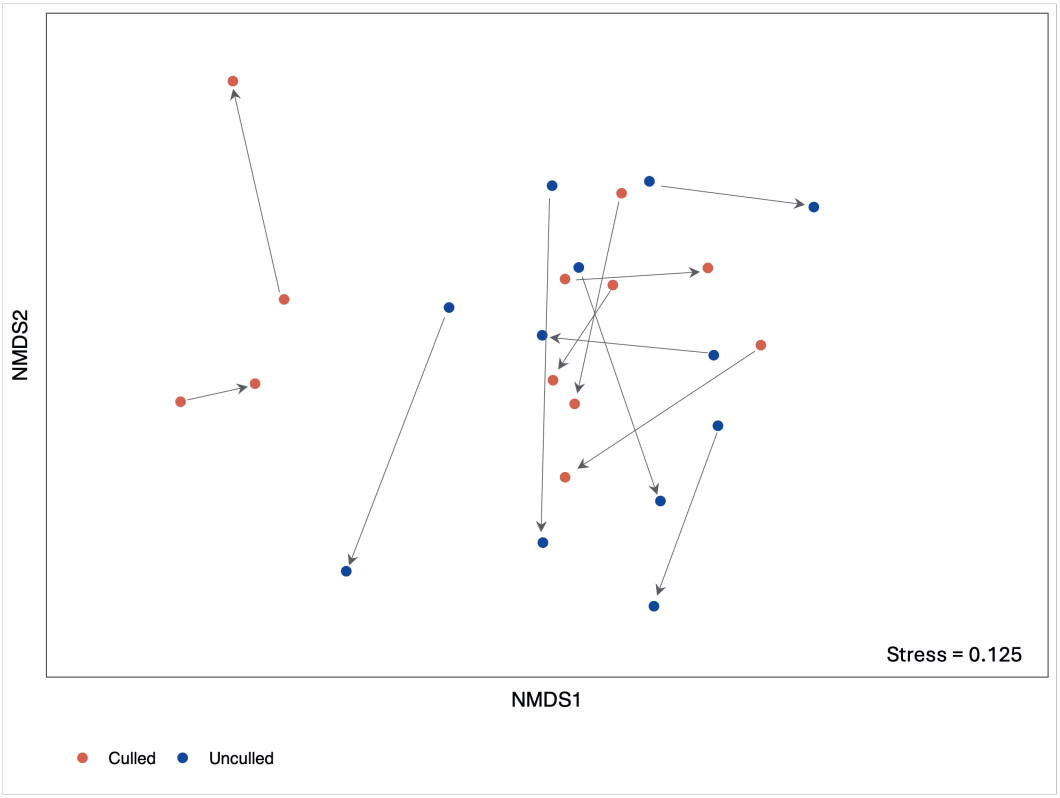

Figure 6. Effect of culling on the fish community from non-metric multidimensional scaling (NMDS) ordination. The plot shows scores of culled (red) and unculled (blue) sites on NMDS1 and NMDS2 at the first (November 2023) and last (October 2024) survey point. Arrows connect dots representing the same site, with the tip of the arrow indicating the points of the last survey. NMDS1 and NMDS2 were computed for the biomass of every fish species, at every site for November 2023 and October 2024 respectively.

4. Discussion

We assessed the impact of lionfish predation on the Mediterranean fish community with an experimental approach for the first time. We repeatedly removed lionfish from six sites on the southern coast of Crete for 11 months, reducing the lionfish biomass compared to six control sites. We found a high influx of new lionfish into culled sites after a cull, which highlights that frequent culling is necessary if the goal is to lower the lionfish density in the Mediterranean. Lowering the lionfish biomass through culling did not result in any difference in the biomass and community composition of local fishes between control and culled sites. A similar fish biomass between treatments in our experiment indicates that lionfish are not significantly decreasing the abundance of local fishes in the Mediterranean to the extent with which they are affecting the fish community of the tropical western

Atlantic. A similar community composition between culled and control sites suggests that lionfish predation is not specific to particular species or size classes in the Mediterranean. We interpret our results in the context of movement patterns of lionfish and local species and differences between the Mediterranean invasion and the western Atlantic one.

During 11 months of monitoring, the lionfish density at the control sites varied between 120 and 150 lionfish/ha. Although this is lower than the 400 lionfish/ha reported in some areas of the western Atlantic, it is remarkably higher than the 2-30 lionfish/ha in the native range and comparable to densities reported in other studies in the eastern Mediterranean (Green and Côté 2009, Kulbicki et al. 2012, McTee and Grubich 2014, Gavriel et al. 2021, Kleitou et al. 2021, 2024, Phillips et al. 2024). While lionfish densities are convenient to compare studies across ranges, we focussed our analyses on lionfish biomass. The amount of prey biomass consumed by lionfish strongly depends on the size of the individuals (Green et al. 2011). Therefore, lionfish biomass, rather than density, is the most important parameter determining the prey consumption of a lionfish population. Overall, culling resulted in a lower lionfish biomass at culled sites compared to controls. We measured comparable lionfish biomass in control and culled sites before culling, as expected from the random assignment of our treatments to the 12 sites. After the first cull, we measured lower biomass in culled sites compared to the control at every survey. In addition, at every cull we removed significantly lower lionfish biomass than at the first cull, confirming that culling lowered the lionfish biomass at the culled sites. Culled biomass suggests that lionfish biomass calculated from transect data underestimated the lionfish biomass at our study sites by approximately two- or three-fold. This did not affect any of our conclusions on differences between lionfish biomass between the two treatments, since we only used biomass from transect data for such comparisons.

Although repeated culling effectively lowered the lionfish biomass in culled sites, it did not affect the composition of the local fish community. Lionfish feed preferentially on demersal and benthic species, which were the primary target of our surveys (Green and Côté 2009, 2014, D'Agostino et al. 2020). Lionfish were also shown to feed preferentially on fishes of small size and of species with shallow body depth in the western Atlantic (Green and Côté 2014). This has the potential to result in a shift in the composition of the fish community of areas with high lionfish biomass in favour of larger size classes and species with deeper bodies. Our results could simply indicate that, in the Mediterranean, lionfish feed on prey with demersal and benthic ecology without any species-specific preference. In this scenario, lionfish are expected to feed on each prey with intensities that depend solely on their relative local abundance. Studies combining stomach content analyses, prey targeted and local availability of each prey species may help clarify this aspect in the future (Green and Côté 2014). A change in the composition of the local fish community could also derive from less intense predation by lionfish on species that are less

associated with the bottom, as opposed to demersal and benthic ones (Zannaki et al. 2019, D'Agostino et al. 2020). We restricted our fish surveys to 3 m above the substrate, which limited the resolution of our data on pelagic species. However, a lack of changes in the community composition is also plausible if pelagic species were accounted for, since they often find refuge on the reef at twilight, the time in which lionfish hunt most actively and could therefore be preyed upon extensively by lionfish (Green et al. 2011, D'Agostino et al. 2020). Our data indicates that any putative lower level of naïveté in Lessepsian species towards lionfish is probably not relevant for prey survival (D'Agostino et al. 2020). If that was the case, we would have found a shift in the fish community composition in favour of Lessepsian species at our control sites.

In the Bahamas, lionfish predation resulted in a reduction of recruit counts of 79% and increases in lionfish densities correlated with a 65% decrease in recruit biomass (Albins and Hixon 2008, Green et al. 2012). In the same area, lionfish removals resulted in increases of prey biomass of up to 70% compared to reefs with high lionfish abundance (Green et al. 2014). In contrast, we found no difference in biomass between control and culled sites, although the power of our experiment was enough to detect changes in the order of magnitude in the Bahamas. Lionfish became invasive in the Mediterranean more recently than in the western Atlantic and were first reported in Crete in 2016, (Phillips and Kotrschal 2021, Bottacini et al. 2024). Therefore, lionfish had been in the study area for about seven years before our study. Studies in the Bahamas were also conducted two to six years after lionfish were reported in high densities in the area, suggesting that time after invasion is probably not what is causing these differences (Albins and Hixon 2008, Green et al. 2014, Bottacini et al. 2024). However, lionfish densities in the Mediterranean are overall lower than those in the western Atlantic (Green and Côté 2009, Kleitou et al. 2021, 2024, Phillips et al. 2024). We selected our experimental sites based on high lionfish densities, but the sites were in areas where the overall lionfish density was probably lower. Therefore, it is possible that prey fish were moving between experimental sites and neighbouring areas with lower lionfish densities. Considering the record lionfish densities in Bahamian coral reefs and the longer history of the invasion in the western Atlantic, the situation might have been different in the studies in the Bahamas (Green and Côté 2009).

In the experiments in the Bahamas, observations were conducted on small, isolated coral patches, and had a strong focus on recruits. This was either because recruits were the only target of surveys or because the strongest effects of lionfish predation were found on recruits and small size classes (Albins and Hixon 2008, Green et al. 2014). This setting probably limited the influence of surrounding areas on the study sites, since recruits are expected to move less than fishes of larger size classes, especially between coral patches

isolated by sand. Yet, when focussing on the smallest size classes in our study, we also found no difference between control and culled sites. The limited information on Mediterranean recruits, their survival rate and their movement patterns comes from the western part of the basin (García-Rubies and Macpherson 1995, Félix-Hackradt et al. 2013). Most of the smallest individuals counted during our surveys were 2-5 cm in total length, while recruits of most Mediterranean species are smaller (García-Rubies and Macpherson 1995). This suggests that the individuals that we surveyed could have been several weeks old, which could have given them enough time to move between study sites and surrounding areas. This is a different scenario from that of studies in the western Atlantic, where frequent monitoring around peaks of recruitment allowed for counts on the youngest recruits (Albins and Hixon 2008, Green et al. 2014). In addition, in our experiment movement of recruits may have been facilitated by the high connectivity of our study sites, which were not completely isolated. Future experiments with frequent surveys centred around peaks of recruitment on isolated rocky pinnacles in the Mediterranean could mitigate this possible drawback. However, it will be challenging to time an experiment around peak of recruitment in the Mediterranean, since this varies strongly depending on the species (García-Rubies and Macpherson 1995). Moreover, the recruits of most species were never found in our transects, suggesting that many species either recruit on other substrates or in shallower areas. More information on eastern Mediterranean recruits and their movement is needed to plan future experiments more accurately.

We recorded rapid and substantial recolonisation of the culled sites in our study. This raises the important question of the origin of the lionfish that were caught after the first cull. Part of these could be individuals that were not caught during previous culls, either because they were undetected due to the high complexity of the substrate or because they were missed while spearing. Short-term culling data suggests that the lionfish missed and undetected were, on average, 18 percent. However, in the last two culls (July 2024 and October 2024), we culled approximately 70 percent of the lionfish that were caught on the first cull. This suggests that approximately 50 percent of the lionfish culled in July 2024 and October 2024 were individuals that entered the study sites in between culls. Recruitment can be excluded as a source of lionfish recolonising our culled sites since the time in between culls was not sufficient for new lionfish recruits to reach the large size of the individuals that we culled (Savva et al. 2020). In addition, we only encountered lionfish recruits (approximately 2 cm in total length) in October and November, both in 2023 and 2024. Local dive centres confirmed that they encounter lionfish recruits only in the autumn. This suggests that the lionfish reproductive season is shorter than predicted in the Mediterranean and confirms that recruitment does not explain recolonisation of the culled sites (Mouchlianitis et al. 2022). Neighbouring areas are the most plausible source of recolonising lionfish and that adult lionfish are less site-attached than previous studies

suggested (Jud and Layman 2012, Akins et al. 2014, Phillips et al. 2024). Culling data indicates that recolonisation happened more intensely during the summer, which matches previous knowledge from the Mediterranean (Kleitou et al. 2021, Huseyinoglu et al. 2024). The drivers of lionfish movements and their seasonal patterns in the Mediterranean remain a mystery that could be solved by future studies tackling movement through acoustic telemetry (Jud and Layman 2012, Akins et al. 2014, Gavriel et al. 2021).

In the experiments in the western Atlantic, weekly or monthly interventions on lionfish densities were necessary to result in effects on the local fish community (Albins and Hixon 2008, Green et al. 2014). While effective and very accurate in an experimental context, such frequent manipulations cannot be implemented as a population control method at a larger scale. In our experiment, we conducted five culls over the course of 11 months, which is a frequency that is possible to maintain through public culling tournaments (Kleitou et al. 2021). However, lionfish recolonisation rates were high and a lack of effect on the local fish community indicates that culling is not an effective solution to invasive lionfish if the goal is to protect the local biodiversity. Measuring the impact of invasive species through experimental approaches is paramount to devise mitigation strategies, especially when the invaded ecosystems are facing multiple threats (Coll et al. 2010). In the eastern Mediterranean, *P. miles* is only one of several Lessepsian species present in high densities and having potential overlapping impacts (D'Agostino et al. 2020, Tosunoğlu et al. 2026). For example, the rabbitfishes *Siganus rivulatus* and *Siganus luridus* were shown to be the cause of barren reefs in the eastern Mediterranean through an exclusion experiment that linked their grazing activity with the reduction in macroalgal cover (Sala et al. 2011). For local species that use macroalgae as spawning ground or nesting material, this could be detrimental in the breeding season (Raventos 2004, Raposeiro and Azevedo 2009). Predation by invasive predators such as lionfish and the cornetfish *Fistularia commersonii* could be exacerbating the effect of reduced substrate complexity on these species. While monitoring efforts are effective at detecting the arrival of exotic species and monitoring their spread in the Mediterranean, our general knowledge of the impact of Lessepsian species is limited (Azzurro et al. 2022a, Bottacini et al. 2024, Azzurro et al. 2025). Our study highlighted that we need a better understanding of the fundamental ecology and biology of local species and invasive ones to study how invasive species interact with an ecosystem. In the context of the lionfish invasion in the eastern Mediterranean, we highlight the (seasonal) movement of lionfish and substrate preference and movement patterns of recruits and juveniles of local species as fruitful research avenues.

Acknowledgements
We thank Anna Schoonhoven, Hannah de Waele, Kees Kalverboer, Cristina Beams and Derian Palma Quirós for their help and assistance in the field.

Ethical statement
The full experimental protocol, including the removal and sampling of wild lionfish through spearfishing, was approved by the Committee for Research Ethics of the Hellenic Centre for Marine Research (permit n. 125/2023, issued on 12th September 2023).

Additional permits
Lionfish culling and sampling using scientific diving and spearfishing tools was authorized by the Hellenic Ministry of Environment and Energy under a sample collection permit (permit n. ΥΠΕΝ/ΔΔΔ/90677/2894, issued on 27th September 2023).

Supplementary material

Table S1. Details of the underwater sites and their location. Size is the surface of the projection of a site to the water surface, measured through a GPS device from the surface. Min. and max. depth are the shallowest and deepest point that were available for surveys on a site.

Site n.	Latitude	Longitude	Size (m^2)	Cluster	Treatment	Min. depth (m)	Max. depth (m)
1	34.969668	25.272387	1390	1	control	6	19
2	34.962512	25.256927	2208	1	culled	4	12
3	34.953681	25.169671	2035	2	control	6	19
4	34.954299	25.164208	2084	2	control	5	11
5	34.953293	25.157573	1985	2	culled	5	16
6	34.953484	25.155473	2318	2	culled	6	13
7	34.938256	25.052984	1954	3	control	5	13
8	34.939506	25.047666	1785	3	culled	6	17
9	34.933144	25.025373	2662	3	culled	9	25
10	34.930832	25.019563	3147	3	control	11	23
11	35.161989	24.425087	1671	4	control	8	22
12	35.165411	24.419752	2142	4	culled	6	30

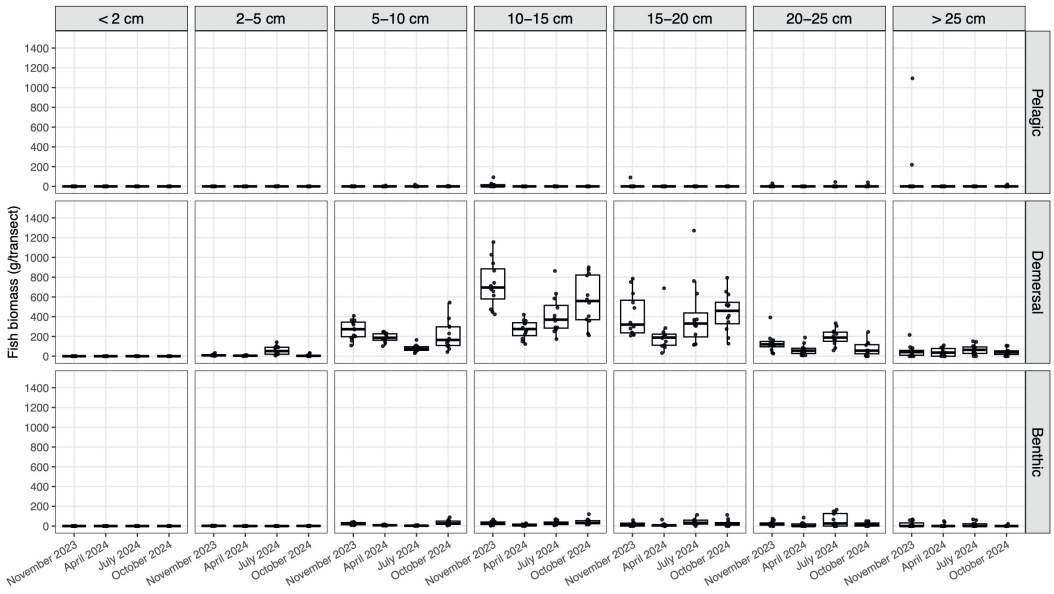

Figure S1. Fish biomass (g/transect) of pelagic, benthic and demersal fishes of each size class and at each survey time in the study. The surface of a transect is 100 m^2.

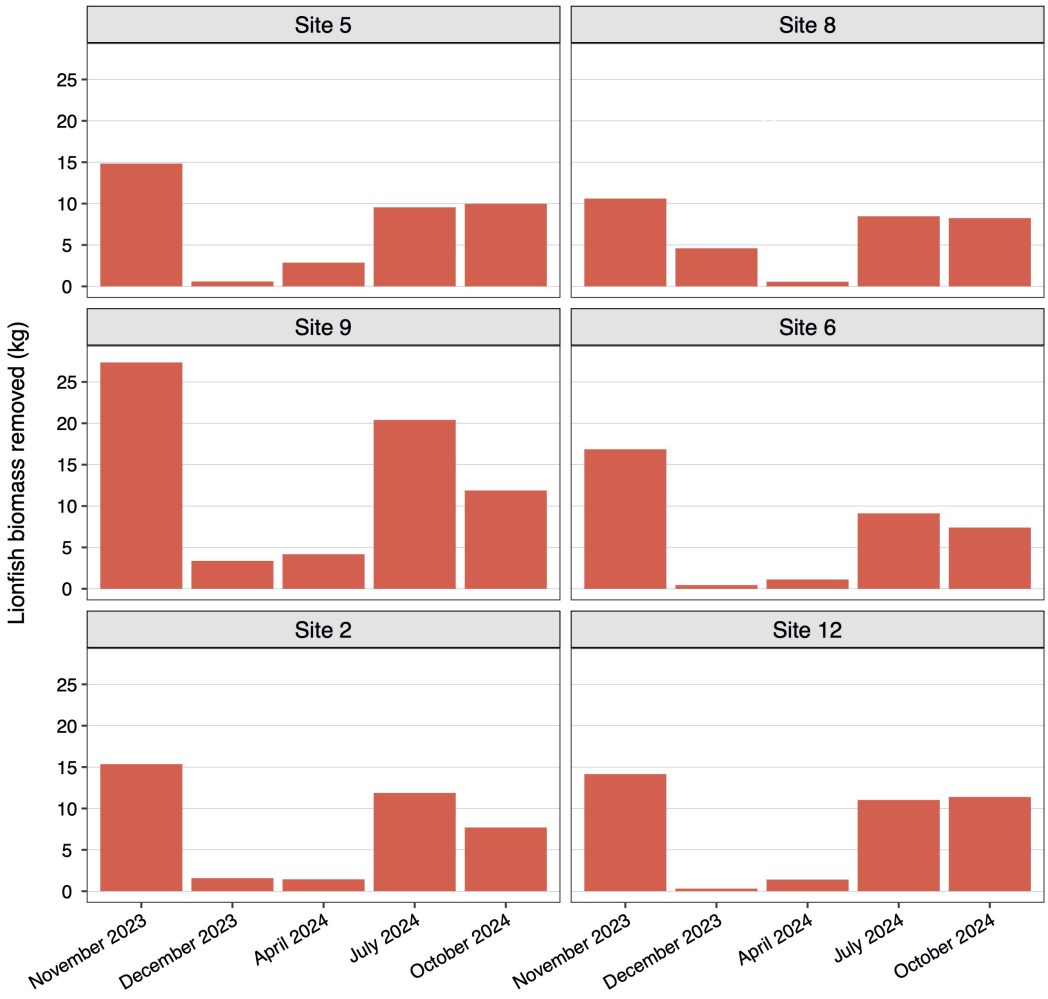

Figure S2. Lionfish biomass (kg) removed from each culled site throughout the study.

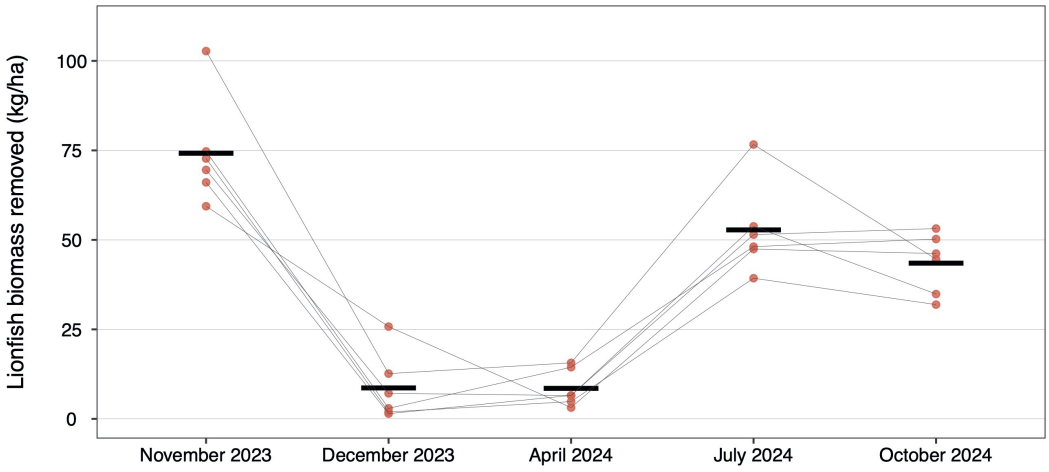

Figure S3. Lionfish biomass (kg/ha) removed from culled sites throughout the study. Kg/ha were obtained by dividing the biomass removed from a site by the surface of that site. Please, note that we used site area for the calculations, but culling was conducted over an area that included a buffer (see materials and methods). Red dots represent the raw data at each of the six culled sites and horizontal lines represent means for each time point. The thin grey lines connect data points from the same site.

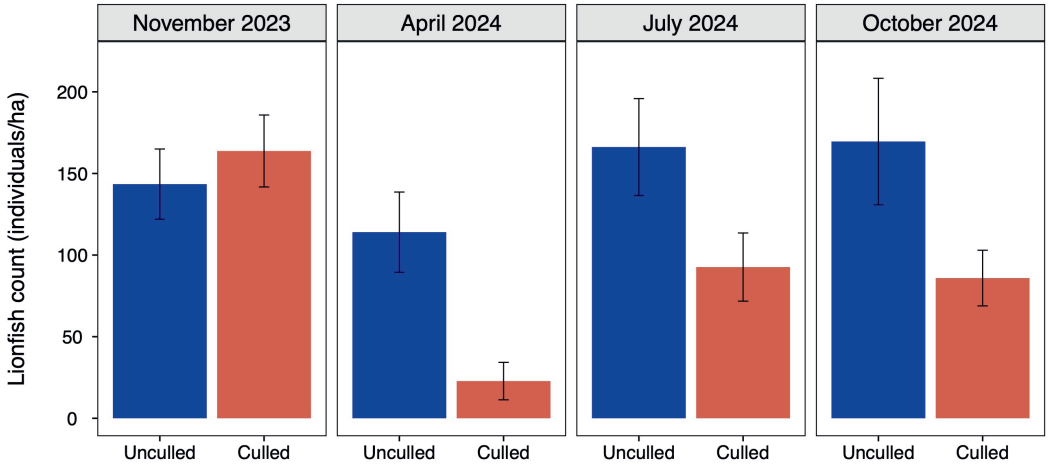

Figure S4. Lionfish counts (individuals/ha) from transect data at culled and unculled sites during the study.

Table S2. Proportion of fish biomass made up by lionfish at culled and unculled sites throughout the study. N is the number of transects.

Survey time	Treatment	Prey (g/100m^2)	Lionfish (g/100m^2)	Total (g/100m^2)	Lionfish relative (%)	n
November 2023	Unculled	1915	183	2098	8.7	69
	Culled	1822	230	2052	11.2	57
April 2024	Unculled	674	169	843	20.0	68
	Culled	948	8	957	0.9	71
July 2024	Unculled	1221	286	1507	19.0	69
	Culled	1537	123	1660	7.4	57
October 2024	Unculled	1710	394	2104	18.7	68
	Culled	1240	165	1406	11.8	69

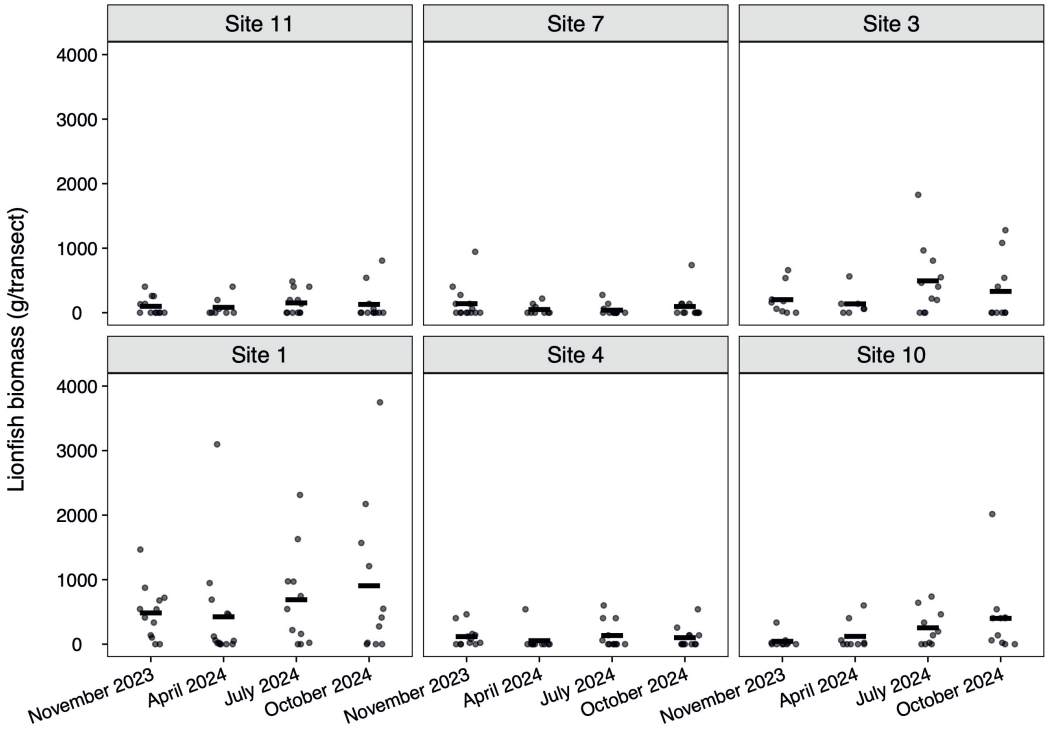

Figure S5. Lionfish biomass (g/transect) from transect data at the control sites throughout the study. The surface of a transect is 100 m^2. One, single value of 5653 g/transect was removed from October 2024 st Site 3 for the sake of visualisation.

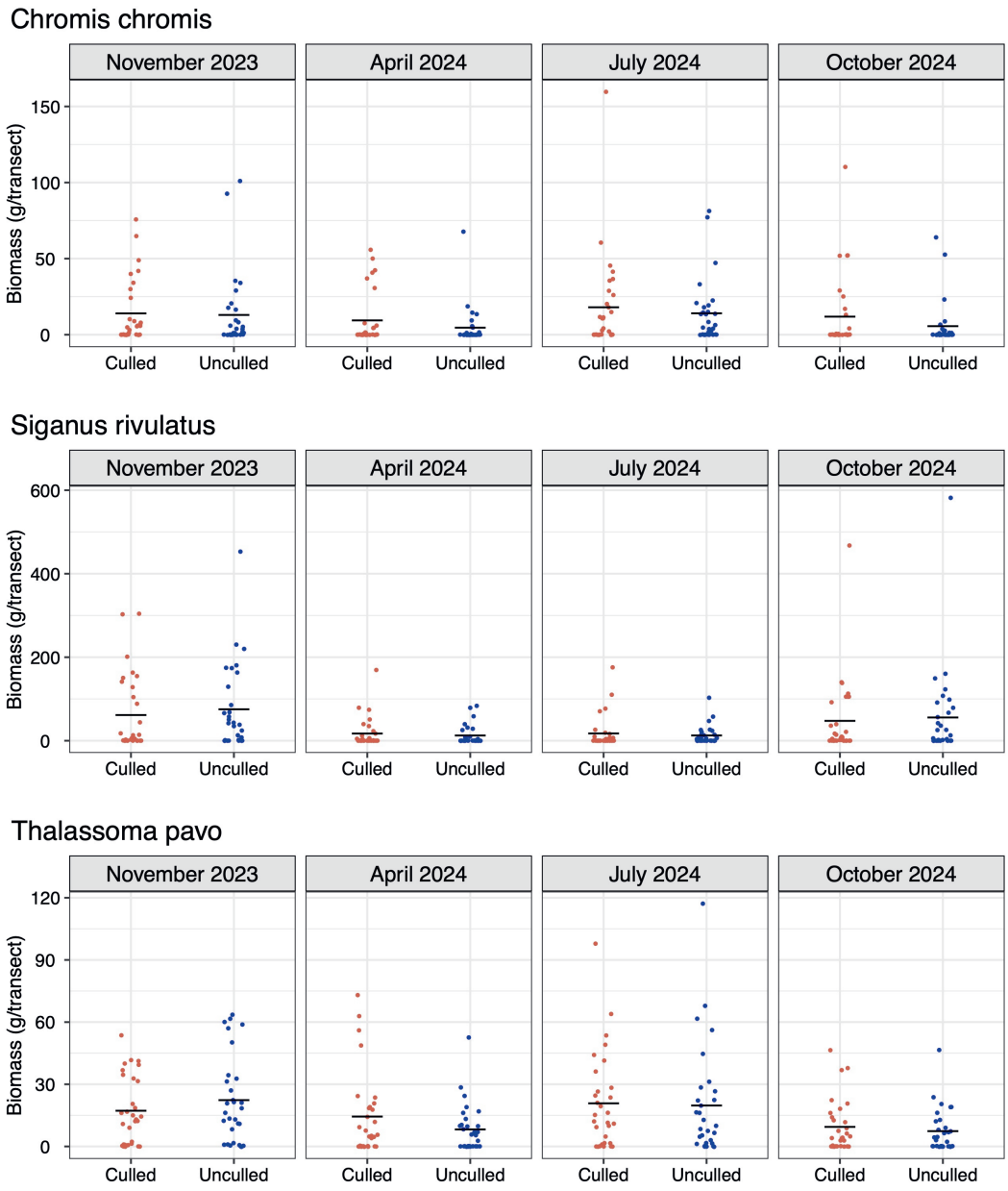

Figure S6. Biomass (g/transect) of three most abundant species at the study sites (*Chromis chromis*, *Siganus rivulatus* and *Thalassoma pavo*) throughout the study. The surface of a transect is 100 m^2.

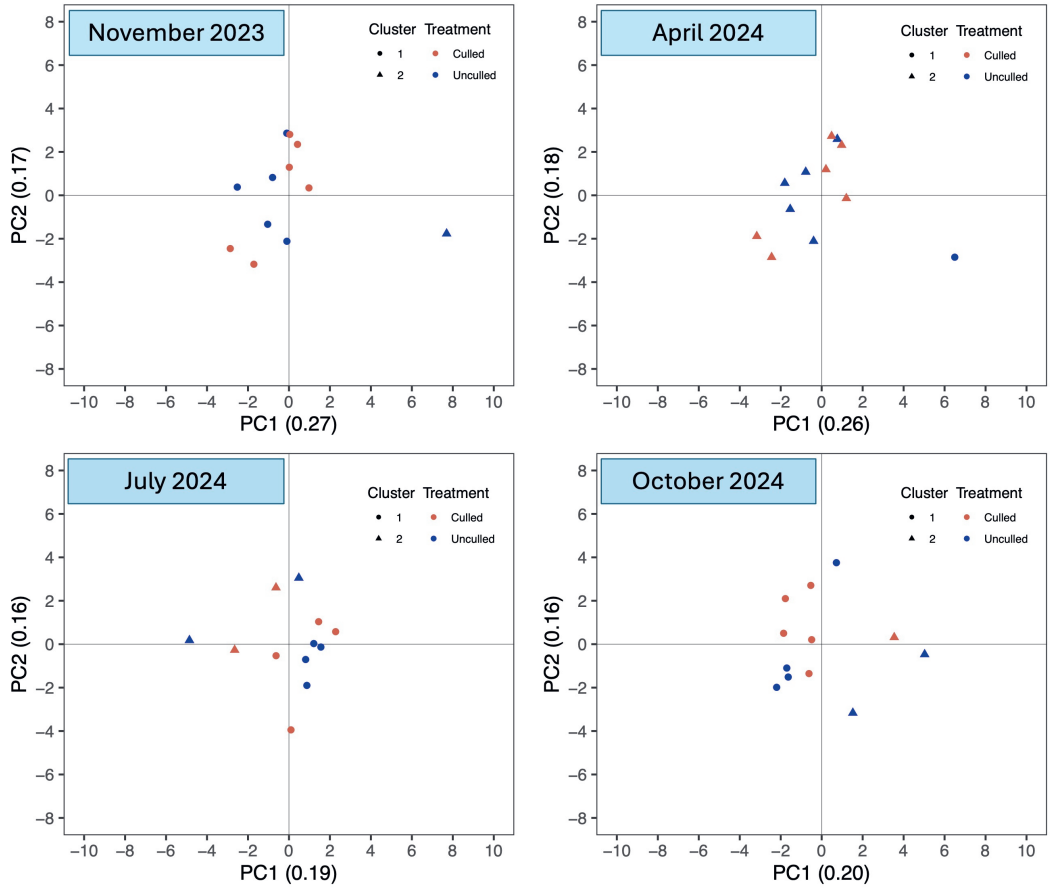

Figure S7. PCA scores of fish biomass along PC1 and PC2 at each of the 12 sites at the four survey times. Numbers in brackets show the proportion of the variance explained by a PC. The colour of a data point represents the treatment (red for culled, blue for unculled), while the shape represents the cluster (circles for cluster 1, triangles for cluster 2) that were assigned through cluster analysis.

Chapter 4 | Camouflage in a moving predator: testing for background choice in stalking lionfish (*Pterois miles*)

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Abstract

Predation is pervasive in the natural world, where predators and prey have evolved spectacular mechanisms to avoid detection and recognition. An example is camouflage through background matching, which allows animals to resemble their background. Animals can move strategically towards backgrounds that match their morphology (background choice) to enhance camouflage. We studied the relevance of background choice in lionfish (*Pterois miles*), conspicuous stalking predators that could take advantage of background matching by resembling corals and other invertebrates in their surroundings. We offered different background combinations to hunting lionfish and we found that they indeed account for their background while hunting, although the response did not support the hypothesis that the preference is determined by background matching. Lionfish deviated towards a rocky background, which closely resembled the substrate where lionfish were caught (rocky reefs in the Mediterranean Sea). We suggest that lionfish showed a preference for a rocky background because in the Mediterranean they live on rocky reefs. While we cannot rule out that background matching played a role in background choice, we suggest that remaining close to a rocky reef while hunting offers lionfish the best combination of easy access to hiding spots and high availability of prey.

1. Introduction

Predation is ubiquitous, as virtually every animal is either predator or prey at some moment of their life cycle. Therefore, predation has a major role in shaping animal evolution as well as ecosystems (Dawkins and Krebs 1979, Reznick and Endler 1982, Magurran 1990, Brönmark and Miner 1992, Ripple and Beschta 2012, Price et al. 2015, Hammerschlag et al. 2019). A predator-prey interaction can only take place after a predator detects and recognises a prey and both parties should benefit greatly from avoiding detection and recognition (Merilaita et al. 2017, Stevens and Ruxton 2019). For a predator, being inconspicuous can delay or reduce reactions in targeted prey, increasing their success. For a prey, being inconspicuous can decrease their chances of being caught or being targeted in the first instance (Pembury Smith and Ruxton 2020). While empirical evidence shows that if prey are camouflaged predators take longer to find them and are less likely to attack them (Morey 1990, De Alcantara Viana et al. 2022), evidence showing that inconspicuousness enhances the success of predators is scarce (Théry and Casas 2002, Brechbühl et al. 2010, Pembury Smith and Ruxton 2020). Predators and prey can detect each other using any combination of their senses and which senses are more prominent depends heavily on the environment and ecological context (Chivers et al. 2001, Hettena et al. 2014, Weissburg et al. 2014). Underwater, chemical and visual cues are the most studied in predator-prey interactions, either alone or in combination (Mathis and Vincent 2000, Chivers et al. 2001, Kelley and Magurran 2003, Weissburg et al. 2014, Fischer et al.

2017). Chemical cues can be used by animals for detection from long distances and tend to be prominent in environments where light is scarce or turbidity is high. Conversely, in the (short) ranges of distances and time relevant for predator-prey interactions, visual cues tend to be more important in clear water (Hartman and Abrahams 2000, Kelley and Magurran 2003). Contrary to chemical cues, the sight of an animal makes it possible for prey to assess its size and properties more easily than it would be possible through chemical cues (Chivers et al. 2001).

Crypsis refers to any mechanism used by living organisms to interfere with detection and recognition. Due to the importance of visual cues in predator-prey interactions, both predators and prey have evolved a spectacular suite of mechanisms to be visually cryptic. Prey that hide to avoid predator attacks (Jennions et al. 2003) or have a partly transparent body (Johnsen 2001, Bagge 2019) are examples of crypsis through interference with visual detection. Camouflage refers to the morphological adaptations that decrease the chance of animals to be detected or recognised (Cuthill et al. 2005, Merilaita et al. 2017, Michalis et al. 2017, Cuthill 2019). In camouflage through background matching animals resemble the background on which they live. Because the visual appearance of many animals is typically inflexible, background matching is often facilitated by background choice; animals choose their background strategically and prefer surroundings that they can match (Stevens and Ruxton 2019). A common assumption is that background matching can be easily impaired by movement (Hall et al. 2013), unless animals move to match moving backgrounds or have colour patterns that evolved specifically to conceal motion signals (Bian et al. 2016, Tan et al. 2024). However, many predators move while hunting. Whether this implies that they cannot camouflage through background matching remains enigmatic as studies on camouflage in predators are scarce (Théry and Casas 2002, Brechbühl et al. 2010, Pembury Smith and Ruxton 2020).

Lionfishes of the species *Pterois miles* and *Pterois volitans* (hereafter, lionfish) are stalking predators and constantly move around the prey that they target. While hunting, lionfish slowly hover over the substrate following individual prey with their pectoral fins fully flared. Stalking can continue for several minutes and often ends with a lionfish striking on a prey from a very short distance and swallowing it whole (D'Agostino et al. 2020). This stalking strategy of lionfish seems unaffected by geographical range or level of prey naïveté (Fishelson 1975, Albins and Hixon 2008, Côté and Smith 2018, D'Agostino et al. 2020). The mechanisms explaining the success of hunting lionfish and how their unique morphology allows them to slowly approach prey without triggering a reaction are poorly understood (Côté and Smith 2018, Bottacini et al. 2024). Lionfish hunt in clear waters, either on coral reefs (Indo-Pacific and Atlantic Ocean) or on rocky reefs

(Mediterranean Sea), where prey should be able to detect or recognise them visually. Understanding the stalking hunting strategy of lionfish will contribute to determining what makes them one of the most successful invasive species in the marine realm (Côté and Smith 2018, Bottacini et al. 2024). Although colourful and complex in appearance, lionfish could become cryptic to their prey through strategic background choices (Marshall et al. 2019). Here, we test the hypothesis that hunting lionfish make background choices that could allow them to camouflage by strategically orientating their body among colourful reef invertebrates. Testing this hypothesis is important to understand the hunting strategy of lionfish and their success as invaders across different habitats. Our experiments contribute to the study of camouflage in a moving predator and the unique hunting strategy and morphology of a highly invasive predator.

2. Materials and Methods

We conducted our study as two separate experiments, one in October-November 2023 (hereafter, coral-blue experiment) and one in March-April 2024 (hereafter, multiple-backgrounds experiment). The two experiments were done with two different groups of lionfish. Since the experiments were conducted completely independently and with different lionfish, we present the results separately. We used the same experimental apparatus and procedures and treated the animals in the same manner in the two experiments, unless stated otherwise.

2.1 Animals and husbandry

The lionfish *Pterois miles* and *Pterois volitans* are native to the coral reefs of the Indo-Pacific Ocean and Red Sea (Kulbicki et al. 2012). The two species are virtually indistinguishable in morphology and appearance and became invasive in the western Atlantic Ocean after releases and escapes from aquaria (Whitfield et al. 2002, Côté and Smith 2018). More recently, *P. miles* have also been invading the Mediterranean Sea after introductions from the Red Sea through the Suez Canal (Kletou et al. 2016, Phillips and Kotrschal 2021, Bottacini et al. 2024). Invasive lionfish have spread throughout the tropical reefs of the western Atlantic and are now abundant and well-established in the rocky reefs of the eastern Mediterranean (Côté and Smith 2018, Bottacini et al. 2024). Lionfish keep expanding their invasive ranges, both in the western Atlantic and in the Mediterranean (Soares et al. 2023, Bottacini et al. 2024).

We caught lionfish (*P. miles*) from the coastal waters of Crete (Greece), where they are well-established as an invasive species. We did this using a combination of SCUBA and free diving with two hand nets from a maximum depth of 18 m, without anesthetising the fish. This resulted in two lionfish populations with a wide range of sizes (25 lionfish in the coral-blue experiment, from 14 cm to 36 cm in total length and 25 lionfish in the multiple-

backgrounds experiment, from 11 cm to 35 cm in total length). Lionfish were stocked in a rectangular communal tank (2.5 m x 1.5 m x 1.5 m) at the Hellenic Centre for Marine Research, Gournes, Greece. The tanks had a constant inflow of water (temperature: 18-20 C, salinity: 35 g/L, pH: 7.4) provided by a borehole and a constant outflow which maintained the water level constant (1.36 m during stocking). The tanks were located outside, under a natural photoperiod of approximately 12 and 11 hours of daylight at the beginning of the coral-blue and multiple-background experiment respectively. The photoperiod gradually changed to reach approximately 10 (coral-blue experiment) and 13 (multiple-background experiment) hours of daylight at the end of the experiments due to seasonality.

At capture, we assigned an identity to each lionfish following an established methodology based on analyses of their unique barred pattern (Phillips et al. 2024). The lionfish were fed daily with gilt-head seabream (*Sparus aurata*) provided by a local fish hatchery (for coral-blue experiment, mean total length = 5.6 cm, n = 50, sd = 0.5 cm and mean total weight = 3.4 g, n = 50, sd = 0.6 g; for multiple-backgrounds experiment, mean length = 5.0 cm, n = 20, sd = 0.5 cm and mean total weight = 2.0 g, n = 20, sd = 0.6 g). Seabream were stocked in a separate cylindrical tank that received water from the same source as the lionfish tanks and were fed daily with commercial pellet. The same seabream (hereafter, prey) used for feeding, were used as prey during the experiments.

2.2 Experimental apparatus

After two weeks of acclimation, we placed an experimental apparatus in one of the corners of the tank (fig. 1a). The apparatus (fig. 1b) consisted of two panels (70 x 50 cm) that allowed us to present combinations of different background combinations to hunting lionfish. We used three patterns on the panels: blue, coral and rock (Fig. 1c). The patterns were two-dimensional, printed representations of corals and rocks in natural size. A frame of PVC pipes at the bottom of the tank ensured that the panels were held at the same distance and angle throughout the study. During trials, a prey was presented at the end of the apparatus inside a clear jar of 8 cm in diameter. The jar was added to or removed from the tank through ropes that could be handled from the side of the tank. We used white pebbles at the bottom of the jar to ensure that it sank and remained stable during a trial. The distance between the entrance of the apparatus and the centre of the prey jar was 80 cm. We placed a white bar at the bottom of the tank to connect the centre of the entrance with the closest part of the prey jar. This white bar marked the closest approach trajectory to the prey jar and had marks every 20 cm to facilitate measurements and calibrations during video analysis.

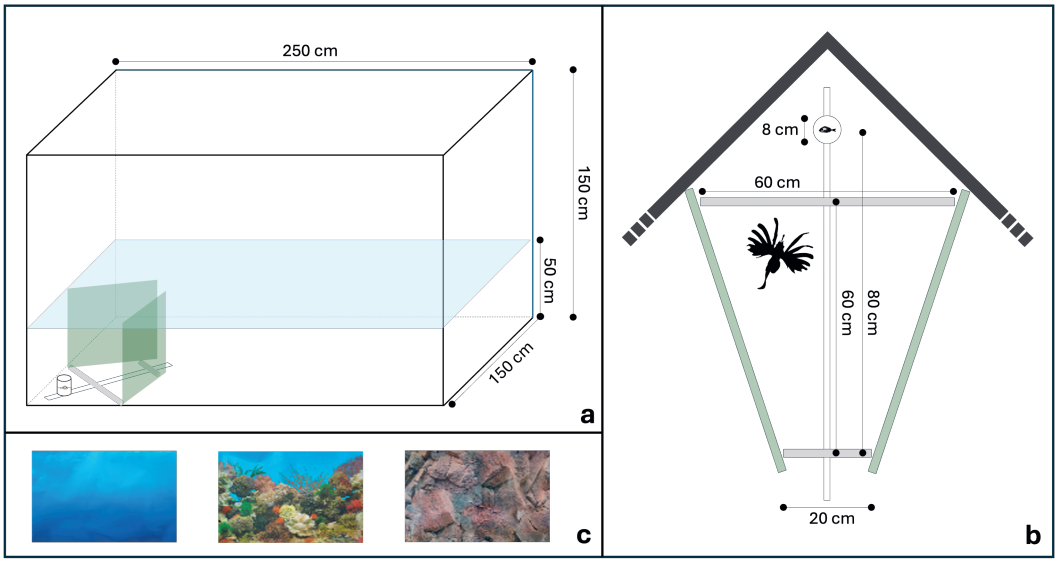

Figure 1. Overview of the tank during the experiments (**a**), measurements and position of the experimental apparatus (**b**) and patterns used as backgrounds (**c**).

The patterns were presented in three background combinations: coral-blue, rock-blue, coral-rock. In the coral-blue experiment, we only tested the combination coral-blue, while in the multiple-backgrounds experiment we tested all three combinations. Each combination was presented over the course of multiple days, using the same image in each treatment. Within each combination, we randomised the side on which each pattern was presented across days. This was done to correct for any laterality in our fish or potential other unknown environmental features that may have affected the lionfish trajectory in the apparatus.

We predicted that hunting lionfish adjust their approach trajectory to present themselves against backgrounds that match their morphology and colouration. Specifically, we expected lionfish to deviate consistently towards coral backgrounds, regardless of the alternative. In choosing our backgrounds, we did not account for the visual system of prey due to the large variety of prey that lionfish feed on (Green and Côté 2014, D'Agostino et al. 2020). We could therefore not measure quantitatively which backgrounds could be best for lionfish to camouflage. Rather, we chose the patterns because they represent realistic backgrounds that lionfish can have while hunting in nature: coral reefs in their native range and the invaded western Atlantic, rocky reefs in the invaded Mediterranean and open water present in any range. We also tested for any effect that might be simply due to colour preference by analysing two additional colour combinations: blue-black and coral-black (see supplementary material).

2.3 Experimental procedures

At the start of an experimental day, we lowered the water level to 50 cm in preparation to the experiments. Preliminary observations ensured that changes in water level did not affect the behaviour of the animals. In this way, lionfish could only enter the apparatus through the entrance. We then lowered the prey jar into the apparatus and waited for a lionfish to enter. A trial started the moment a lionfish (focal lionfish) entered the apparatus. In the cases when another lionfish approached the apparatus while the focal lionfish was approaching a prey, we placed a metal net (gate) at the entrance to prevent the second lionfish from entering and interfering with the trial. Lionfish are typically not impacted by manipulations in their surroundings. However, to exclude any interference with the swimming trajectory, the gate was only lowered after the focal lionfish reached the middle length of the apparatus (i.e., approximately 40 cm). In pilot trials we found that this operation did not interfere with the behaviour of focal lionfish and prey. A trial ended when the focal lionfish struck on the plastic jar, when it lost interest in the prey, or after five minutes from the start of the trial. We defined loss of interest based on the position on the orientation of the pectoral fins of the focal lionfish, flared and perpendicular to the body of the fish when hunting and to parallel to its body when not hunting. At the end of a trial, we removed the prey jar from the apparatus and filmed the focal lionfish on either side with a camera on a PVC handle for individual identification. After filming, we gently guided the lionfish outside of the apparatus. Before any subsequent trial, we replaced the prey in the jar with a new individual that had never been involved in the experiments. Every trial was filmed by a camera (GoPro Hero 7 Black) held on top of the apparatus by a PVC frame. The same camera model was used for the videos for individual identification at the end of a trial.

2.4 Video analyses

We analysed a total of 273 trials (80 in the coral-blue experiment and 193 in the multiple-backgrounds experiment). This was after filtering the videos and discarding trials that were unsuitable to analyse (46 in the coral-blue experiment and 18 in the multiple-backgrounds experiment). We decided to discard a video whenever it was impossible to identify the focal lionfish or whenever there was some accidental misalignment or malfunction in some of the elements of the apparatus. The videos were analysed with the free, open-source software Tracker (6.1.5) and the identification of our individuals was done visually from the individual identification videos (Phillips et al. 2024). For every trial, we determined the position of the lionfish in the apparatus in an X-Y coordinate system: X represents the position of the lionfish along the reference bar and Y represents the orthogonal distance of

the lionfish from the bar. We determined the lionfish X and Y coordinates every 1 second of video (measurement points), starting from the moment a lionfish entered the apparatus. These measurements were taken based on the midpoint of a line connecting the lionfish eyes. The Y position was then standardised by interpolation at the following X coordinates: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70 and 75 cm. This was done to ensure that we had Y measurements that were equidistant along the X axis (one every 5 cm) and that every trial had the same maximum number of data points (i.e., 15 X coordinates and corresponding Y coordinates). We also measured the body orientation of the lionfish, defined as the angle between the main axis of the lionfish body and the reference bar. Data on the lionfish's body orientation was collected and interpolated in the same manner as Y coordinates. For each measurement point of lionfish position, we also scored Y coordinate of the prey inside the jar.

2.5 Statistical analyses

We analysed whether approaches deviated significantly from the shortest approach trajectory: a lionfish swimming straight towards the prey along the reference line. We tested this on the interpolated Y values and body angles for each X value to test whether they significantly deviated from 0. In the multiple-backgrounds experiment, analyses were done separately for each background combination. Y coordinates were first corrected for the Y coordinate of the prey inside the jar, which was subtracted from the Y value of the lionfish to calculate the lionfish's relative Y coordinate. For each response variable and background combination, we fitted a generalised linear mixed model (GLMM) with X coordinate as a fixed predictor and lionfish identity random predictor. When appropriate, we added an interaction term between the X coordinate and lionfish identity (random slope) and the random effect of trial (nested in lionfish identity) (Santon et al. 2023). We used the models to make predictions on our response variables and considered these statistically significant if their 95% confidence interval did not overlap with 0. We used R 4.4.1 (R Core Team 2024) for all statistical analyses and data visualisations.

3. Results

In the coral-blue experiment, we observed 17 lionfish participating in 80 trials. Lionfish only deviated significantly from the shortest trajectory towards the prey jar at the end of their approach (1.06 cm at x = 70, CI: 0.00, 2.13) but changed their body orientation at multiple points in their trajectory (2.03° at x = 40, CI: 0.26, 3.83; -3.03° at x = 65, CI: -4.84, -1.27; -5.30 at x = 70, CI: -7.36, -3.27) (fig. 2). In the multiple-backgrounds experiment, we observed 22 lionfish participating in 193 trials. For most background combinations, lionfish did not deviate significantly from the shortest trajectory towards their prey (fig. 3). When given the choice between coral and rock, lionfish deviated towards rock (-1.56 cm at x = 5, CI: -2.76, -0.40; -1.78 cm at x = 10, CI: -2.96, -0.62; -2.03 cm at

x = 15, CI: -3.18, -0.87; -2.26 cm at x = 20, CI: -3.43, -1.09; -2.30 cm at x = 25, CI: -3.47, -1.14; -2.36 cm at x = 30, CI: -3.53, -1.21; -2.17 cm at x = 35, CI: -3.36, -1.01; -1.99 cm at x = 40, CI: -3.15, -0.83; -1.85 cm at x = 45, CI: -3.05, -0.69; -1.55 cm at x = 50, CI: -2.73, -0.41; -1.34 cm at x = 55, CI: -2.54, -0.16) and changed their body angle towards coral (2.81° at x = 30, CI: 0.23, 5.29; 3.07° at x = 35, CI: 0.46, 5.74; 3.18 at x = 40, CI: 0.57, 5.72; 3.06° at x = 45, CI: 0.50, 5.62; 2.63° at x = 50, CI: 0.10, 5.24; 3.19 at x = 55, CI: 0.53, 5.75) (fig. 3). Similarly, lionfish deviated towards rock at the end of their approach when given the choice between blue and rock (-5.03 cm, CI: -7.96, -2.07 at x = 75) (fig. 3). Additional comparisons showed no drastic differences in coral-black and blue-black combinations (see supplementary material).

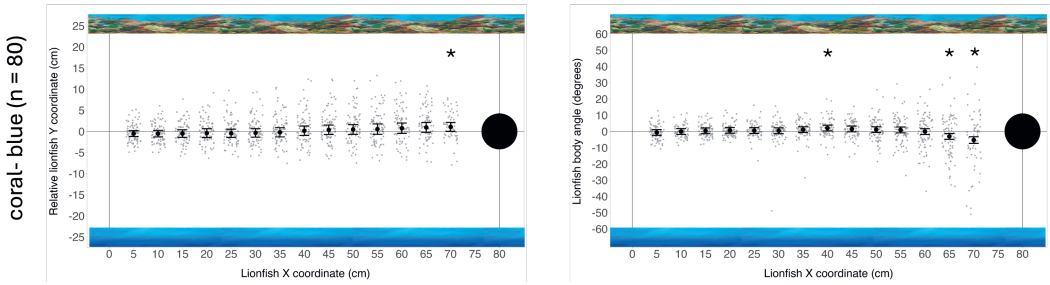

Figure 2. Deviations in relative distance (**left**) and body angle (**right**) from closest approach trajectory in lionfish approaching prey in the **coral-blue experiment**. The black circle at X = 80 represents the prey jar and the grey dots show the (jittered) raw data of the relative Y coordinate and body orientation of lionfish at every X value. The black dots show the model predictions with their 95% confidence intervals and asterisks highlight confidence intervals that do not overlap with 0.

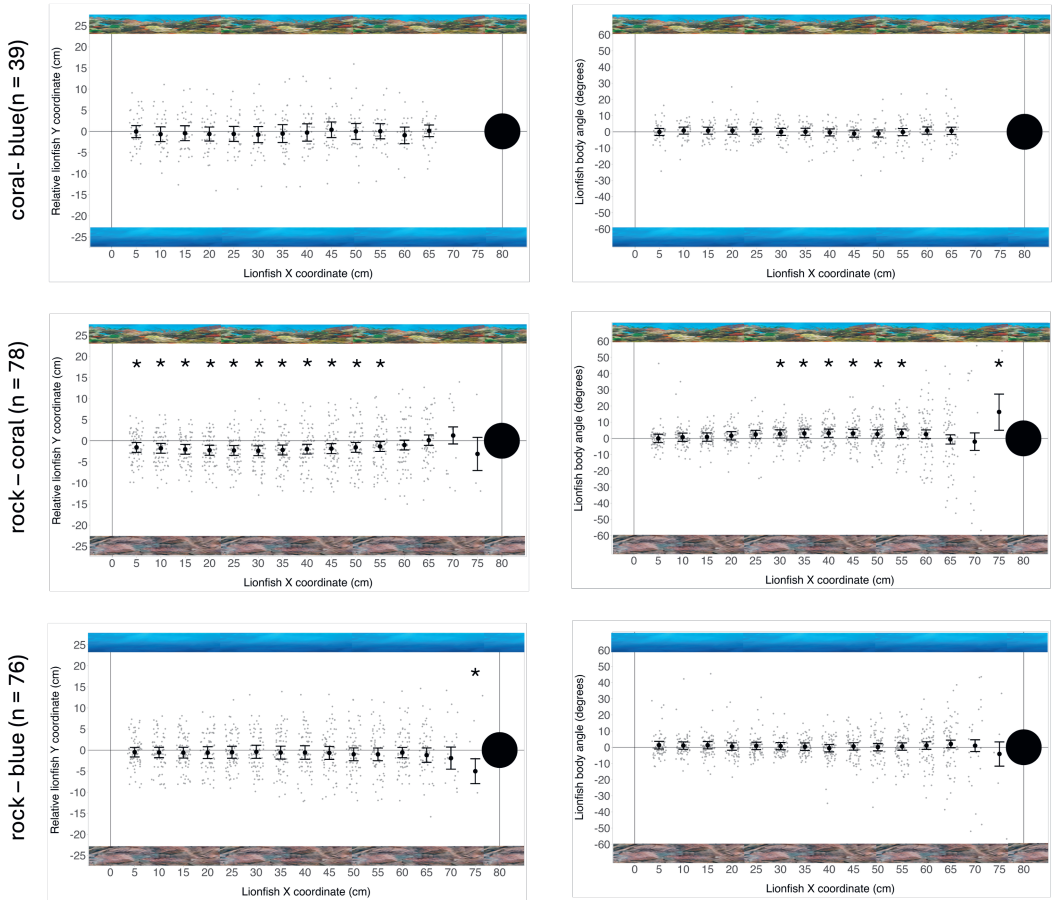

Figure 3. Deviations in relative distance (**left**) and body angle (**right**) from closest approach trajectory in lionfish approaching prey with different background combinations in the **multiple-backgrounds experiment**. The black circle at X = 80 represents the prey jar and the grey dots show the (jittered) raw data of the relative Y coordinate and body orientation of lionfish at every X value. The black dots show the model predictions with their 95% confidence intervals and asterisks highlight confidence intervals that do not overlap with 0.

4. Discussion

We analysed the trajectory of lionfish approaching prey with different background combinations and found that lionfish adjust their trajectory according to their surroundings. Contrary to our predictions, lionfish did not deviate consistently towards a colourful and complex coral background but deviated towards a rocky background when the alternative was a coral background and showed no consistent deviations with other background combinations. Lionfish showed no consistent changes in trajectory when they had a choice between blue and black background or between a coral and a blue or black background, suggesting that colour and pattern complexity by themselves do not explain the deviations observed in our experiments.

We suggest that deviations towards rocky backgrounds are driven by a preference for the substrate that the lionfish in our study were most familiar with. We caught lionfish in the Mediterranean and our individuals had no experience with corals or similarly colourful structures (Bussotti and Guidetti 2011, La Mesa et al. 2011, Kleitou et al. 2021). In the Mediterranean, hunting close to rocks is probably favourable for lionfish as their prey are found on this substrate, which they typically move towards to seek shelter from predators (Bussotti and Guidetti 2011, La Mesa et al. 2011, Zannaki et al. 2019). In addition, lionfish are almost exclusively found on rocky habitat in the Mediterranean, usually partly hidden in crevices (Phillips et al. 2024). Remaining close to potential hiding places offered by rocks could be a way for lionfish to protect themselves from potential predators through hiding. There is a paucity of information on what predators lionfish may have and they are unlikely to be preyed upon as adults (Côté and Smith 2018, Bottacini et al. 2024). This suggests that higher prey availability and proximity to hiding places offered by rocky reefs is probably why lionfish deviated towards rocky backgrounds in our experiments.

We conducted our experiments in the daylight and at 50 cm of depth. In the wild, lionfish are crepuscular hunters and often live and hunt at depths deeper than 10 m (Cure et al. 2012, Côté and Smith 2018, D'Agostino et al. 2020, Gavriel et al. 2021). In these conditions, light intensity and colours are altered by water selectively filtering out different wave lengths at different depths. In particular, the red component of the light spectrum is almost completely filtered out from depths of 10 m and the characteristic red bars of lionfish tend to acquire a brown or dark grey tone (Warrant and Johnsen 2013, Marshall et al. 2019). Therefore, in natural circumstances, a rocky reef with swaying algae could offer lionfish more opportunities to camouflage than the blue open sea. In our experiment, it is possible that lionfish showed a preference for the background that would allow them to best camouflage in the wild (i.e., grey rocks, as opposed to colourful corals or blue). This aspect could be tackled in future experiments by analysing the reaction (or lack of thereof) of prey when approached by lionfish with backgrounds of the same structural complexity, but different colour compositions reflecting those found at different depths. In future experiments, the choice of backgrounds could account for the visual system of prey and should include more than one pattern per treatment to control for any reaction that predator or prey might show against a specific pattern.

There may be mechanisms other than background matching making lionfish visually cryptic to prey. For instance, rather than specifically matching their background, lionfish could resemble inanimate objects such as algae or coral branches drifting in the current (mimicry). This would allow them to be successful regardless of their background and could explain their success across habitats and ranges (Cure et al. 2012, Green et al. 2012,

D'Agostino et al. 2020). If lionfish were heavily relying on camouflaging with coral backgrounds, they would not be as successful in the eastern Mediterranean, where structurally complex corals are rare or absent at depths shallower than 30 m (Bussotti and Guidetti 2011, La Mesa et al. 2011, Kleitou et al. 2021). In addition, given the preference of lionfish for demersal and benthic prey (Green et al. 2011, Green and Côté 2014, Zannaki et al. 2019, D'Agostino et al. 2020), it is likely that lionfish are seen against the blue background of the open sea by most of their prey in natural situations and mimicking floating objects can be predicted to be more effective than background matching in this type of scenario as well. It is challenging to test for the relevance of mimicry experimentally, since it is usually impossible to determine whether a prey detected a predator without recognising it or did not detect it in the first place (Skelhorn et al. 2010). This challenge is especially important in a context where prey are naïve to lionfish and are likely to not recognise them as a threat, even when detected (D'Agostino et al. 2020).

Our study contributes to investigating camouflage in motion: lionfish move as they approach their prey through their stalking strategy (Cure et al. 2012, D'Agostino et al. 2020), a different strategy from that of ambush predators such as scorpionfishes found in their same family (Scorpaenidae) (Marshall et al. 2019, John et al. 2023, 2024). Theory suggests that movement can easily break camouflage (Stevens and Ruxton 2019) and this could explain why lionfish did not deviate towards the most colourful and complex backgrounds in our experiments; camouflage through background matching is not effective in a fish that, while hunting, moves relatively to its background. There is evidence suggesting that stick insects (*Extatsoma tiaratum*) can enhance their camouflage by moving, since they match the movement pattern of the twigs on which they live (Bian et al. 2016). While, marine invertebrates do sway under the action of currents, this is a rhythmic movement that may simply be too different from a lionfish swimming in a trajectory that follows that of their prey. In our experiments, the backgrounds were not moving but future experiments could investigate the effect of moving corals on the hunting strategy of lionfish. This could be done either by using real or realistic imitations of invertebrates in a context where different currents can be applied or by presenting backgrounds in a virtual reality apparatus where the movement of structures can be manipulated (Vidal et al. 2023).

A mechanism that has been suggested to apply specifically to camouflage in motion is motion dazzle: high-contrast patterns composed of repeated elements such as lines or stripes may help animals camouflage when they are moving (Scott-Samuel et al. 2011, Stevens and Ruxton 2019, Lovell et al. 2024). Clear examples of such patterns in the natural world are lionfish bars and zebra (*Equus zebra*) stripes, which seem to make these animals conspicuous in their environments but that could make them more difficult to detect when they are moving. Experiments with humans suggest that the trajectory and

speed of moving objects is more difficult to determine compared to that of moving objects with a uniform colour pattern (Scott-Samuel et al. 2011, Lovell et al. 2024). This could make it difficult for prey to determine whether a lionfish is approaching them and at what speed. Motion dazzle could also explain the unusual colour pattern of lionfish, which differs markedly from that of sit-and-wait scorpionfishes known to rely on camouflage (John et al. 2023, 2024). Virtual presentations of lionfish with different colour patterns could exploit the lionfish-prey system as a model to investigate this fascinating mechanism from the perspective of a predator (Johnson and Basolo 2003, Watve and Taborsky 2019). Similarly to mimicking floating objects, motion dazzle could work regardless of lionfish surroundings.

Finally, our experiments differ from others in that we looked at camouflage as a mechanism that can increase success in predators. Experiments on other animals (including predators), tend to look at camouflage from the perspective of animals trying to evade predation (Phillips et al. 2017, Smithers et al. 2018, Twort and Stevens 2023). However, theory suggests that predators and prey could both benefit from camouflage (Merilaita et al. 2017). This area of study deserves more experiments and dedicated experimental set-ups, ideally on animals that are both predator and prey, to disentangle the relative importance of camouflage in becoming better at preying as opposed to evading predation (Brechbühl et al. 2010, Rodríguez-Morales et al. 2018).

In conclusion, lionfish account for their background when hunting and change their approach trajectory towards their prey according depending on available backgrounds. The main factor underpinning these changes in trajectory could be a preference in lionfish to hunt close to the substrate that they are most familiar with, which could offer them better foraging opportunities and lower risk of predation. Future experiments should test for alternative mechanisms that prevent detection and recognition in lionfish prey such as mimicking floating objects and motion dazzle. These studies will help elucidate how the unique appearance of lionfish evolved and what makes them so successful at interacting with their prey. Experiments like these are paramount to pinpoint what makes such a great predator and what allows them to be so successful at predation and invasion across contexts. In addition, studying these mechanisms will give insights on the potential benefits of camouflaging as a predator and on camouflage in moving animals, two fields of study that can benefit from more empirical evidence.

Acknowledgements

We are grateful to the nursery facility Castellorizo S.A. (Agios Andreas branch) for providing us with the prey fish used in our experiments and thank Dana van den Akker,

Lieke van de Geest, Cristina Beams and Kees Kalverboer for helping us catch the lionfish that were used in our experiments.

Ethical statement

Maintenance of wild lionfish in captivity and experiments on their interactions with prey was approved by the ethics committee of the Hellenic Centre for Marine Research (permit number: 121/29.08.2023).

Additional permits

Capture of live lionfish by SCUBA and free divers was allowed by the local authorities through a sample collection permit (permit number: 6PΔΛ4653Π8-I9P).

Supplementary material

We tested for any deviations in trajectory that might be simply due to colour preference by analysing two additional colour combinations: blue-black and coral-black. Blue and black backgrounds both consisted of plain, uniform colours, so any change in trajectory in the trials with this background combination would have indicated that colour can, in itself, affect the trajectory of lionfish. Conversely, we expected lionfish in trials with coral-black to deviate their trajectory towards coral, similarly to those in coral-blue. Lionfish showed a small deviation and change in body orientation at the beginning of their approach when given the choice between blue and back (1.49 cm, CI: 0.42, 2.53 and -3.12°, CI: -5.46, -0.86 at x = 5) (fig. S1). Lionfish also showed a change in body angle towards black when the alternative was coral (5.32°, CI: 0.30, 10.38 at x = 70) (fig. S1).

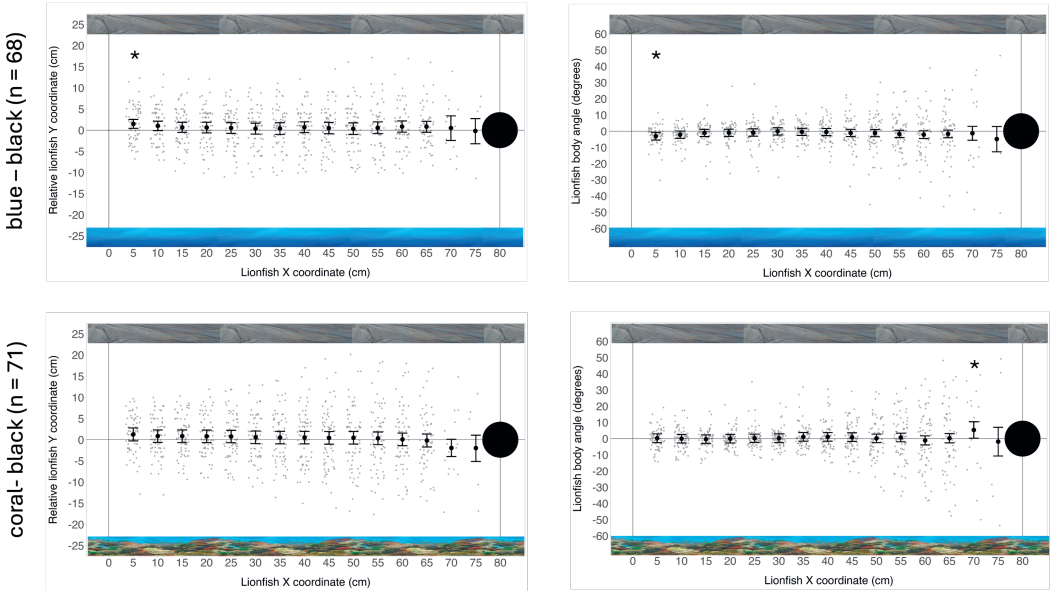

Figure S1. Deviations in relative distance (**left**) and body angle (**right**) from closest approach trajectory in lionfish approaching prey with different background combinations including **black backgrounds**. The black circle at X = 80 represents the prey jar and the grey dots show the (jittered) raw data of the relative Y coordinate and body orientation of lionfish at every X value. The black dots show the model predictions with their 95% confidence intervals and asterisks highlight confidence intervals that do not overlap with 0.

Chapter 5 | Lionfish (*Pterois miles*) blow directed jets of water to manipulate prey behavior

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Abstract
Predation is pervasive in the natural world and shapes evolution of predators and prey. Numerous behavioral adaptations allow predators to manipulate prey behavior, resulting in higher success in predator-prey interactions. The lionfishes *Pterois miles* and *Pterois volitans* are highly effective predators and their invasiveness is attributed to their high hunting success. How lionfish attain such a high predation effectiveness remains enigmatic. Lionfish are stalking predators and show a peculiar behavior while approaching their prey fishes: they actively blow directed jets of water at them. This is the only known example of a predator blowing jets of water at mobile prey underwater. The causes and consequences of jet-blowing remain unknown. Here, we tested the hypothesis that lionfish use jets of water to re-orientate their prey and facilitate head-first capture. We conducted two separate experiments with two different groups of lionfish and prey species. We show that lionfish catch more prey head-first when blowing and that prey exposed to jets orientate towards an approaching lionfish, with smaller lionfish being more likely to blow. However, blowing was not determined by the initial orientation of prey and was largely driven by lionfish size. In addition, in one of the experiments, blowing lionfish struck from a further distance, possibly due to prey swimming towards them in response to blowing. Our results show that jet-blowing allows lionfish to manipulate the behavior of their prey by re-orientate them to facilitate swallowing, providing the first evidence that jet-blowing can be used to manipulate the behavior of mobile prey.

1. Introduction

Predation is wide-spread in the natural world and has a major role in shaping animal evolution and ecosystem functioning (Reznick and Endler 1982, Magurran 1990, Hammerschlag et al. 2019). Predators and prey are involved in evolutionary arms races and constantly adapt to increase success in their interactions (Dawkins and Krebs 1979, Brönmark and Miner 1992, Price et al. 2015). There are prominent examples of behavioral adaptations that allow predators to increase their success by manipulating their prey before capture. For instance, archerfish (*Toxotes jaculatrix*) blow jets of water through the air to make their insect prey fall from branches above the water level or underwater to expose hidden food items, triggerfishes (Balistidae) turn sea urchins upside down to expose their more vulnerable body parts and pufferfishes (Tetraodontidae) blow water to excavate prey buried in the sand (Frazer et al. 1991, Wainwright and Turingan 1997, Gerullis and Schuster 2014, Dewenter et al. 2017). In these examples, manipulation physically displaces prey to facilitate capture or consumption. However, predatory fishes can also manipulate their preys' behavior. For instance, anglerfishes (Lophoioidei) lure prey by moving a bioluminescent dorsal fin ray (Maile 2023) and the Livingston's cichlid (*Nimbochromis livingstonii*) mimics a dead fish at the bottom of lake Malawi to attract cichlid prey

(McKaye 1981). In these latter examples, a prey is lured within striking distance by a predator body part or the whole animal mimicking a food source.

Fish predators tend to swallow prey head-first to reduce swallowing time and the risk of prey escape (Morris et al. 1956, Reimchen and Douglas 1984, Hoyle and Keast 1987, Reimchen 1991). The fin rays of many bony fishes have evolved into defensive spines that point towards their tail (Price et al. 2015). If swallowed head-first, these spines bend backwards along the body of the prey while, if swallowed tail-first, defensive spines are more likely to bend forward and get stuck in the predator's mouth, inflicting injuries and increasing swallowing time (Reimchen 1991). Head-first swallowing is wide-spread among piscivores and in terrestrial predators it often involves post-capture prey manipulations to orientate a prey fish head-first before swallowing (Reimchen and Douglas 1984, Reimchen 1991). However, most predatory fishes are limited in post-capture manipulations. This is because they can only use their mouth to manipulate their prey and they can often not re-orientate their prey without releasing it, which increases the risk of escape (Morris et al. 1956, Hoyle and Keast 1987). Therefore, predatory fishes tend to catch their prey fishes in a head-first position, a manner that requires no post-catch handling to swallow them head-first (Deelder 1951, Morris et al. 1956). Swallowing prey head-first tends to be more important when the size is of a prey is large relative to that of the predator (Reimchen 1991, Price et al. 2015).

The lionfishes *Pterois miles* and *Pterois volitans* (hereafter, lionfish) have a unique hunting strategy: they slowly stalk their prey with their pectoral fins fully flared for a long time before striking and catching them through water suction created by opening and protrusion of their mouth (Muller et al. 1982, Green et al. 2011, Cure et al. 2012, Côté and Smith 2018, D'Agostino et al. 2020). Lionfish are highly invasive and prey naïveté has a role in their success as predators in their invasive ranges (Cure et al. 2012, Côté and Smith 2018, D'Agostino et al. 2020, Bottacini et al. 2024). However, as lionfish show the same hunting strategy in their native range, prey naïveté alone cannot explain the evolution of their stalking hunting strategy (Cure et al. 2012, McCormick and Allan 2016, Côté and Smith 2018). Lionfish are large and conspicuous and it is unlikely that they can become inconspicuous through camouflage (Marshall et al. 2019, Bottacini et al. 2025). It therefore remains unclear how such a conspicuous animal manages to slowly approach its prey without triggering an antipredator response (Côté and Smith 2018).

Lionfish blow directed jets of water at their prey fish while they approach them (Albins and Lyons 2012, Cure et al. 2012). This is, to our knowledge, the only example of a predator blowing water jets at prey that could flee from them (Albins and Lyons 2012). Jet-blowing has only been reported on lionfish approaching fishes (Albins and Lyons 2012), although

lionfish are known to also feed on invertebrates, especially when young (Valdez-Moreno et al. 2012, Côté and Smith 2018, Zannaki et al. 2019). It has been suggested that lionfish blow at their prey to orientate them head-first for more efficient swallowing, as fishes tend to orientate themselves towards the source of a current (positive rheotaxis) and could therefore orientate to face the source of the water jet (Arnold 1974, Albins and Lyons 2012). Alternatively, blowing may allow lionfish to stun their prey by overstimulating their lateral line (Albins and Lyons 2012). These two hypotheses are not mutually exclusive; blowing may both orientate prey for head-first capture and reduce anti-predator behavioral responses through overstimulation of a prey's lateral line. Studies investigating these hypotheses are lacking and the causes and consequences of jet-blowing in lionfish remain unknown.

Here, we tested the hypothesis that lionfish blow jets of water at their fish prey to re-orientate them and increase the chance of head-first capture. We offered live prey to lionfish in a controlled environment and analyzed their interactions through video analysis. We predicted that (i) when blowing, lionfish would capture a greater proportion of prey head-first compared to when not blowing, (ii) lionfish would blow more frequently at prey that are not orientated towards them and that (iii) smaller lionfish would be more likely to blow because they were dealing with relatively larger prey. In addition, we predicted that (iv) prey that are blown at would turn to orientate themselves towards an approaching lionfish. On the contrary, prey that are not blow at should not change their orientation during an approach. Our predictions are based on the available knowledge on prey handling in piscivorous predators and rheotaxis in fishes. Our hypotheses assume that swallowing prey head-first allows lionfish to prevent their defensive spines to interfere with swallowing, which should be more problematic for relatively small predators.

2. Materials and Methods

We conducted two separate experiments, using two different species of prey: European seabass (*Dicentrarchus labrax*, hereafter, seabass) and gilt-head seabream (*Sparus aurata*, hereafter, seabream). We conducted the first experiment in July 2024 (seabass experiment) and the second one in October-November 2024 (seabream experiment). The experiments were done with two distinct groups of lionfish. We used the same experimental apparatus and procedures and treated the animals in the same manner in the two experiments, unless stated otherwise.

2.1 Animals and husbandry

We caught lionfish (*P. miles*) from the coastal waters of Crete (Greece), where they are well-established as an invasive species (Bottacini et al. 2024). We did this using a combination of SCUBA and free diving with two hand nets to a maximum depth of 18 m,

without anesthetizing the fish. This resulted in two lionfish groups with a wide range of sizes (27 lionfish in the seabass experiment, from 11.4 cm to 32.3 cm in total length and 20 lionfish in the seabream experiment, from 15.9 cm to 24.4 cm in total length). Lionfish were stocked in a rectangular communal tank (2.5 m x 1.5 m x 1.5 m) at the Hellenic Centre for Marine Research, Gournes, Greece. The tanks had a constant inflow of water (temperature: 20-22 C, salinity: 35 g/L, pH: 7.4) provided by a borehole and a constant outflow which maintained the water level constant (during stocking, 1.36 m of depth). We placed rocks as enrichment along one of the short sides of the tank and a cylindrical fiberglass arena close to the other short side (fig. 1a). At capture, we assigned an identity to each lionfish through pictures of their unique barred lateral pattern and measured their total lengths to the closest millimeter (Chaves et al. 2016, Phillips and Kotrschal 2021). The lionfish were fed daily with seabass (5-9 cm in total length) in the first experiment and gilt-head seabream (3-7 cm in total length) in the second experiment. Seabass and seabream were provided by a local hatchery facility and stocked in a separate cylindrical tank that received water from the same source as the lionfish tanks. Seabass and seabream were fed daily with commercial pellet and were also used as prey during the experiments.

2.2 Experimental set-up and procedures

The trials took place inside the experimental arena (150 cm in diameter and 75 cm in height, fig. 1b). The arena had a circular hole (entrance) of 10 cm in diameter located at 10 cm above the bottom of the tank. Through the entrance, lionfish could access the arena and participate in a trial. The bottom of the arena was covered with a layer (2 cm) of fine sand, which allowed for a uniform color and high contrast between fish and bottom. We placed two bolts at 8 cm from each other at the center of the arena, to have a size reference for subsequent video analyses. Every trial was filmed by three cameras: a Sony HDR-CX625 (9.2 megapixels, 16:9) fixed above the lionfish tank (2.5 m from water level) and two GoPro Hero 7 Black cameras (filming settings: 2.7 K, 60 FPS, linear) fixed at the top of the arena just below the water surface. The Sony camera could film the entire arena and was used to track the movement and orientation of lionfish and prey during trials. Footage from the two GoPro cameras was used to determine whether lionfish were blowing.

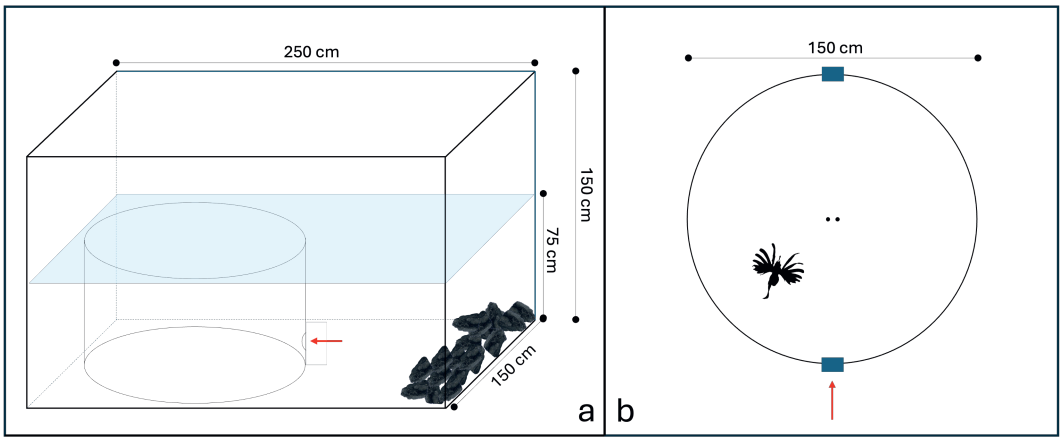

Figure 1. Overview of the set-up for behavioral observations on lionfish-prey interactions. Panel a shows the communal tank and **panel b** shows the top-view of the experimental arena. Red arrows show the entrance to the arena. In panel a, the blue surface indicates the water level during the trials and the grey rocks show the location of the enrichment. In panel b, the two black dots represent the screws that we used for scale and the two blue rectangles show the position of the two GoPro cameras.

During the acclimation phase (at least two weeks) and when not running trials, lionfish could freely enter the arena from the entrance or from above. At the beginning of an experimental day, we lowered the water level of tank to the height of the arena (75 cm), so that it could not be entered from above. We then closed the entrance through a transparent trap door so that no lionfish could enter and placed a prey at the center of the arena. After one minute of prey acclimation, we opened the door and waited for a lionfish (focal lionfish) to enter. Lionfish decided whether to participate in a trial and were often observed waiting outside the arena for the door to be opened. As soon as the focal lionfish entered the arena, we closed the entrance with the door. We checked that this operation did not interfere with the focal lionfish or prey behavior in pilot trials. A trial started when the focal lionfish entered the arena and ended when the lionfish captured the prey, lost interest or the prey escaped from a strike. We only analyzed trials in which the prey was not moving when a lionfish started swimming towards it in a hunting posture. This was done to minimize changes in lionfish approach angle caused by moving prey. At the end of a trial, the focal lionfish was filmed on either side of the body with one of the GoPro cameras for later identification and gently guided outside the arena.

2.3 Video analyses

We analyzed videos of the trials to measure the behavior and orientation of lionfish and prey during a lionfish approach. An approach started when the focal lionfish maintained a visual orientation of ≤90 degrees towards the prey for five seconds or longer. With this condition, we ensured that the variables that we scored were part of a predator-prey

interaction, with the focal lionfish specifically targeting the prey. This excluded the time during which a lionfish simply exploring the arena. An approach ended when the lionfish captured the prey, lost interest or the prey escaped from a strike. Therefore, there was only one approach within each trial. We defined loss of interest when a lionfish was not visually directed towards the prey within an angle of 90 degrees for more than 5 seconds. We discarded videos in which we detected poor quality of the footage or malfunctions of the apparatus during the analysis (< 10% in both experiments). We only analyzed approaches in which the prey did not swim more than its own body length during an approach (88% in the seabass experiment and 60% in the seabream experiment). This resulted in 167 videos in the seabass experiment and 215 videos in the seabream experiment. Videos were analyzed using the software Tracker (version 6.1.6, January 2024).

For each approach, we recorded lionfish identity, prey size, blowing behavior, strike success, catch orientation, strike distance, lionfish-prey angle. Lionfish identity was based on the unique lateral pattern of lionfish individuals and was determined by analyzing the lateral videos filmed at the end of every trial (Chaves et al. 2016, Phillips and Kotrschal 2021). Prey size was measured with a ruler to the closest millimeter before placing the prey in the arena. Blowing behavior (present or absent) was defined based on the characteristic rhythmic moving pattern of the gill covers during an approach (Albins and Lyons 2012). Strike success was determined based on whether a lionfish caught the prey (success) or not (failure). Catch orientation was defined as head-first, side-first or tail-first depending on the orientation at which the prey was caught (fig. 2a). Strike distance was defined as the distance between the tip of the lionfish snout and the closest part of the prey at the moment of strike. Lionfish-prey angle was defined as the absolute angle between two lines perpendicular to the lines connecting the eyes of the animals (fig. 2b). Lionfish-prey angle was measured at start of an approach (start angle) and at the moment a lionfish struck (end angle). Data from the seabream experiment showed that most of the change in angle during an approach is due to the prey changing orientation, while lionfish tend to approach following a straight trajectory (fig. S1). The outcome of a strike was recorded as a success or failure based on whether the prey was caught or not.

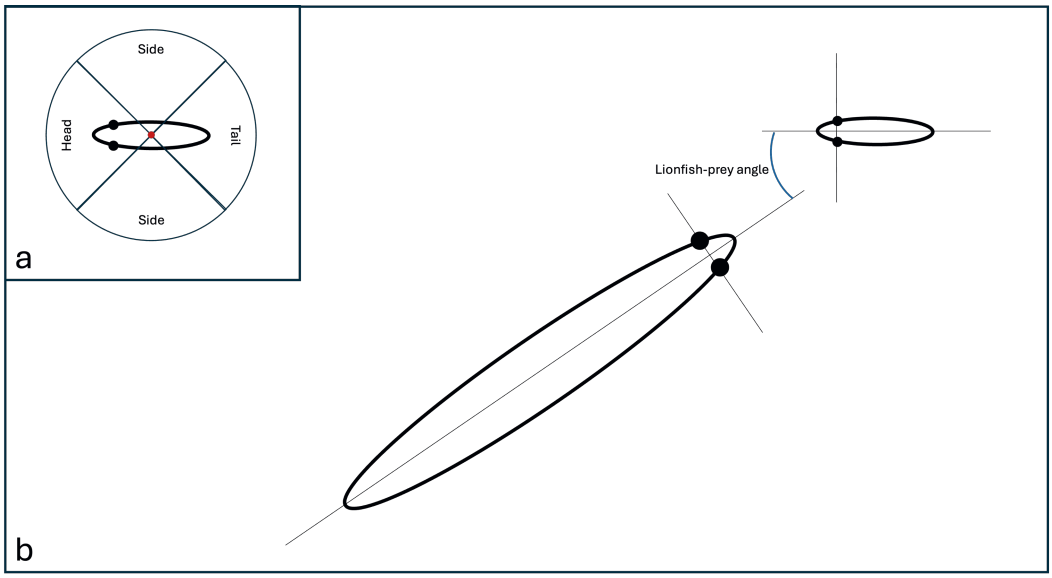

Figure 2. Overview of the variables scored from videos on lionfish and prey orientation. Panel a shows the definition of catch orientation and **panel b** shows the definition of lionfish-prey angle. Oval shapes represent prey (small ovals) and lionfish (large oval), with black dots representing the animals' eyes. In panel a, the red dot represents the prey's body center and the four quadrants represent the angles that were used to score whether a prey was caught head-, tail, or side-first. In panel b, the thin black lines mark the longitudinal and transversal body axes of the animals and the blue line marks the lionfish-prey angle. A screenshot from one of the videos used during data collection is available in the supplementary material (fig. S2).

2.4 Statistical analyses

To test whether blowing increased the probability of head-first captures, we tested whether the proportion of head-first captures differs between approaches with blowing and without blowing (Catch_orientation ~ Blowing + (1|Lionfish_ID), family = binomial). We defined non-head-first catches as the sum of side- and tail-catches. To investigate what factors predict whether a lionfish blew during an approach, we modelled the proportion of blowing approaches as a function of absolute lionfish-prey start angle and lionfish size (Blowing ~ Start_angle + Lionfish_size + (1|Lionfish_ID), family = binomial). To test the effect of blowing on prey orientation, we modelled the change in lionfish-prey angle during an approach as a function of blowing and start angle (Angle_change ~ Blowing + Start_angle + Blowing:Start_angle + (1|Lionfish_ID), family = gaussian). We defined a change in lionfish-prey angle as the difference between the absolute start angle and the absolute end angle. Therefore, positive values of change in angle indicate a net change of the prey orientation towards a lionfish, while negative values indicate a net change in orientation away from a lionfish. Based on our prediction, we expected no change in angle in approaches without blowing and a change in angle equal to the initial angle in approaches with blowing. We modelled strike distance as a function of blowing to test for the

possibility that lionfish could strike from a further distance in approaches with blowing (Strike_distance ~ Blowing + (1|Lionfish_ID), family = gamma in the seabass experiment and family = gaussian in the seabream experiment). To test whether blowing increases lionfish hunting success, we modelled the strike success rate as a function of blowing (Strike_outcome ~ Blowing + (1|Lionfish_ID), family = binomial). Because there were no failures in the seabream experiment, we only modelled strike success in the seabass experiment.

Data from the two experiments were always modelled separately. We used R 4.4.1 (R Core Team 2024) for all statistical analyses and data visualizations. We fitted generalized linear mixed models (GLMMs) using the package 'glmmTMB' (Brooks et al. 2017), choosing distributions and link functions based on the nature of our response variables and adjusting them when necessary to meet model assumptions (Santon et al. 2023). We accounted for multiple observations on the same individual lionfish by adding lionfish identity (Lionfish_ID) as a random factor in every model. We included interaction terms when they were biologically meaningful (Santon et al. 2023). We did not account for date or order of trials for each individual, since there was no obvious pattern in the participation rate of lionfish throughout the studies (fig. S3 and S4). We checked for violations of the model assumptions using the package 'DHARMa' (Hartig 2022). We extracted test statistics and p values with the package 'car' (Fox et al. 2001) and we interpreted differences as statistically significant when their associated p value was lower than 0.05 or when their 95% confidence interval did not overlap with 0. Full summary tables for each model are available in the supplementary material (tab. S1-S5). We used the package 'tidyverse' (Wickham et al. 2019) for data visualizations.

2.5 Ethical note

The experimental protocol for the maintenance of wild lionfish in captivity and experiments on their capture of live prey was approved by the ethics committee of the Hellenic Centre for Marine Research (decision number: 125/12.09.2023).

3. Results

Jet-blowing had a strong effect on the proportion of head-first captures in both experiments (fig. 3, GLMM, $X^2 = 25.4$, d.f. = 1, $p < 0.001$ in the seabass experiment and $X^2 = 40.2$, d.f. = 1, $p < 0.001$ in the seabream experiment). When a lionfish blew, a prey was 11.9 (95% CI: 4.5, 31.2) and 7.8 (95% CI: 4.14, 14.7) times more likely to be caught head-first than when the lionfish was not blowing in the seabass and seabream experiments respectively. The angle between lionfish and prey at the start of an approach had no effect on whether a

lionfish blew in either the seabass or seabream experiment (fig. 4, GLMM, $X^2 = 0.16$, d.f. = 1, $p = 0.69$ in the seabass experiment and $X^2 = 0.37$, d.f. = 1, $p = 0.54$ in the seabream experiment). Large lionfish were less likely to blow than small ones in both the seabass (fig. 4, GLMM, $X^2 = 21.7$, d.f. = 1, $p < 0.001$) and seabream (fig. 4, GLMM, $X^2 = 5.8$, d.f. = 1, $p = 0.016$) experiment. The majority of the lionfish that participated in our experiments blew at least once: 23 of 26 in the seabream experiment and 17 our of 20 in the seabass experiment (fig. 5). In the seabass experiment, prey turned towards a lionfish when they were approached with blowing. The angle between lionfish and prey did not change as a function of the initial angle in approaches without blowing (fig. 6, β_1: 0.18, 95% CI: -0.02, 0.38) while it did when lionfish blew (fig. 6, $\beta_1 = 0.74$, 95% CI: 0.56, 0.92). In the seabream experiment, prey turned towards a lionfish in approaches with or without blowing, but the change in orientation was larger in when a lionfish blew (fig. 6, $\beta_1 = 0.87$, 95% CI: 0.74, 0.99 in blowing approaches and $\beta_1 = 0.59$, 95% CI: 0.42, 0.76 in approaches without blowing). Strike distance was not affected by blowing in the seabass experiment (fig. 7, GLMM, $X^2 = 0.16$, d.f. = 1, $p = 0.69$), while it was longer when lionfish blew in the seabream experiment (fig.7, GLMM, $X^2 = 43.5$, d.f. = 1, $p < 0.001$, effect size: 3.1 cm, 95% CI: 2.20 cm, 4.04 cm). In the seabass experiment, the strike success rate was not different between blowing and non-blowing approaches (fig. 8, GLMM, $X^2 = 0.04$, d.f. = 1, $p = 0.82$). In the seabream experiment, the strike success rate was 100% in both blowing and non-blowing approaches.

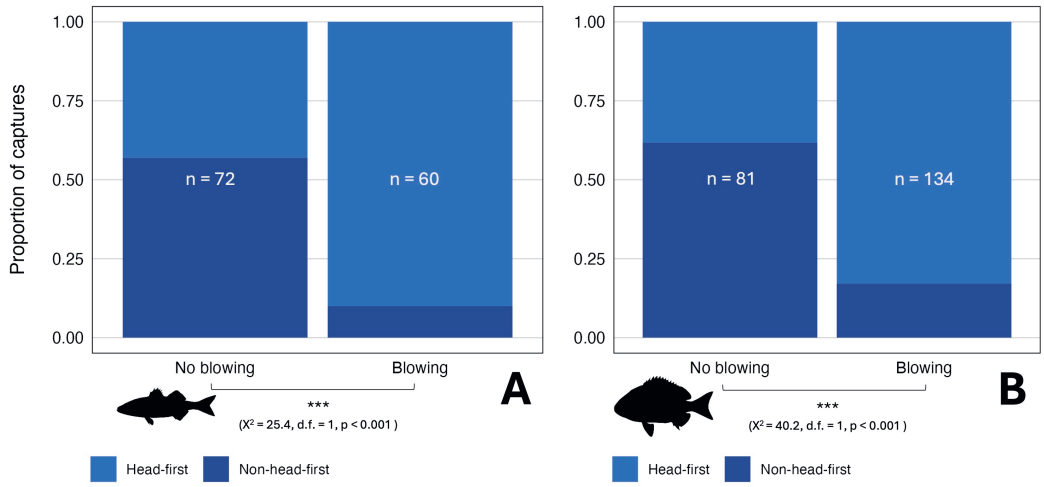

Figure 3. Effect of blowing on the proportion of head-first captures. Proportion of head-first captures in the seabass (**panel A**) and seabream (**panel B**) experiment in blowing and non-blowing trials (n indicates the sample size). Non-head-first captures include both lateral captures and tail captures.

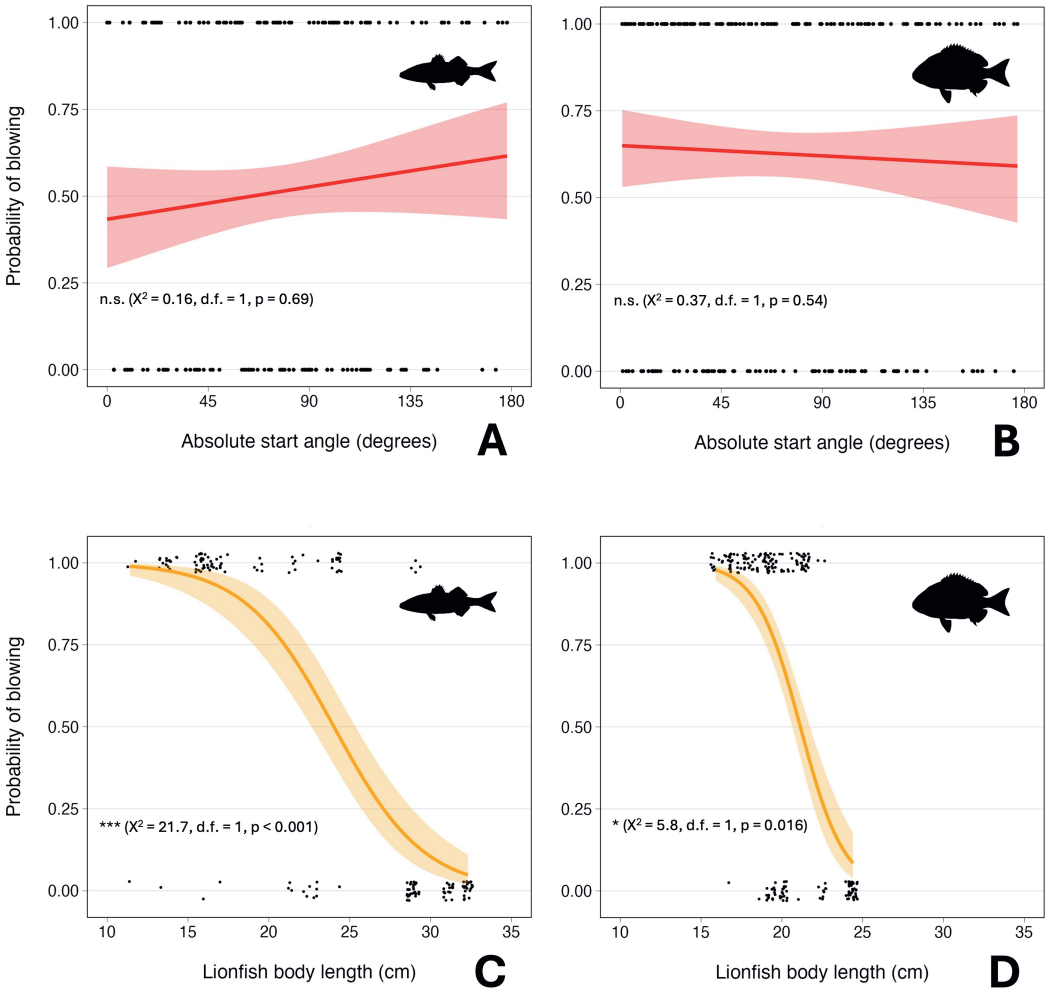

Figure 4. Effect of lionfish-prey start angle and lionfish length on blowing probability. Probability of a lionfish blowing as a function of absolute lionfish-prey (**panels A-B**) start angle and lionfish total length (**panel C-D**) in the seabass and seabream experiment. Absolute start angle varies from 0° (prey facing a lionfish) and 180° (prey orientated in the complete opposite direction as a lionfish). Dots represent (jittered) raw data points.

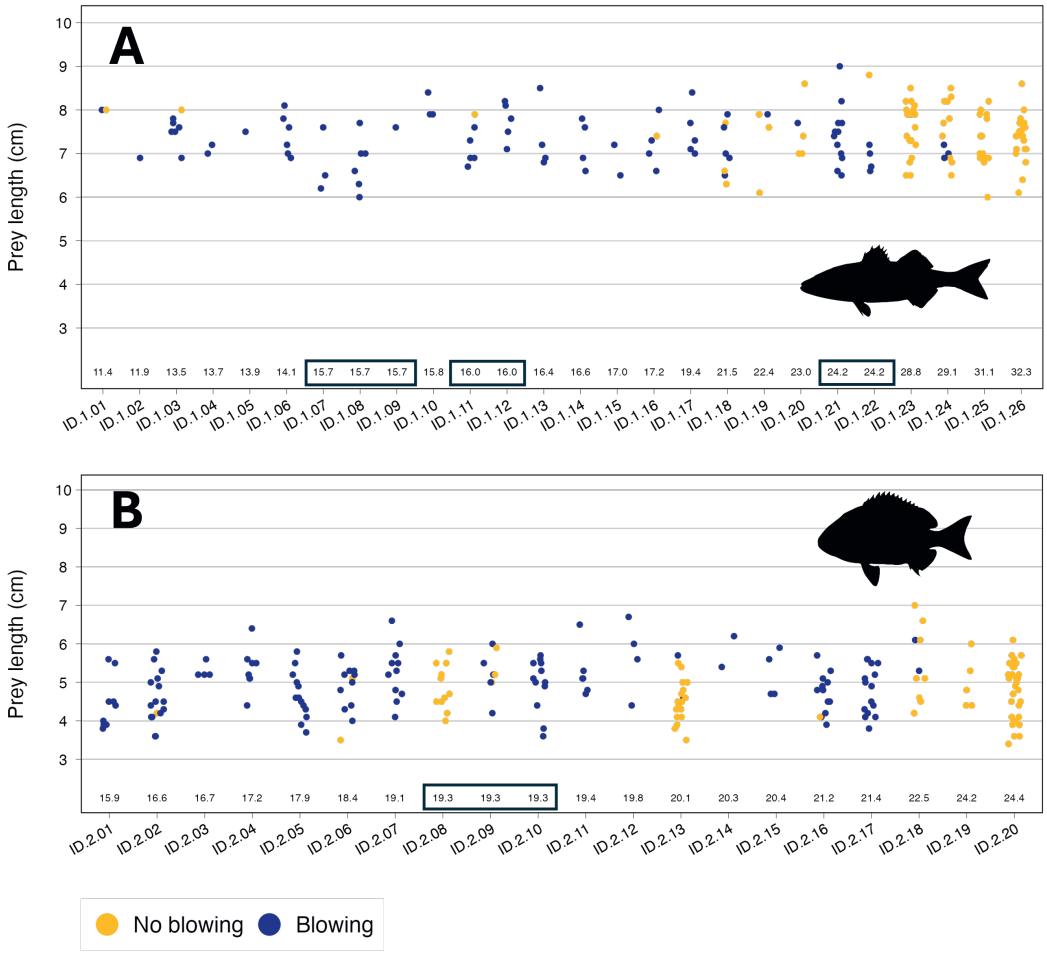

Figure 5. Individual variation in blowing behavior among lionfish. Differences in blowing behavior among lionfish individuals when presented with similar-sized prey in the seabass (**panel A**) and seabream (**panel B**) experiment. Dots represent (horizontally jittered) raw data points of prey sizes offered to each lionfish, color-coded based on whether a lionfish blew at them. The horizontal axes show the identities of the lionfish participating in trials. The numbers along the horizontal axes associated with each identity show the total length of the lionfish in cm. Lionfish of the same total length within each experiment are highlighted with a rectangle.

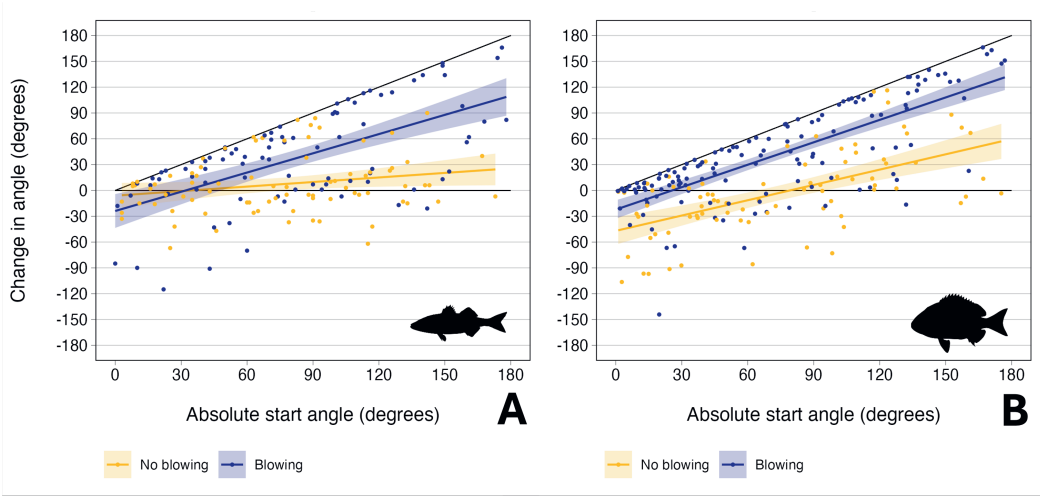

Figure 6. Effect of blowing on lionfish-prey angle. Absolute change in lionfish-prey angle during approaches in the seabass (**panel A**) and seabream (**panel B**) experiment. Horizontal axes show the absolute lionfish-prey start angle (0° represents prey and lionfish facing each other and 180° represents a prey orientated in the complete opposite direction as a lionfish). Positive changes in lionfish-prey angle represent changes in prey orientation towards a lionfish, while negative changes represent changes in orientation away from a lionfish. Black lines show the predicted response when prey were blown at (β_1= 1) and the predicted response when prey were not blown at (β_1= 0). Dots represent raw data points and shaded areas around the lines represent 95% confidence intervals.

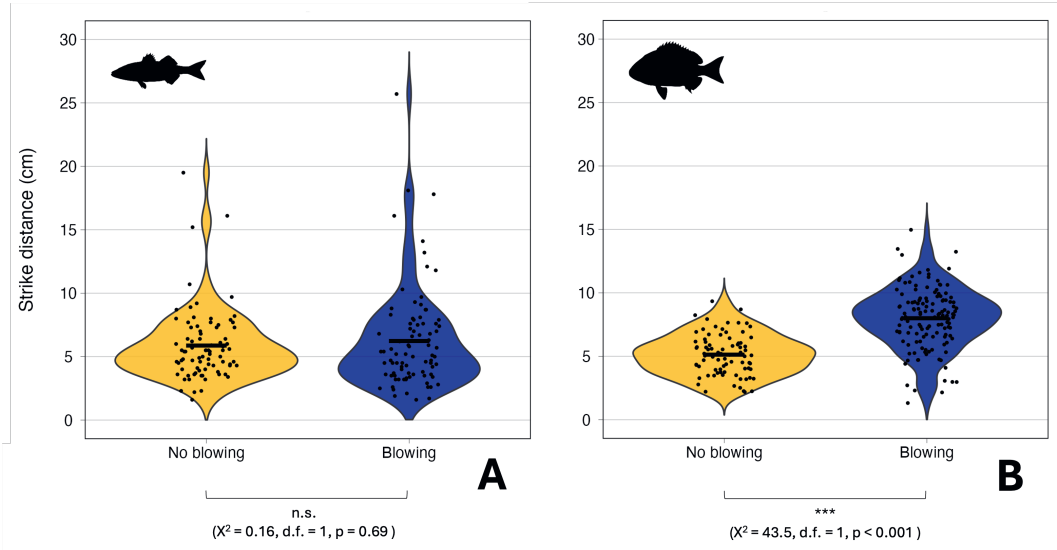

Figure 7. Effect of blowing on strike distance. Strike distance in the seabass (**panel A**) and seabream (**panel B**) experiment in blowing and non-blowing trials. Strike distance was defined as the distance between lionfish

and prey at the moment a lionfish struck. Horizontal bars in the violin plots represent means and dots represent (horizontally jittered) raw data points.

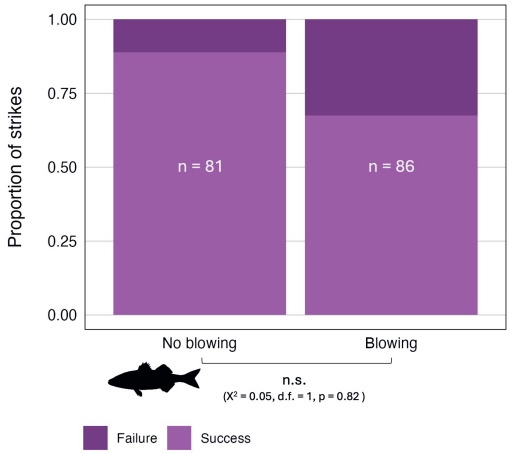

Figure 8. Effect of blowing on lionfish strike success rate. Proportion of successful and unsuccessful strikes in blowing and non-blowing approaches in the seabass experiment (n indicates the sample size). There were no failures in the seabream experiment.

4. Discussion

We tested possible causes and consequences of jet-blowing in lionfish. We predicted that blowing would result in a higher proportion of head-first capture by decreasing lionfish-prey angle through prey re-orientation. The proportion of head-first captures was higher in trials where lionfish blew at their prey, supporting this prediction. The strike success rate was not different between blowing and non-blowing approaches, suggesting that blowing does not increase the hunting success of lionfish, but probably provides post-capture benefits such as easier swallowing. Prey that were blown at turned towards the approaching lionfish, showing that blowing is used to reduce the lionfish-prey angle. Similar responses can be expected in a variety of lionfish prey, since positive rheotaxis is highly conserved across taxa in fishes (Arnold 1974, Coombs et al. 2020). Lionfish produce jets of water in pulses (Albins and Lyons 2012), which resulted in the same behavioral response in prey (i.e., positive rheotaxis) that would be expected if fish were reacting to a current with constant speed, which is more common in natural and experimental contexts (Arnold 1974). In the seabass experiment, there was no change in orientation when lionfish approached prey without blowing. However, in the seabream experiment prey turned towards a lionfish when they were approaching without blowing, although the change in angle was lower than in approaches with blowing. This could be interpreted as the result of predator inspection or as part of more general exploratory behavior towards a novel object entering a simple, empty arena (Dugatkin 1992). Alternatively, it is possible that the

water movement generated by lionfish swimming with their flared pectoral fins is sufficient to generate a response in prey. The different response by prey to non-blowing lionfish in our experiments could be due to species-specific differences or size differences between prey in the two experiments. Targeted behavioral assays of seabream and seabass juveniles are needed to explore the potential of such species-specificities underlying the subtle differences we observed.

Our first prediction on the causes of jet-blowing was that lionfish would blow more often with large absolute lionfish-prey start angles. Our results do not support this prediction. This is especially surprising considering the results showing that blowing contributes to prey reorientation. We predicted that blowing would be most advantageous when prey are not orientated towards them because in that case a change in prey orientation should have a larger effect on how easy it becomes to swallow. Conversely, considering that blowing probably has a metabolic cost (Schlegel et al. 2006), lionfish should prefer not to blow if prey do not need to be reoriented. Our second prediction on the causes of jet-blowing was that larger lionfish would be less likely to blow. Our results show that lionfish size had a large influence on whether a lionfish blew during an approach, with small lionfish blowing more frequently than large ones. This confirms previous observations indicating that blowing lionfish are generally smaller than non-blowing ones (Albins and Lyons 2012). Prey size was held constant regardless of lionfish size in our experiments. Therefore, larger lionfish were dealing with relatively smaller prey and facing a lower risk of injuries during swallowing, decreasing potential benefits of blowing (Reimchen 1991, Price et al. 2015). Our data provides tentative support to the hypothesis that the ratio between lionfish and prey size could be more important than absolute lionfish size; in the seabream experiment, we used slightly smaller prey (4.9 cm vs 7.4 cm). This could explain why the largest lionfish blowing in the seabream experiment was smaller (22.5 cm) than the largest lionfish blowing in the seabass experiment (29.1 cm). As the variation in lionfish size was much larger than that in prey size, it was mostly lionfish that drove this ratio in our experiments. It was therefore not possible to test for any effect of lionfish/prey size ratio on blowing.

The effect of lionfish size on blowing could also be interpreted in terms of differences in return of energetic investment between small and large lionfish. This is based on the assumption that there is a metabolic cost associated with blowing (Schlegel et al. 2006). Prey size being equal, a successful strike will give a large lionfish a relatively lower energetic benefit, which may not justify the cost of blowing while approaching. This is especially relevant considering that any unit increase in mouth diameter should result in a cubic increase in the volume of blown water and, therefore, a cubic increase in the amount

of energy needed to produce a water jet with the same velocity. To study this trade-off, we would need to quantify the exact costs of blowing and swimming in hunting lionfish. Regardless of the exact costs and benefits involved, if blowing is determined by the principle of return of investment, the ratio between lionfish and prey size, rather than absolute predator size, could be a better predictor of whether lionfish blow. Future experiments with strategic combinations of lionfish and prey sizes could be conducted to test for the effect of the relative size of prey on blowing. When controlling for the ratio between lionfish and prey size, the value in eating a prey can be controlled to be the same regardless of the size of a lionfish. Such experiments will also control for the confounder of larger lionfish having a lower risk of injury while swallowing; if the size ratio is controlled for across lionfish sizes, there can be the same risks of injury while swallowing a prey in smaller and larger lionfish.

The two largest lionfish in both our experiments did not blow in any trial, which could suggest that blowing might be a behavior typical of juveniles that is then gradually abandoned during development. The morphology and body proportions of lionfish change significantly as they grow, including in their head and mouth region (Rojas-Vélez et al. 2024). Therefore, it is possible that large lionfish simply lose the ability to blow due to morphological changes in their mouth. However, this is not likely to explain the whole range of variation in blowing in our experiments for four reasons. First, several lionfish blew only in some of their approaches, indicating that there are also other factors triggering blowing apart from the mere ability of lionfish to blow. Second, in the seabass experiment we had two lionfish around 29 cm in total length that occasionally blew, while the two largest lionfish in the seabream experiment never blew although they were considerably smaller (around 24 cm). Although this inference is based on very few individuals, if blowing depended on morphological traits, we would expect a clearer size boundary between blowing and non-blowing lionfish. Third, past observations report blowing in lionfish of estimated lengths of up to 30 cm (Albins and Lyons 2012). Fourth, because jet-blowing probably evolved from coughing, it should requires similar buccal movements and there is no reason to hypothesize that large lionfish could lose the ability to cough (Wainwright and Turingan 1997). Therefore, we argue that blowing is within the behavioral repertoire of lionfish of any size. It is noteworthy that we found major individual differences in blowing behavior. These differences are particularly striking when looking at individuals of the same size. For instance, there were three lionfish of exactly 19.3 cm in total length in the seabream experiment. One of these blew in all the trials, one blew in none and one blew only occasionally, despite being offered prey of the same species and of comparable size. The trials took place over multiple days, making any effects related to hunger and motivation unlikely to explain these individual differences.

Most of our results were very similar for two independent experiments, which were done with two different prey species and two distinct groups of lionfish. Observations from video analyses showed that prey that are blown at start swimming against the current produced by a lionfish. Based on this observation, we predicted that swimming prey should start moving towards the lionfish when blowing is interrupted at the moment of strike. This could allow blowing lionfish to strike from a further distance. When we tested for differences in strike distance, it was longer in blowing approaches compared to non-blowing ones in the seabream experiment. The mean strike distance in blowing trials (8.2 cm) was very close to 9.6 cm, which is the distance reached by water jets (Albins and Lyons 2012). We did not find the same effect in the seabass experiment. This could simply be explained by differences in swimming speed or reactiveness between the two different prey species. What goes against this reasoning is that positive rheotaxis is highly conserved across fishes and one of its main functions is that fishes can swim against a current and avoid displacement (Arnold 1974, Coombs et al. 2020).

Lionfish feed on a variety of prey and have been observed to blow more or less frequently depending on the species that they target (Albins and Lyons 2012, Green and Côté 2014, Zannaki et al. 2019, D'Agostino et al. 2020). Part of this variation could be explained by the presence (or lack) of defensive structures in prey and their body proportions. Deeper-bodied prey are usually more difficult to swallow and the same is true for prey with defensive structures (Brönmark and Miner 1992, Price et al. 2015). In our experiments, both prey species had defensive spines and their body proportions were similar. Future experiments could test for differences between prey with or without defensive spines to test whether lionfish blow more at defended prey. We fed 30 rockpool shrimp (*Palaemon sp.*) to the same lionfish that we tested in the seabream experiment and we observed no blowing. This could be because *Palaemon* shrimp are benthic organisms and could therefore react to currents differently from fishes (Berglund 1982, Carvalho et al. 2017, Deli et al. 2018). For instance, a current might not displace or reorientate them at all or stimulate them to hold tighter to the substrate, making capture by lionfish more difficult. To our knowledge, there is no information on the behavioral responses of *Palaemon* shrimp to currents or their antipredator responses. The different behavior of lionfish towards shrimp could be studied in future experiments to investigate how consistent it is across species invertebrates with different ecology. These experiments should first investigate how the specific species of invertebrates that are tested react to a current.

Alternative hypotheses to prey reorientation may also explain jet-blowing in lionfish. For example, pulsed water jets might momentarily impair prey fishes by overstimulating

their lateral line (Albins and Lyons 2012), and lionfish could potentially blow chemical cues that distract or confuse their prey. These hypotheses are not incompatible with the idea that lionfish use jets to reorient prey, nor should they be viewed as mutually exclusive. Multiple mechanisms may operate simultaneously, producing additive effects on prey behavior. However, that lionfish blow chemical cues at their prey seems unlikely, since there is no indication that they may possess specific glands that could serve this purpose (Côté and Smith 2018, Bottacini et al. 2024). To test this hypothesis the chemical composition of blown water needs to be characterized (Chua et al. 2016). We consider the possibility that jets of water can overwhelm their prey lateral line more plausible (Mogdans 2019). A first approach to testing this hypothesis could be measuring the effects of artificially produced jets of water on various behaviors in fishes. Any stunning effect mediated by the lateral line can be expected to interfere with behaviors and functions that go beyond antipredator responses. Such studies could include a manipulative component, where the sensitivity of the fish lateral line is interfered with pharmacologically (McHenry et al. 2009).

Our results show that jet-blowing causes behavioral changes in fish prey, initiating swimming and positive rheotaxis in fish that were not moving. To our knowledge, this is the only example of a predator manipulating prey behavior through jet-blowing. While blowing jets of water is a behavior found in many distantly related fishes, it is usually used to displace, turn or dig out immobile prey (Frazer et al. 1991, Wainwright and Turingan 1997, Dewenter et al. 2017). In all these species, jet-blowing likely evolved from coughing, a behavior that is used by fishes to expel voluminous objects from their mouth (Wainwright and Turingan 1997). In addition, positive rheotaxis is extremely widespread in fishes (Arnold 1974, Coombs et al. 2020). Why is jet-blowing not more common among fish predators, then? The answer could lie in the peculiar stalking hunting strategy of lionfish, which allows them to approach prey up to an extremely short distance before striking (Green et al. 2011, Green and Côté 2014). Blowing creates a water current that only reaches an extremely short range (Albins and Lyons 2012). Most predators would be detected and recognized before their prey can be influenced by their water jets. However, this explanation is unlikely since lionfish water jets reach a maximum distance of approximately 10 cm, which is shorter than the approximately 25 cm of mean closest approach distance that naïve prey tolerate in the invaded Mediterranean (D'Agostino et al. 2020). In addition, lionfish blow more often in their native range, where prey are not naïve and should keep a longer distance from an approaching lionfish compared to the invaded western Atlantic (Albins and Lyons 2012, Cure et al. 2012, D'Agostino et al. 2020).

We are the first to report jet-blowing in lionfish from the Mediterranean, showing that this behavior is also present in *P. miles* and is not exclusive of *P. volitans*, the predominant species of invasive lionfish in the western Atlantic (Hamner et al. 2007). Lionfish caught a

larger proportion of prey head-first in approaches with blowing. Prey that were blown at actively turned towards an approaching lionfish and started swimming against the current. This makes lionfish the first example of predators using jet-blowing to manipulate the behavior of mobile prey, rather than to physically move or displace immobile ones. Jet-blowing may also allow lionfish to strike on their prey from a closer distance. We observed large variation between and within individual lionfish in blowing behavior. Lionfish did not blow more often on prey that were not orientated towards them. While lionfish size explained some of the variation in blowing behavior observed in our experiments, the factors determining whether lionfish blow while approaching their prey remain largely unknown. We suggest that the ratio between lionfish and prey size, the presence of defensive structures and the body proportions of prey are studied in future experiments on the causes of jet-blowing in lionfish. Such experiments should consider the variation among lionfish individuals and could also include invertebrate prey.

Acknowledgements
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Ethical statement
The experimental protocol for the maintenance of wild lionfish in captivity and experiments on their interactions with prey was approved by the ethics committee of the Hellenic Centre for Marine Research (decision number: 125/12.09.2023).

Additional permits
Capture of live lionfish by SCUBA and free divers was allowed by the local authorities through a sample collection permit from the Hellenic Ministry for Environment and Energy (permit number: 6ΡΔΛ4653Π8-Ι9Ρ).

Supplementary material

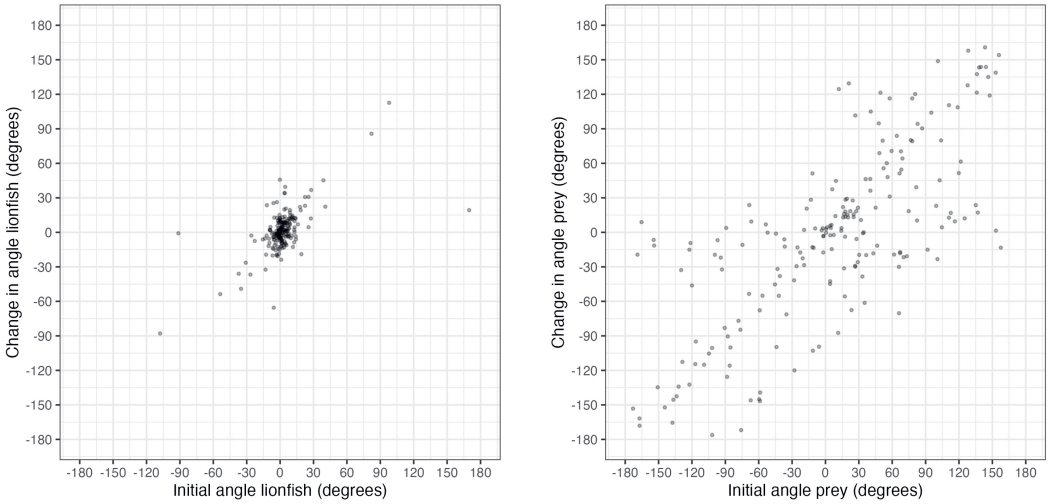

Figure S1. Contribution of lionfish and prey to the total change in angle during approaches in the seabream experiment. The panel on the **left** shows the change in angle of lionfish during an approach, relative to the initial lionfish-prey angle. The panel on the **right** shows the change in angle of prey during an approach, relative to the initial lionfish-prey angle.



Figure S2. Stills from videos that were used for data collection. Panel A was taken from one of the videos from the cameras mounted on the side of the arena. These videos were used to determine whether a lionfish

was blowing or not. **Panel B** was taken from the central camera mounted above the arena. These videos were used to determine angles and distances during an approach. In both videos, a lionfish is approaching a prey with fully flared pectoral fins. Panel B also shows the two cameras mounted on the side of the arena and some lionfish in the common area. The two screws used to calibrate the software for size estimations are visible in both stills.

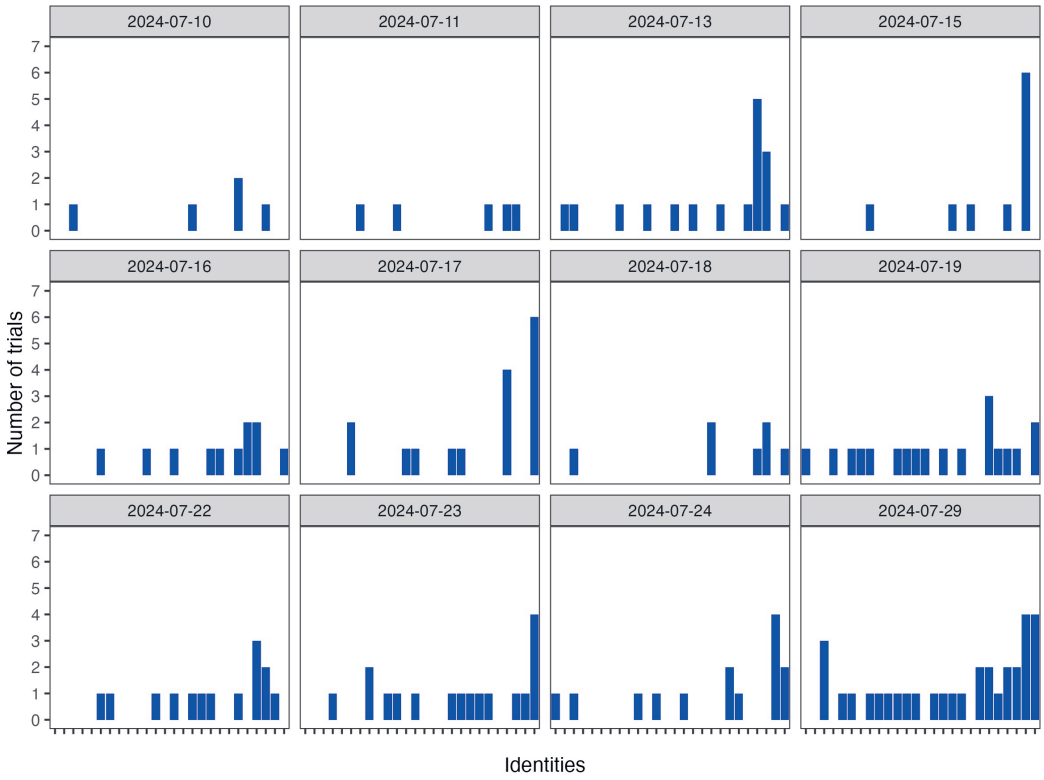

Figure S3. Participation rate of individual lionfish during the seabass experiment. Each mark on the x axis represents an individual and the blue bars show the number of trials in which they participated every day. Dates are shown in the grey labels.

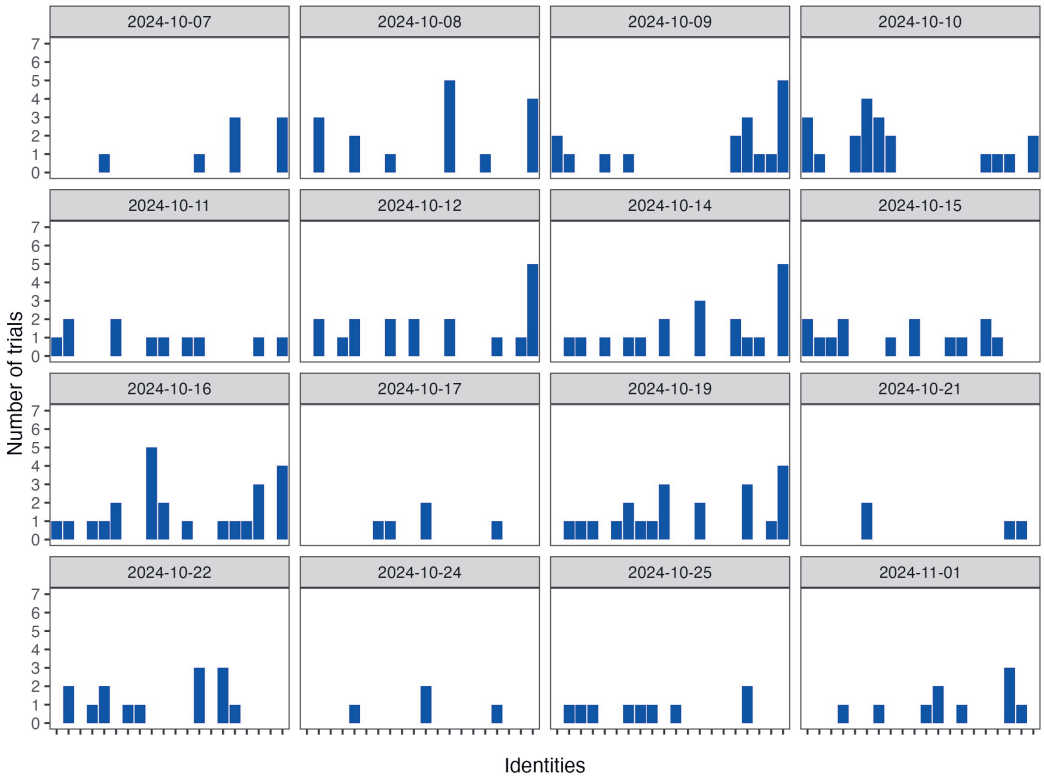

Figure S4. Participation rate of individual lionfish during the seabream experiment. Each mark on the x axis represents an individual and the blue bars show the number of trials in which they participated every day. Dates are shown in the grey labels.

Table S1. Full tables for the models on the **effect of blowing on the proportion of head-first captures** in the seabass and seabream experiment.

Seabass experiment				
Family	binomial (logit)			
Formula	Catch_orientation ~ Blowing + (1|Lionfish_ID)			
	Random Effects			
Groups	Name	Variance	Std. Dev.	
Lionfish_ID	(Intercept)	2.42E-08	0.0001555	
Number of observations	132			
Lionfish_ID	24			
	Fixed Effects			
	Estimate	Std. Error	z value	p value (>|z|)
Intercept	-0.280	0.238	-1.175	0.240
Blowing	2.477	0.492	5.037	4.74E-07
Seabream experiment				
Family	binomial (logit)			
Formula	Catch_orientation ~ Blowing + (1|Lionfish_ID)			
	Random Effects			
Groups	Name	Variance	Std. Dev.	
Lionfish_ID	(Intercept)	0	0	
Number of observations	215			
Lionfish_ID	20			
	Fixed Effects			
	Estimate	Std. Error	z value	p value (>|z|)
Intercept	-0.478	0.229	-2.091	0.037
Blowing	2.052	0.324	6.341	2.29E-10

Table S2. Full tables for the models on the **effect of lionfish size and initial angle on the proportion of blowing approaches** in the seabass and seabream experiment.

Seabass experiment				
Family	binomial (logit)			
Formula	Blowing ~ Start_angle + Lionfish_size + (1|Lionfish_ID)			
		Random Effects		
Groups	Name	Variance	Std. Dev.	
Lionfish_ID	(Intercept)	2.28E+00	0.00016	
Number of observations	167			
Lionfish_ID	26			
		Fixed Effects		
	Estimate	Std. Error	z value	p value (>|z|)
Intercept	9.925	2.043	4.859	1.18E-06
Start_angle	0.001	0.004	0.399	0.690
Lionfish_size	-0.420	0.090	-4.655	3.24E-06
Seabream experiment				
Family	binomial (logit)			
Formula	Blowing ~ Start_angle + Lionfish_size + (1|Lionfish_ID)			
		Random Effects		
Groups	Name	Variance	Std. Dev.	
Lionfish_ID	(Intercept)	17.31	4.16100	
Number of observations	214			
Lionfish_ID	20			
		Fixed Effects		
	Estimate	Std. Error	z value	p value (>|z|)
Intercept	36.909	14.800	2.494	0.013
Start_angle	0.003	0.004	0.612	0.54
Lionfish_size	-1.710	0.712	-2.401	1.63E-02

Table S3. Full tables for the models on the **effect of blowing on the change in angle by prey during an approach** in the seabass and seabream experiment.

Seabass experiment				
Family	gaussian (identity)			
Formula	Angle_change ~ Blowing + Start_angle + Blowing:Start_angle + (1|Lionfish_ID)			
	Random Effects			
Groups	Name	Variance	Std. Dev.	
Lionfish_ID	(Intercept)	4.83E-09	6.95E-05	
Number of observations	167			
Lionfish_ID	26			
	Fixed Effects			
	Estimate	Std. Error	z value	p value (>|z|)
Intercept	-6.115	8.711	-0.702	4.83E-01
Blowing	-17.778	12.266	-1.449	0.147
Start_angle	0.176	0.101	1.747	0.081
Blowing :Start_angle	0.568	0.136	4.189	2.80E-05
	Estimate	SE	Lower.CI	Upper.CI
Blowing	0.745	0.091	0.567	0.922
No-blowing	0.176	0.101	-0.021	0.374
Seabream experiment				
Family	gaussian (identity)			
Formula	Angle_change ~ Blowing + Start_angle + Blowing:Start_angle + (1|Lionfish_ID)			
	Random Effects			
Groups	Name	Variance	Std. Dev.	
Lionfish_ID	(Intercept)	1.26E-05	0.003546	
Number of observations	214			
Lionfish_ID	20			
	Fixed Effects			
	Estimate	Std. Error	z value	p value (>|z|)
Intercept	-46.974	7.599	-6.182	6.34E-10
Blowing	24.741	9.411	2.629	0.009
Start_angle	0.593	0.087	6.825	8.76E-12
Blowing :Start_angle	0.276	0.108	2.543	1.10E-02
	Estimate	SE	Lower.CI	Upper.CI
Blowing	0.869	0.065	0.742	0.996
No-blowing	0.593	0.087	0.423	0.763

Table S4. Full tables for the models on the **effect of blowing on strike distance** in the seabass and seabream experiment.

Seabass experiment				
Family	gamma (log)			
Formula	Strike_distance ~ Blowing + (1|Lionfish_ID)			
	Random Effects			
Groups	Name	Variance	Std. Dev.	
Lionfish_ID	(Intercept)	4.15E-02	0.204	
Number of observations	167			
Lionfish_ID	26			
	Fixed Effects			
	Estimate	Std. Error	z value	p value (>|z|)
Intercept	1.737	0.088	19.658	<2e-16
Blowing	0.041	0.101	0.403	6.87E-01
Seabream experiment				
Family	gaussian (identity)			
Formula	Strike_distance ~ Blowing + (1|Lionfish_ID)			
	Random Effects			
Groups	Name	Variance	Std. Dev.	
Lionfish_ID	(Intercept)	0.934	0.966	
Number of observations	214			
Lionfish_ID	20			
	Fixed Effects			
	Estimate	Std. Error	z value	p value (>|z|)
Intercept	5.087	0.423	12.041	< 2e-16
Blowing	3.100	0.470	6.598	4.16E-11

Table S5. Full table for the model on the **effect of blowing on strike success** in the seabass and seabream experiment.

Seabass experiment				
Family	binomial (logit)			
Formula	Strike_success ~ Blowing + (1|Lionfish_ID)			
	Random Effects			
Groups	Name	Variance	Std. Dev.	
Lionfish_ID	(Intercept)	1.56E+00	1.247	
Number of observations	167			
Lionfish_ID	26			
	Fixed Effects			
	Estimate	Std. Error	z value	p value (>|z|)
Intercept	1.352	0.667	2.027	0.043
Blowing	-0.201	0.880	-0.228	8.19E-01

Chapter 6 | General discussion

With my thesis, I contribute to the study of the ecology of lionfish, one of the most invasive species in the marine realm. In **chapter 2**, I provide an overview of the knowledge that was available on the Mediterranean invasion at the start of my project and present an update on lionfish distribution in the Mediterranean. We show that our knowledge on lionfish in the Mediterranean is in its infancy and that lionfish continue to expand in the basin. In **chapter 3**, I present a study in which we experimentally tested for the impact of lionfish on the Mediterranean fish community for the first time. Although we lowered the lionfish density at experimental sites through regular removals, this had no effect on the local fish biomass and community composition compared to control sites. This suggests that culling at the intensity and rate conducted during public tournaments cannot be considered a biodiversity conservation measure. In **chapter 4**, I present experiments on background choice, a potential mechanism through which lionfish could avoid detection by their prey. We show that, although lionfish select their background when hunting, their preference is probably not determined by background matching. In **chapter 5**, I show a study in which we investigated the functions of jet-blowing, a unique component of the stalking hunting strategy of lionfish. We show that lionfish blow jets of water at their prey to re-orientate them and catch them more easily head-first. In the first two sections of this general discussion, I place my findings in a broader context and suggest future research avenues on the invasion ecology of lionfish in the Mediterranean and on lionfish predation ecology. In the third section, I present remaining open questions on lionfish ecology. In the final section, I suggest that future quantitative and experimental studies should also focus on other Lessepsian species to leverage them as a broader study system to understand bio-invasions in marine ecosystems.

The spread and impact of invasive lionfish in the Mediterranean Sea

In **chapter 2**, we found a fast westward and northward expansion of lionfish in the Mediterranean between 2021 and 2023. In 2023, lionfish were reported from the whole eastern Mediterranean. Since the publication of this chapter, lionfish have become more abundant along the Ionian Italian coast and have further expanded northwards into the Adriatic (Azzurro et al. 2025). Except for some individual sightings in the western Mediterranean (e.g., Spain, Sardinia), lionfish remain confined to the eastern part of the basin and are following the typical westward and northward invasion pattern of Lessepsian species (Phillips and Kotrschal 2021, Azzurro et al. 2025). At the moment, the lionfish invasion has three fronts in the Mediterranean: the Strait of Sicily, the Strait of Messina and the transition area between southern and Northern Adriatic Sea, at the latitude of the cities of Ancona and Split (Azzurro et al. 2025). Whether lionfish will further expand these fronts remains to be seen as most Lessepsian species remain confined to the eastern Mediterranean (Azzurro et al. 2013). The distinctive morphology of lionfish makes them

suitable for monitoring through citizen science initiatives and collaborations with the numerous diving centres operating on the Mediterranean coast (Phillips and Kotrschal 2021, Bottacini et al. 2024, Azzurro et al. 2025). Although they offer little information on lionfish densities, such efforts have the advantage of comprising the whole Mediterranean and should be continued in the future to monitor any range expansions. Future monitoring would benefit from complementary studies on the quantification of the density of lionfish through standardised protocols for underwater visual census in various areas of the Mediterranean.

Lionfish have been considered invasive for a decade in the eastern Mediterranean, where they reached far higher densities than in their native range (Kulbicki et al. 2012, Kletou et al. 2016, Kleitou et al. 2024, Phillips et al. 2024). Therefore, it is important to expand the scientific focus from monitoring to ecological studies, where interactions of lionfish with the local species and their impact on the ecosystem are accounted for (Zannaki et al. 2019, D'Agostino et al. 2020, Huseyinoglu et al. 2024). This is especially important since resources have been directed towards control initiatives with the goal of protecting the local biodiversity, without assessing the impact of lionfish (Kleitou et al. 2021, Savva et al. 2024). In **chapter 3**, our aim was two-fold: testing for the impact of invasive lionfish on the Mediterranean fish community and assessing whether lionfish removals benefit the local biodiversity. With that aim, we tested the hypothesis that removing lionfish through regular spearfishing would result in an increase in the biomass of local fishes and in a shift in the community composition. We found that, although regular removals lowered lionfish densities, this had no effect on the biomass and community of local fishes compared to control areas. Because lionfish prey extensively on Mediterranean fishes, we interpret our results as the consequence of the exchange of local fishes between our sites and surrounding areas, which had lower lionfish biomass (Zannaki et al. 2019, D'Agostino et al. 2020). Information available on the home range of some of the species in our study area suggests that this may be the case (Alós et al. 2011, 2012, La Mesa et al. 2012).

Future experiments on the effects of lionfish on the fish community of the Mediterranean could be designed in areas that are isolated enough for the local fish populations to have lower influence from surrounding areas. An experiment on isolated reefs would complement experiments from the western Atlantic, where the clearest effects of lionfish on the local fish community have been shown on isolated reef patches with limited to no exchange of fishes in the species that were surveyed (Albins and Hixon 2008, Green et al. 2012, 2014). However, results from such experiments would be difficult to generalise in the Mediterranean, where continuous reefs are the norm. Another way of approaching these experiments in the future is culling areas that are larger than the home

range of local fishes. Such efforts would require large culling teams and the involvement of volunteers. Additional insights could come from an experiment in continuous reefs similar to the one that we conducted, but at a time point when lionfish densities are higher and more homogeneous. This is also a reason why it would be important to quantitatively monitor lionfish densities at a smaller scale than previously done (Bottacini et al. 2024, Azzurro et al. 2025). The chance of monitoring the impact of invasive species at different timepoints in the invasion process represents an opportunity in invasion ecology, where impacts are often measured at the peak of invasions. Information from different timepoints in the invasion is also largely missing from the western Atlantic, where studies on the impact of invasive lionfish were concentrated in a narrow time span (but see Ballew et al. 2016) and started when the lionfish densities had probably already reached their peak (Albins and Hixon 2008, Green and Côté 2009, Green et al. 2012, 2014).

In chapter 3, we removed lionfish with a method and at a rate that are comparable to those employed in public culling tournaments in the Mediterranean (Savva et al. 2024). Therefore, an important conclusion that can be drawn from our study is that culling at the rate conducted during public tournaments is unlikely to provide benefits to the local fish community of invaded areas in the Mediterranean. More frequent culls could counteract re-colonisation and could certainly reduce the lionfish density more effectively. However, this would be difficult to scale and can only be done for research purposes, making it an unsuitable method for management purposes. There are two further complication to account for when considering culling as a control measure in the Mediterranean: there are many areas in the sea (e.g., deep waters) that are not accessible during culling and the Suez Canal will remain open, resulting in constant recolonisation. Therefore, lionfish removals will never have a definitive effect and would require continuous efforts and investments to keep lionfish densities below established thresholds (Frazer et al. 2012, Green et al. 2014, Malpica-Cruz et al. 2016). This shows how different marine invasions are from the situation in the few islands where eradication of invasive species was successful: the vastness, connectivity and inaccessibility of large part of marine water bodies makes monitoring and control measures less effective than on land (Genovesi 2005, Jones et al. 2016). Overall, this stresses the importance of focussing on preventing new invasions, rather than trying to control them.

We need more evidence on the ecological impact of invasive species, particularly from experimental approaches that can show causality (Ricciardi et al. 2013, Katsanevakis et al. 2014). Lionfish have become a model for such studies for a number of reasons. First, there is a lot of information available on their spread (Côté et al. 2013, Soares et al. 2022, 2025, Azzurro et al. 2025). Second, lionfish are relatively easy to manipulate in density (Albins and Hixon 2008, Green et al. 2014). Third, it is a remarkable opportunity to have the same invader in two separate invaded ranges, in completely different ecological circumstances.

Although relatively common in freshwater, where ecosystems are less connected, this is rare in marine invasions (Nunes et al. 2014, Melotto et al. 2020, Abreo et al. 2026, Kiesecker and Blaustein 2026). Fourth, in both the Atlantic and Mediterranean invasion, lionfish are expanding and colonising areas with different ecology and local communities. In the Mediterranean, lionfish are expanding in the Adriatic, which is ecologically very different from the eastern Mediterranean (Coll et al. 2010). In the western Atlantic lionfish are expanding along the Brazilian coast and have crossed the Amazon-Orinoco plume, an important biogeographic barrier source of different fish communities (Soares et al. 2022, 2025). These range expansions provide further opportunities to test for the impact of the same species in different contexts. Finally, in the Mediterranean, predation by lionfish is likely only one component of a much larger set of changes. In the areas that we surveyed for the experiment presented in chapter 3, up to 40% of the biomass (excluding lionfish) was composed of Lessepsian fishes. The impact of most Lessepsian species has never been studied and could complement studies on lionfish with examples from species that are taxonomically and ecologically different (Kalogirou et al. 2007, Sala et al. 2011).

The stalking hunting strategy of lionfish

Lionfish are crepuscular, generalist predators that hunt on a variety of prey (Cure et al. 2012). Although stomach content analyses report a proportion of invertebrates, the diet of lionfish is primarily composed of small demersal and benthic fishes (Valdez-Moreno et al. 2012, Harms-Tuohy et al. 2016, Zannaki et al. 2019). Lionfish have a stalking hunting strategy: they slowly follow moving prey with their pectoral fins fully open for a long time before striking and swallowing them whole (Kulbicki et al. 2012, D'Agostino et al. 2020). When they target prey that are not moving, lionfish show the same hunting strategy that they use for moving prey and slowly approach fishes up to a close distance before striking (Albins and Lyons 2012). The stalking hunting strategy of lionfish has elements that are typical of both ambush and pursuit strategies, but it also has major differences. Similarly to pursuit predators, lionfish have an active approach to their prey. At the same time, once prey display an antipredator response, lionfish do not chase them to outswim or outmanoeuvre them. Similarly to ambush predators, lionfish strike before their prey exhibit any anti-predator response, but do not passively wait for their prey to spontaneously enter their strike range (Cure et al. 2012, D'Agostino et al. 2020).

An important question on the hunting strategy of lionfish is how they manage to approach their prey up to a distance that can be as short as 10-20 cm without triggering an antipredator response (D'Agostino et al. 2020). For a prey to react to a predator, two important processes must take place: detection and recognition. Detection occurs when a

prey perceives cues of the presence of a predator in the environment (Kelley and Magurran 2003). After detection, prey must recognise the danger associated with a predator (Brown 2003, Kelley and Magurran 2003). Recognition can only ensue detection and it has a key role in determining if and how a prey will react to an animal approaching. For example, a coral reef fish may detect both a herbivore and a predator in the surroundings, but will only react to a predator after recognising that it represents a threat to its survival. Camouflage, in its various forms, is a prominent example of a mechanisms that animals use to interfere with detection and recognition (Merilaita et al. 2017). For instance, through background matching, predators can become less detectable against their background and mimicry can allow animals that are detected to be recognised as non-dangerous because they resemble non-threatening objects (Cuthill et al. 2005, Cuthill 2019). In **chapter 4**, we tested the hypothesis that lionfish interfere with detection in their prey through background matching. Although conspicuous and colourful in appearance, lionfish could camouflage when they hunt on coral reefs, where the colour and complexity of invertebrates in their surroundings could match their appearance (Marshall et al. 2019). We expected hunting lionfish to choose a coral reef background as opposed to backgrounds with a uniform colour or a rocky reef. We found that lionfish did not show a preference that supports the hypothesis that they choose their background to camouflage. Although we did not test whether and how lionfish are detected by their prey, lionfish feed on bottom dwelling fishes and we can expect them to be often forced to approach them with the uniform background of the blue water. In this case, interference with recognition, rather than detection, may be more relevant.

In invaded ranges, prey may not recognise lionfish as predators due to naïveté (Anton et al. 2016, D'Agostino et al. 2020). However, lionfish use the same stalking hunting strategy across ranges, suggesting that prey naïveté alone cannot explain the fundamental principles of how lionfish hunt (Cure et al. 2012). Two phenomena that could be relevant for the barred pattern of lionfish are flicker fusion and motion dazzle. Flicker fusion occurs when objects with high-contrast patterns move at a sufficient speed to conceal their shape against their background (Umeton et al. 2017, 2019). On the other hand, motion dazzle makes it difficult to predict trajectory and speed of objects with high-contrast, broken patterns (Scott-Samuel et al. 2011). The relevance of these phenomena in animals could benefit from more empirical evidence, since motion dazzle has only been shown to be effective on human subjects and flicker fusion has only been shown to work in prey (Stevens et al. 2011, Umeton et al. 2019). In general, the evidence for direct benefits of camouflage in predator-prey interactions is skewed towards prey, although camouflage is regularly presented as a mechanism that can benefit both predators and prey (Merilaita et al. 2017). Studies on lionfish morphology and its role in their interactions with prey could be an important contribution to our knowledge on the benefits of camouflage for predators. A possible approach could be to use video presentations or virtual reality set-ups, where

the lionfish approach, morphology and colouration are changed strategically to test for the relevance of flicker fusion or motion dazzle (Umeton et al. 2019, Vidal et al. 2023). Experiments with virtual presentations would also allow for adjustments of colours and brightness to simulate the twilight light in which lionfish hunt (Cure et al. 2012).

Regardless of what mechanisms are at play in their stalking strategy, lionfish approach prey up to a remarkably close distance before striking in the wild (20-50 cm) (D'Agostino et al. 2020). While approaching their prey, lionfish occasionally blow jets of water at them (Albins and Lyons 2012, Cure et al. 2012). In **chapter 5**, we report blowing in lionfish from the Mediterranean for the first time and present the first experiment aimed at studying the causes and consequences of blowing. Before our experiment, jet-blowing had only been described in lionfish from the western Atlantic and the functions of this behaviour were unknown (Albins and Lyons 2012). We show that lionfish blow at their prey to orientate them towards their mouth, resulting in a higher proportion of head-first captures in blowing approaches. Lionfish use jet-blowing to trigger positive rheotaxis, a highly conserved response to water currents in fishes; prey perceive the water jets as a current and orientate towards its source (i.e., the lionfish mouth). Similarly to other fish predators, capturing prey head-first could allow lionfish to swallow their fish prey more easily, reducing injuries and escapes (Morris et al. 1956, Reimchen and Douglas 1984, Hoyle and Keast 1987, Reimchen 1991). Future experiments could test the benefits of head-first capture in lionfish by measuring whether prey that are caught head-first are swallowed in shorter time. In our experiment, this was not possible because the prey were relatively small and the swallowing process was difficult to quantify by behavioural observations.

The benefits of jet-blowing for lionfish raise the question of why this behaviour did not evolve in more fish predators. While blowing is now well documented in lionfish across ranges, I am not aware of other predators directing water jets at mobile, fish prey. Observing fish hunting for mobile prey underwater is challenging, because observers can easily interfere with the interaction by startling either predator or prey. This certainly contributes to lionfish remaining the only example of predators blowing jets of water at fish prey. Especially for predatory fishes, jet-blowing could be an advantageous way to orientate prey head-first before capture. Terrestrial animals achieve this by using post-capture manipulations, which would result in increased risk of prey escape in predatory fishes (Reimchen and Douglas 1984, Reimchen 1991). Furthermore, jet-blowing probably evolved from coughing, which is highly conserved among fishes and could therefore become an addition to the behavioural repertoire of many species (Wainwright and Turingan 1997). Probably, jet-blowing did not evolve more often because predators can rarely approach prey close enough for jet-blowing to be effective. We did not quantify how

far water jets reach in our study, but a previous study indicates that they were detectable at a maximum distance of approximately 10 cm, confirming that jet-blowing can only be effective once a predator has approached a prey very closely (Albins and Lyons 2012).

Open questions on lionfish ecology

Studying invasive lionfish represents an opportunity to learn more about invasions in the marine environment, where invasive predators are rare and replicated invasions developing in parallel in different seas are even less common. There are major questions on lionfish ecology that remain open. Answering such questions could lead to important insights on what makes a successful invader in marine environments. Moreover, while some of these aspects of lionfish ecology are often considered mere natural history, they should always be viewed in light of their reflections on invasion ecology. Lionfish are amenable to laboratory experiments and are site-attached, easy to observe and identify at the individual level underwater. We should leverage the lionfish invasions to conduct more empirical studies, as it would answer questions that have been largely neglected in marine invasions.

Are prey populations adapting to invasive lionfish through evolution?

Both Mediterranean and western Atlantic fishes were shown to be naïve to lionfish (Anton et al. 2016, D'Agostino et al. 2020). This was expected since neither basins has native lionfishes and lionfish morphology and hunting strategy do not overlap with those of local predators (Anton et al. 2020). It follows from the definition of prey naïveté that it can be counteracted by evolutionary adaptations; although prey may not recognise a novel predator due to a lack of coevolutionary history, they might evolve to recognise it through generations (Strauss et al. 2006, Anton et al. 2020). Although evolutionary adaptations are generally not expected to evolve rapidly in marine environments due to their high connectivity, selection pressures from invasive species can result in prey evolving antipredator responses within a few generations (Nunes et al. 2014, Anton et al. 2020, Melotto et al. 2020). Despite lionfish being invasive for more than two decades in the western Atlantic, there is only one study testing for potential adaptations of local fishes to lionfish. The study was conducted in areas that had been invaded three and seven years before the experiment on adults of a territorial damselfish, without analysing specific antipredator responses (Kindinger 2015). Future studies on adaptations to lionfish should be conducted in prey from areas with a larger gradient of generations of coexistence with lionfish and controlling for the experience of the individuals tested. Such studies will be beneficial to the larger field of invasion ecology, where experiments on naïveté are often conducted in early phases of invasions, when prey are expected to show the highest level of naïveté (Anton et al. 2020).

Future experiments could take advantage of areas around the invasion fronts of lionfish to test how naïve individuals react to an approaching lionfish in a controlled environment. Such experiments require prey from populations of the same species that have co-existed for different lengths of time with lionfish. For instance, numerous fish species that are preyed upon by lionfish are found throughout the whole Mediterranean. Lionfish, on the other hand, have been abundant for more than a decade in the eastern part of the basin, have only recently colonised the central part and are completely absent in the western part (Azzurro et al. 2025). Prey from these three areas could be compared in their response to lionfish to assess whether generations of coexistence with lionfish are resulting in adaptive changes in prey. Collecting eggs and rearing larvae in captivity would ensure that the fish tested have no experience with lionfish or any other stimulus fish that is used in the experiments. Two candidate prey species are *Apogon imberbis* and *Chromis chromis*, which are abundant across the whole Mediterranean and preyed upon extensively by lionfish (Zannaki et al. 2019, D'Agostino et al. 2020). *C. chromis* is a substrate spawner and males of *A. imberbis* are mouth brooders (Picciulin et al. 2004, Mazzoldi et al. 2008). This could make the collection of eggs possible through the placement of artificial, retrievable egg-laying substrate for *C. chromis* and capture of mouth brooding males of *A. imberbis*. Rearing the larvae would yield completely naïve juveniles, which could be tested in a controlled environment for how they react to an approaching lionfish as opposed to control native predators and non-predators. A similar approach could be implemented with fishes from areas in the northern Brazilian coast, where lionfish are also expanding (Soares et al. 2022, 2025).

Experiments comparing captive-reared prey of the same prey species will also provide an opportunity to test for naïveté towards lionfish while controlling for two major confounders that have, thus far, not been accounted for. First, no experiment has worked on completely naïve prey, making it impossible to quantify how much of the responses measured were the result of genetic naïveté (Black et al. 2014, Anton et al. 2016, D'Agostino et al. 2020). Second, previous experiments mostly compared naïve and non-naïve individuals of different species. For instance, Mediterranean prey were compared in their response to an approaching lionfish with Lessepsian species, which have coevolved with lionfish and various prey species from the Atlantic were compared with taxonomically close species from the native range of lionfish (Anton et al. 2016, D'Agostino et al. 2020). While important, these efforts do not allow to assess the proportion of behavioural differences that could have simply be due to intrinsic differences among the species compared or to different levels of direct experience with lionfish among individuals of

different species. Working with captive-reared individuals from the same species would eliminate both confounders.

Are individual prey adjusting to invasive lionfish through learning?

In invasion ecology, prey naïveté is regarded as an evolutionary phenomenon that results in a lack of antipredator response in prey (Strauss et al. 2006, Sih et al. 2010). However, predator recognition in fishes is not entirely innate, but has a learned component: individuals can learn during their lifetime which organisms pose a threat to their survival (Brown 2003, Kelley and Magurran 2003). Learned predator recognition is prominent in marine fishes, where individuals that have experienced encounters with a predator throughout their life react more promptly to the predator in future encounters. This is an adaptive mechanism in marine fishes, which have a bipartite life cycle: they are born as pelagic larvae and spend a long time drifting in the open sea before settling in the coastal environment as young recruits (Barth et al. 2015). This can result in young fishes recruiting in far and different environments from where they were spawned. Therefore, being able to rapidly learn through experience which organisms threaten their survival is important for the success of recruits. Recruits of marine fishes that have not encountered predators can learn with a single conditioning event which animals pose a threat to their survival (Mitchell et al. 2011). As a consequence, local fishes could be learning that lionfish are dangerous, regardless of whether they coevolved with them. Testing learned predator recognition could help elucidate whether this is a mechanism relevant in the lionfish invasions.

Tests on learned predator recognition could be conducted on naïve individuals of a prey species from the Mediterranean or the western Atlantic. Such experiments should compare the learning performance of prey towards lionfish and towards other native predators (McCormick and Allan 2016). Similarly to experiments on prey naïveté, studies on learned predator recognition will greatly benefit from the use of recruits that have had no experience with predators prior to the experiments. Studying learning as a mechanism for prey to cope with lionfish as a new predator can help elucidate what impact lionfish could have on the fish populations in invaded areas. In addition, these studies would also indicate that in highly invaded seas such as the Mediterranean, local species could also show similar plasticity towards other invasive predators, without having to go through evolutionary processes. In general, learning could be a more effective and faster way for prey to cope with a new, invasive predator; while evolution requires multiple generations, plastic responses take place within one.

What are the natural enemies of lionfish in their native range?

Reports of predation on lionfish are extremely rare in their invaded ranges, which is often presented as support for the hypothesis that lionfish have no enemies. This would, in turn, be a major component of their success as an invasive species (enemy release hypothesis) (Côté and Smith 2018, Bottacini et al. 2024). However, which factors control lionfish populations in their native range remain unknown. Direct observations of lionfish predation in the field will likely remain anecdotal and sporadic in the future, since predation is a rare phenomenon to observe underwater and the low density of lionfish in the native range makes it even more challenging (Cure et al. 2012, Kulbicki et al. 2012). Lionfish are highly venomous and large fish, which makes it unlikely that any predator feeds consistently on adults. This has led to the hypothesis that it could be parasites limiting their populations in invaded ranges. However, the parasite loads of lionfish in their native range, while higher than in invasive ranges, are unlikely to decrease their fitness to the point of explaining the large difference in lionfish density observed between invaded and native ranges (Loerch et al. 2015, Sellers et al. 2015, Tuttle et al. 2017).

In alternative to predation and parasitism, lionfish densities could be limited by competition in their native range. The Indo-Pacific ocean is highly biodiverse and has a completely different species composition from the eastern Mediterranean and the Caribbean (Myers et al. 2000, Hughes et al. 2002, Coll et al. 2010). Competition could come from other species of lionfish, which are completely absent in Caribbean and Mediterranean, but also from other meso-predators. Future efforts could quantify the exact ecological and trophic niche that lionfish occupy in the Indo-Pacific Ocean to test how much competition they can be expected to face compared to the invaded ranges. Interesting insights into potential control factors could come from areas in the western Atlantic where lionfish have naturally declined in recent years (Benkwitt et al. 2017, Debrot et al. 2023). Future studies should investigate the origin of these declines and continue monitoring lionfish densities in the most eastern part of the Mediterranean to track the long-term population dynamics in areas that have been surveyed since the start of the invasion (Kleitou et al. 2021, 2024). Although challenging, studying factors controlling lionfish populations in the Indo-Pacific is important because it will give us insights into whether lionfish could find enemies in invaded ranges. In addition, knowing which enemies lionfish have in the Red Sea can also help us predict potential scenarios in the future of the Mediterranean invasion, where the source of invasive lionfish (i.e., the Suez Canal) could also become the source of control organisms.

What are the drivers of lionfish aggregations?

Lionfish are often found in large aggregations composed of several individuals resting in close proximity to each other and the drivers of the formation of these groups are still unknown (Kletou et al. 2016, García-Rivas et al. 2017, Hunt et al. 2019). Lionfish could be a social species, where individuals are attracted towards conspecifics, but it could also be that multiple lionfish are attracted to the same shelters, becoming part of a group as a consequence (Hunt et al. 2019). Lionfish within an aggregation do not interact in any apparent way, suggesting no social drivers to lionfish aggregations (García-Rivas et al. 2017). Lionfish are also found clustered around habitats that offer hiding opportunities and the first experimental study on lionfish aggregations from the western Atlantic points at habitat complexity as the main ecological driver of lionfish aggregations (Hunt et al. 2019, Phillips et al. 2024). However, lionfish preferred a shelter close to a conspecific as opposed to one without a conspecific in a captive setting, suggesting that social drivers may also play a role in the formation of aggregations (Holmes et al. 2025). Although social and ecological drivers are not mutually exclusive and could both have a role in the formation and maintenance of lionfish aggregations, they have not been accounted for in the same experiment (Hunt et al. 2019, Holmes et al. 2025). Future experiments could elucidate whether lionfish are socially attracted to conspecifics by manipulating social and structural complexity, either in the wild or in large aquaria.

Understanding the drivers of lionfish aggregations will have both applied and fundamental implications. From an applied perspective, it could result in potential population control methods based on large-scale, commercial fishing. At present, the most common control method for invasive lionfish is spearfishing by SCUBA and free divers, which has several limitations and is difficult to scale (Malpica-Cruz et al. 2016, Kleitou et al. 2021). Taking lionfish fishing to a commercial scale necessitates specific traps and nets that can be used by commercial fishers. Preliminary traps have been developed and tested, but could benefit by either being placed in strategic areas where lionfish aggregate or by including attractants based on the cues that lionfish use to aggregate naturally (Harris et al. 2020, Tosunoğlu et al. 2026). From a fundamental perspective, studying lionfish aggregations will contribute important knowledge on their basic ecology and aspects that might be key to their invasion success. For instance, lionfish within aggregations tend to have similar and relatively large sizes, which may be the result of individuals grouping for reproductive purposes (García-Rivas et al. 2017). Behavioural observations on aggregations in which the sex of the individuals is known could shed light on reproductive aspects of lionfish.

When, where and how do lionfish spawn?

Lionfish have never been observed spawning (Morris 2009, Côté and Smith 2018). Speculations on their reproductive behaviour as well as timing and frequency of spawning events are based on observations on closely related species or dissections of gonads (Fishelson 1975, Savva et al. 2020, Mouchlianitis et al. 2022). This is a major knowledge gap, because reproduction could be the key to understanding ecological traits of lionfish that are currently poorly understood. For example, aggregating with conspecifics could be important in lionfish, which are relatively poor swimmers, to have easy access to reproductive opportunities. Another factor that could benefit from observations of spawning lionfish is the identification of natural enemies: searches and speculations on potential lionfish enemies have focussed on adult lionfish, while the phase when coral reef fishes are most susceptible to mortality is before or soon after recruitment (Almany and Webster 2006, Phillips and Kotrschal 2021). It is possible that lionfish predation in the native range is mostly restricted to eggs or larvae and that a lack of observations on these life stages is the cause of the apparent paucity of lionfish natural enemies. Lionfish are a widely studied species and also attract the interest of recreational divers and aquarists. Yet, no observations of spawning lionfish have ever been reported, indicating that anecdotal observations of spawning will remain unlikely.

Rather, more refined methodologies should be used to determine when and where lionfish spawn. Monitoring large lionfish aggregations through cameras arrays could be an approach, but it would come with limitations of scalability and lack of coverage during dark hours (Boom et al. 2014, Mouy et al. 2023). On the other hand, acoustic telemetry allows to accurately and continuously track fishes over large distances and, when combined with biologging, insights into animal behaviour (Martorell-Barceló et al. 2024, Meira et al. 2024). Once the timing and location of spawning events are known, targeted observations could be conducted in the field. Observing lionfish spawning could lead to important behavioural observations and would also allow for the collection of fertilised eggs. This would pave the way for experiments on the development of eggs and larvae and the identification of potential predators. Laboratory approaches based on hormonal stimulation of spawning and *in-vitro* egg fertilisation could be used to observe spawning or obtain fertilised eggs (Mylonas et al. 2016, Ramos-Júdez et al. 2019). However, such efforts would not provide information on how spawning occurs naturally, preventing possible observations or speculations on natural enemies.

The Lessepsian invasion as a model system to understand marine invasions

In the Mediterranean Sea, the lionfish invasion is only a small component of a larger, more dramatic phenomenon; the Lessepsian invasion (i.e., species invading the Mediterranean from the Red Sea). The source of Lessepsian species is the Suez Canal, a prominent example of a human development project that was planned without accounting for its ecological impact. Scientific efforts on the Lessepsian invasion have been largely focussed on descriptive monitoring of the arrival and spread of new species (e.g., first sightings, range expansions) or modelling and predictive approaches (e.g., species distribution models). While important, these efforts are not fully exploiting the opportunities offered by an ongoing natural experiment where multiple invasions are developing in parallel. Applying quantitative and experimental approaches to key questions that remain open on the Lessepsian invasion could result in important insights that can be generalised to marine bio-invasions. These include the conditions that make an ecosystem susceptible to invasions, the factors that make certain species highly invasive and how invasive species adapt and adjust to new environments during the course of an invasion. While there are Lessepsian organisms in virtually every taxon, this part of the discussion has a focus on fishes. This is because fishes are the organisms that have been best documented in the context of the Lessepsian invasion due to their conspicuousness, relatively well-resolved taxonomy and the possibility to access fishery data.

A late invasion: why did Lessepsian species not colonise the Mediterranean earlier?

The Suez Canal has been open since 1869, raising the question of why Lessepsian species have been colonising the Mediterranean mostly in the last few decades (Galil et al. 2014, Arndt and Schembri 2015). This is a long-standing question in the context of the Lessepsian invasion and answering it could elucidate the factors that make ecosystems more susceptible to invasions. Climate change is a factor that is often presented as the main driver of this delayed invasion; the temperate/subtropical Mediterranean has become warmer, becoming more hospitable for tropical species from the Red Sea (Lejeusne et al. 2010, Azzurro et al. 2019, D'Amen and Azzurro 2020). There are several arguments in supporting of climate change being the cause of the recent increase in Lessepsian organisms. For many species, a long time elapsed between their first sighting and the moment when they became invasive, suggesting that they might have had access to the Mediterranean since the initial days of the canal, but could establish only later due to warming conditions (Rilov et al. 2004, Azzurro et al. 2016). In addition, a meridionalisation process is also occurring in the Mediterranean: species that are native to the basin but used to be confined to its warmest parts are now expanding their distribution and have become more common in colder areas (Deidun et al. 2010, Lejeusne et al. 2010, Righi et al. 2020).

Given that climate change is contributing to alterations in the distribution of thermophilic native species, it could be having the same effect on introduced species.

While it is intuitive to think of global warming as a contributor to the establishment of tropical species in a temperate environment, there are several confounders that limit definitive conclusions. In the early days of the Suez Canal, the area around its mouth had low salinity due to the freshwater provided by the Nile delta. In addition, lakes with high salinity and pH ('bitter lakes') were present along the Suez Canal. The Nile delta and bitter lakes could have limited migrations through the canal by providing salinity barriers (Rilov and Galil, 2009). The construction of the Aswan dam in 1970, in combination with enlargements of the canal (multiple enlargements, between 1956 and 2025) have affected these salinity barriers; the dam lowered the amount of water coming from the Nile and enlargements of the canal have diluted bitter lakes (Galil et al. 2015, 2017). Moreover, enlargements have been made to cater for more intense traffic, which could further favour the arrival of Lessepsian species through ballast water or ship fouling (Williams et al. 1988).

Climate change, removal of salinity barriers and increased ship traffic can all be predicted to have increased the arrival Lessepsian species in recent decades and with the available information it is impossible to establish which factor has been the most prominent. While these factors could all be acting in concert, pinpointing why the intensity of invasions has increased could allow us to develop potential mitigation strategies. If climate change is the main driver, it will be impossible to mitigate the effect of the Suez Canal through local interventions. However, if changes in salinity barriers are the cause, restoration of such barriers could be considered, although this would certainly involve major investments and significant changes in the territories in the area. Alternatively, if ship traffic is the main driver of the spread of invasive species through the Suez Canal, treatment of ballast water could be considered to reduce propagule spread (Tsolaki and Diamadopoulos 2010). This would only be effective in case ballast water is the main vector, which deserves further investigation; it is still debated whether Lessepsian species enter the Mediterranean aided by ballast water or through unaided passive or active migration (Arndt and Schembri 2015). Part of the answer to these questions could come from analyses of the water parameters in the canal and ballast water. Such analyses could be combined with biological surveys to determine the presence of propagules to disentangle the relative roles of ballast water and the canal itself in the spread of Lessepsian species. Once the conditions of the Suez Canal and ballast water are determined, they could be recreated in physiological experiments aimed at determining how tolerant Red Sea species are to those conditions (Behrens et al. 2017). In similar experiments, simulations of the conditions in

the bitter lakes or at the mouth of the Suez Canal at different times in history could elucidate the extent to which these barriers prevented invasions in the early days of the Suez Canal.

A unidirectional invasion: why are anti-Lessepsian species so rare?

Organisms that enter the Red Sea from the Mediterranean are known as anti-Lessepsian and are extremely rare compared to Lessepsian ones (Rilov and Galil, 2009). The question of why anti-Lessepsian species are so rare has attracted scientific attention since the beginning of the study of the Lessepsian invasion (Golani 1999). Answers to this question could provide insights into the factors that make ecosystems more susceptible to invasions, an ultimate question in invasion ecology (Kolar and Lodge 2001, Catford et al. 2009). So far, answers have been only speculative and have resulted in the generation of hypotheses that have remained largely untested. The 'empty niche hypothesis' is based on the recent biogeographical history of the Mediterranean, which could have resulted in several unoccupied ecological niches (Catford et al. 2009). The recent biogeographical history of the Mediterranean is due to the Messinian salinity crisis, an event that led to complete desiccation of the Mediterranean 5.6 million years ago. This resulted in the extinction of virtually all fish species in the Mediterranean and subsequent recolonisation from the Atlantic when the Strait of Gibraltar reopened (Duggen et al. 2003, Azzola et al. 2026). Species from the Red Sea that occupy the empty niches of the Mediterranean could be facilitated in their establishment by low competition. The empty niche hypothesis could be tested by quantitatively defining which ecological niches Lessepsian species occupy in the Mediterranean and whether such niches were indeed unoccupied at their arrival or were conquered by Lessepsian species through competition (Golani and Galil 1991, Sala et al. 2011). Support to the empty niche hypothesis would indicate that basins with more recent biogeographical history are more susceptible to invasions due to the presence more empty niches. Importantly, this would suggest that niches becoming empty due to other stressors (e.g., overfishing) can also make seas more susceptible to invasions.

According to the 'topicalisation hypothesis', climate change is favouring the establishment of Lessepsian species; similarly to other water bodies worldwide, the Mediterranean is becoming warmer, thereby becoming ecologically more similar to the Red Sea (Lejeusne et al. 2010). This could be facilitating the establishment of Res Sea species in the Mediterranean in two ways: conditions are becoming more hospitable to Lessepsian organisms and less hospitable for native ones (Marras et al. 2015, D'Amen and Azzurro 2020). On the other hand, rising temperatures in the Red Sea are not expected to facilitate the establishment of Mediterranean species, which would explain why anti-Lessepsian organisms are extremely rare. While intuitive, the extent to which warming of the Mediterranean waters is favouring tropical species and harming local ones has not been assessed experimentally. The tropicalisation hypothesis could be investigated through

physiology experiments in the laboratory testing the effect of different environmental conditions on Red Sea species (Malloy and Targett 1991, Lejeusne et al. 2010, Behrens et al. 2017, Claus et al. 2025). Such studies should not only manipulate temperature, but also salinity, which has been shown in genetic studies to be a possible major contributor in the success of Red Sea species colonising the Mediterranean (Bernardi et al. 2016, 2024, Azzurro et al. 2022b). Including Mediterranean species in similar studies could also highlight to what extent changing conditions in the Mediterranean are decreasing the fitness of local species. Support for the tropicalisation hypothesis would indicate that temperate waters are more likely to be invaded by tropical species in the future.

What makes an invader: why are only some Red Sea species colonising the Mediterranean?

While there are numerous species from the Red Sea that have colonised the Mediterranean, there are many that have not. This is puzzling, especially when we consider that for many Lessepsian species there are others that are taxonomically, morphologically and ecologically similar in the Red Sea. For example, *Pterois radiata* is a lionfish species that is as abundant as *P. miles* in the Red Sea and seems to occupy very similar habitat and niches (Kulbicki et al. 2012, Gavriel and Belmaker 2021). Yet, *P. radiata* has never been reported in the Mediterranean (Bottacini et al. 2024). Another example comes from goatfishes from the *Parupeneus* genus: *Parupeneus forsskali* is the only *Parupeneus* species that has invaded the Mediterranean, despite having three congenerics with similar ecology in the Red Sea (*Parupeneus macronemus*, *Parupeneus cyclostomus* and *Parupeneus rubescens*) (Khalaf and Kochzius 2002, Evagelopoulos 2020). Although in many cases we would need more detailed information on the ecology and life history of closely related species to quantitatively establish their ecological similarity, it is remarkable that taxonomically close species vary so drastically in their invasiveness. Previous studies have investigated how ecological and morphological traits correlate with success in the context of the Lessepsian invasion, but have not considered species that have never become invasive in the Mediterranean (Belmaker et al. 2013, Azzurro et al. 2014, Arndt and Schembri 2015).

A first aspect to study is whether similar species have equal opportunities to enter the Mediterranean. In this context, we should start viewing the area of the Gulf of Suez as a distinct area from the rest of the Red Sea. Coral reefs are limited in the Gulf of Suez and other factors such as intense ship traffic and anthropogenic effects might have resulted in a fish community that is different from that of the overall Red Sea (Golani 1999). A ‘gulf bias’ could be acting as a filter for Lessepsian organisms by giving species that are

overrepresented in the Gulf of Suez a higher chance of entering the Mediterranean. Future studies analysing differences in the fish community between the Gulf of Suez and the rest of the Red Sea could help elucidate this aspect. Experiments are also needed to clarify the relative role of ballast water and unaided movement of propagules through the Suez Canal in the colonisation of the Mediterranean by Lessepsian organisms. For instance, experiments in captivity could measure the fitness of similar species in conditions that simulate the Red Sea, the Mediterranean and ballast water or the water in the Suez Canal. While correcting for any gulf bias, such studies could explain to what extent the conditions encountered before entering the Mediterranean explain the differences in in accessibility to the Mediterranean among closely related species.

There are several species that were sighted multiple times in the Mediterranean, but never became invasive (Al Mabruk et al. 2021b, Azzurro et al. 2026, Bottacini et al. 2026). This suggests that differences in accessibility to the Mediterranean are unlikely to be the only explanation to why certain species never invade the Mediterranean and that at least some exotic species are prevented in their establishment. Therefore, future studies should also investigate what makes certain Red Sea species more suitable to Mediterranean conditions. There are several reasons why some Red Sea species could be more successful at colonising the Mediterranean than others: (i) they could be naturally more suited to Mediterranean conditions, (ii) they could be evolve faster under the selection posed by local conditions and (iii) they could be more plastic and adjust rapidly. The suitability of different Red Sea species to Mediterranean conditions could be assessed by studying how their physiology and biology are affected by Mediterranean conditions in experiments on fishes in captivity (Malloy and Targett 1991, Lejeusne et al. 2010, Behrens et al. 2017, Claus et al. 2025). The role of evolution in Lessepsian species is a largely unexplored field of research. Genetic studies on species that successfully established in the Mediterranean revealed some degree of selection (Azzurro et al. 2022b, Bernardi et al. 2024). However, conclusions on whether this makes certain species more invasive than others is limited by a lack of information on the genetics of the numerous species that never established. Studies on the role of plastic adjustments to Mediterranean conditions could show whether differences in ecological or physiological plasticity could be explaining different levels of invasiveness of Red Sea species (Tonella et al. 2018, Rolla et al. 2020, Glassic et al. 2023). Studies on plasticity should compare species that are taxonomically and ecologically close, with representatives that became invasive and representatives that did not. Such studies should look at individuals sampled from the Red Sea, to control for any effect of selection. Studying why only certain species are colonising the Mediterranean is important because it could elucidate the factors that make certain species more invasive than others and could also contribute to understanding the extent to which invasive species change as a result of an invasion in marine contexts.

Variation among invaders: why do Lessepsian species differ in spread and colonisation speed?

A lot of variation exists in the success and colonisation spread of different Lessepsian species that have established in the Mediterranean. Similar insights into what makes a highly invasive species and how invasions change the ecology of organisms could come from studying such variation. Certain species were reported from across the whole Mediterranean within a few years from their introduction, while others have spread more slowly or have occupied a more limited range (Rilov and Galil, 2009, Azzurro et al. 2013, 2016). Additionally, some Lessepsian species have reached extremely high densities and have completely replaced local species from similar ecological niches, while others have remained rarely sighted and are only reported at low densities (Sala et al. 2011, Huseyinoglu et al. 2024, Kleitou et al. 2024). Similarly to the question of what prevents certain Red Sea species from establishing in the Mediterranean, this question can be tackled at three different levels. First, there could be intrinsic differences among species in their success at spreading across a new range. This could be true regardless of whether species move within the Mediterranean aided by ship traffic or not. For example, intrinsic differences could originate from life history traits. The two cardinalfishes (Apogonidae), *Cheilodipterus novemstriatus* and *Apogonichthyoides pharaonis* are abundant and have been present for more than two decades in the extreme east of the Mediterranean (Goren et al. 2010, Çiçek and Avsar 2015, Al Mabruk et al. 2021a, Huseyinoglu et al. 2024). Yet, they have not reached high densities in the central Mediterranean. Both species are mouthbrooding, which could be a reason explaining their slow and limited spread compared to broadcast spawners (Luiz et al. 2024). Similar studies to those conducted to pinpoint traits associated with invasiveness could leverage information available on the life history of slow and fast colonisers (Belmaker et al. 2013, Azzurro et al. 2014, Arndt and Schembri 2015). Second, some species could evolve faster and adjust to Mediterranean conditions more rapidly. Genetic studies suggest that there is selection on certain genes in Lessepsian species (Azzurro et al. 2022b, Bernardi et al. 2024). Future genetic studies on multiple species should test whether this is a factor that makes certain species more invasive than others. Third, certain Lessepsian species may be adjusting faster than other species through plasticity. Experimental approaches comparing plasticity in diet, ecology or physiology of fast and slow colonisers could elucidate this aspect (Tonella et al. 2018, Rolla et al. 2020, Glassic et al. 2023). Such experiments should compare individuals of fast- and slow-spreading Lessepsian species sampled from the Red Sea to test whether plasticity correlates with colonisation rate.

A less permeable interoceanic canal: where is the Panama Canal different from the Suez Canal?

Similarly to the Suez Canal, the Panama Canal (opened in 1914) connects two water bodies that had remained naturally separated for millions of years: the Caribbean Sea and the eastern Pacific Ocean (Castellanos-Galindo et al. 2020). The waters on the two sides of the Panama Canal have markedly different species composition (Porter 1974, Floeter et al. 2008). At the same time, the two sides of the Panama Canal are tropical environments, suggesting that species from one basin could easily thrive in the other basin after introduction. Yet, the Panama Canal has not originated any major biological invasion and only a few, mostly cryptobenthic species have been reported to have migrated through the canal (Castellanos-Galindo et al. 2025). This is probably because of a large, artificial freshwater lake (Lake Gatun) at the centre of the Panama Canal (Castellanos-Galindo et al. 2020). The lake is an ecosystem in itself and provides fishery services to the villages in the area (Hübel et al. 2026). Qualitative speculations from the situation in the Panama Canal suggest that, in the context of the Lessepsian invasion, the removal of salinity barriers could have been the main driver of the increase in Lessepsian invasions in recent decades. This would also be coherent with genetic studies showing that successful Lessepsian species are undergoing selection for osmoregulation genes (Bernardi et al. 2016, 2024, Azzurro et al. 2022b). In addition, the example of the Panama Canal suggests that ballast water may not be as important in the introduction of invasive species through artificial marine canals: if exotic species travelled primarily through ballast water, more invasions should be expected through the Panama Canal, which is also traversed by large cargo ships. To reach more definitive conclusions, we should investigate the dynamics of ballast water (i.e., loading and unloading in fresh vs seawater) of ships that move through the system of locks of the Panama Canal.

Recently, the Panama Canal has been enlarged and marine invasive species in Lake Gatun are increasingly regarded as an issue (Muirhead et al. 2015, Hübel et al. 2026). Marine species now represent the majority of the fish biomass fished in the lake and future changes in the hydraulics of the area should be monitored closely for their potential to result in species crossing the canal (Castellanos-Galindo et al. 2025). It is important to note that Panama Canal and Suez Canal are different in one major aspect: while the Suez Canal is at sea level, the Panama Canal is not; Lake Gatun is at 26 m of altitude and a system of locks prevents massive exchanges of sea water between its two sides (Castellanos-Galindo et al. 2025). This means that, in practical terms, information from the factors preventing invasions through the Panama Canal are unlikely to be directly applicable to the Suez Canal for mitigation strategies. Comparisons between the Suez and Panama Canal should be seen as interesting in the broader context of invasion ecology as they could generate insights on

what causes interoceanic marine invasions and how they can be prevented when planning future development efforts.

Concluding remarks

Lionfish are expanding in the Mediterranean, where they have not reached densities comparable to the western Atlantic. Studying lionfish in the Mediterranean is important, since the invasion is still developing and understudied. No experimental study has yet demonstrated that lionfish have an impact on the local fish community of the Mediterranean.

The stalking hunting strategy of lionfish remains poorly understood. Jet-blowing helps lionfish catch their prey head-first once they are within striking range. How lionfish manage to approach a prey up to an extremely close distance without triggering an antipredator response remains a mystery.

Although lionfish are the best studied invasive species in marine environments, major gaps remain on fundamental aspects of their ecology. Such gaps concern both invasion ecology (e.g., impact on the recently invaded Mediterranean, adaptations and adjustments to lionfish in their prey) and basic biology (e.g., spawning, hunting strategy, aggregating behaviour).

More studies from the native range of lionfish are needed on their fundamental ecology. Studying lionfish in their native range is necessary to understand what aspect of their ecology are being affected by the invasion process.

We should not stop studying the lionfish invasion in the Atlantic, since phenomena such as prey adaptations are best studied after a long time from the establishment of an invasive species. In fact, some of the questions on invasive lionfish remain open because they can only be studied later in the invasion, when scientific and public interest on invasive species tends to decline.

In the Mediterranean, we should see lionfish as a flagship species in the dramatic phenomenon of the Lessepsian invasion. Many Lessepsian species have spread and reached densities comparable to those of lionfish and could therefore have significant impacts on

the ecosystem. However, most information available on these species is currently limited to their spread and distribution, with very limited experimental studies.

The Lessepsian invasion should be seen as a natural experiment that can be leveraged to understand bio-invasions in the marine environment. Studying the Lessepsian invasion with an experimental approach will contribute to answering long-standing questions such as what makes an ecosystem prone to invasions, what makes certain species highly invasive and how colonising a new ecosystem affects the ecology of a species.

Finally, invasive species are often part of a whole suite of anthropogenic effects on ecosystems. This is especially important to consider when we present invasive species as a problem and we take a solution-oriented approach to studying them. We should never exclude the possibility that anthropogenic stressors such as climate change, pollution and overexploitation of resources are the main contributors to ecosystem degradation world.

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Summary

Invasive species are species that spread across a new range with a high colonisation rate, a process that human activities often promote by transporting species across continents, whether intentionally or accidentally. Invasive species are regarded as one of the most serious environmental problems of our time, because they can cause economic damage, environmental alteration, habitat loss, disease spread and extinctions. Invasive predators can be particularly harmful and are a major cause of animal extinctions. One of the best documented bio-invasions is the lionfish (*Pterois miles/volitans*) invasion in the western Atlantic Ocean. Native to the Indo-Pacific Ocean and Red Sea, lionfish reached the coasts of Florida as a result of aquarium releases around 1985 and became invasive about a decade later. Since then, they have spread across the whole Caribbean Sea and along the tropical and temperate eastern coast of America and keep expanding along the Brazilian coast today. Lionfish were soon considered one of the most invasive marine species and had a significant impact by preying on the local fishes. In 2016, a lionfish invasion began in the Mediterranean Sea, which lionfish had reached in 1991 through the Suez Canal. The Suez Canal is an artificial canal connecting the Mediterranean with the Red Sea and is the source of two thirds of the invasive species reported in the Mediterranean. Since 2016, lionfish have spread across the whole eastern Mediterranean, alarming the local governments and scientific community. The Mediterranean invasion is largely understudied, especially in comparison with the western Atlantic one. Moreover, their effectiveness as predators is often presented as one of the key aspects of lionfish invasiveness. At the same time, many aspects of their hunting strategy are poorly understood. With this thesis, I contribute to the study of invasive lionfish in the Mediterranean Sea and study important aspects of lionfish predation ecology.

In **chapter 2**, I review the knowledge on lionfish in the Mediterranean Sea that was available at the start of my project and highlight knowledge gaps, both on the Mediterranean invasion and on lionfish ecology in general. I also present an update on the spread of lionfish in the Mediterranean. Our results show that lionfish continue to expand in the Mediterranean and that interviewing local dive centres is a fruitful approach to track their invasion at a scale that covers the whole basin. In the Mediterranean invasion, studies on how lionfish interact with their prey at the individual level are scarce and studies on the impact that they have on the local biodiversity are completely lacking.

In **chapter 3**, I present an experiment in which we tested the impact of lionfish on the Mediterranean for the first time. Lionfish feed extensively on local fishes and studies from the western Atlantic suggest that they have the potential to deeply affect the local fish

community. We removed lionfish at six underwater sites (culled sites) for almost a year and compared the biomass and community composition of culled sites with that of six sites, at which we did not remove lionfish. We show that, although they lowered the lionfish density, lionfish removals did not have positive effects on the local fish community. Our experiment also represents an assessment of the effectiveness of culling as a means to protect the biodiversity from lionfish predation. In our experiment, we culled at a rate and intensity similar to those of public culling initiatives. Therefore, our experiment also shows that culling can be considered only a way of reducing lionfish densities, but should not be considered a tool for biodiversity conservation.

In **chapter 4**, I present the results of a study where we looked at a potential mechanism that lionfish may use to approach their prey up to an extremely close distance: camouflage through background matching. We tested this by studying background choice, a mechanism through which animals choose backgrounds that match their colour pattern. In the wild, lionfish could be approaching their prey from a colourful and complex background (e.g., corals and other marine invertebrates) and be less conspicuous than they would be against a featureless background (e.g., blue, open water). In a laboratory setting, we analysed the approach trajectory of lionfish in an apparatus where they could choose their background while approaching a prey. We show that, although lionfish account for their background when hunting, they do not do this in a way that would allow them to camouflage through background matching in nature.

In **chapter 5**, I present a study in which we investigate the causes and consequences of jet-blowing behaviour in lionfish for the first time. While approaching their prey, lionfish occasionally blow jets of water at them. We tested the hypotheses that lionfish do this because prey perceive the water jets as a current, which they will orientate towards, allowing lionfish to catch them more easily head-first. We show that blowing lionfish catch a higher proportion of prey head first and that prey do indeed reorientate in response to jet-blowing to face a lionfish. Other fishes blow jets of water, take advantage of the physical power of the jets: triggerfishes and pufferfishes blow to dig prey out of sand and archerfish blow to hit insects so they fall into the water and they can eat them. On the other hand, lionfish blow to trigger a behavioural reaction in their prey, which actively reorientate to face them.

In the general discussion, I place the findings from the previous chapters in a more general context and suggest future research avenues on lionfish predation ecology and the Mediterranean invasion. I also identify knowledge gaps that still remain on the general ecology of lionfish across ranges. Not only will filling such gaps help us understand the invasiveness of lionfish, but it will also lead to important insights in fields such as predation ecology or group living. Invasive lionfish are a unique opportunity in the study of invasive species in marine environments: two invasions are developing in two completely different

body of water, where lionfish are expanding to the point that they are colonising sub-basins with distinct ecology. This offers the opportunity to make comparisons that are not possible in all the cases where we have a species that is invasive in only one place. Finally, in the Mediterranean Sea, lionfish are only one of dozens of species that enter the Mediterranean through the Suez Canal (Lessepsian invasion). I propose research areas in the context of the Lessepsian invasion that could turn it into a model for invasion ecology in marine ecosystems.

Authorship statement

Chapter 1 *General introduction*. I wrote this chapter independently. My supervisors Alexander Kotrschal, Reindert Nijland and Patrick Jansen read a first draft and shared their thoughts on its general coherence and logical structure. Where relevant, I incorporated their input in preparation of the final version.

Chapter 2 *Lionfish (Pterois miles) in the Mediterranean Sea: a review of the available knowledge with an update on the invasion front*. I conceptualised the experiment with my daily supervisor AK, with whom I also developed the methods. I collected and analysed the data from the dive centres. I reviewed the literature and wrote the first draft of the chapter. My daily supervisor AK helped me improve the draft, after which I sent it to the rest of co-authors (BJAP, RN, PAJ, MN), who commented on it. I implemented their input and prepared the draft for submission to the peer-reviewed journal.

Davide Bottacini, Bart J. A. Pollux, Reindert Nijland, Patrick A. Jansen, Marc Naguib, Alexander Kotrschal

Chapter 3 *Regular removals lower invasive lionfish (Pterois miles) biomass, but have no effect on the fish community of the Mediterranean Sea.* I conceptualised the experiment with my supervisors (AK, PJ, BJAP, RN), with whom I also developed the methods. I collected the data with the help of LvdG, DvdA, SN, LdJ, GS, YJJK and EV during four field expeditions. I analysed the data myself and wrote the first draft of the chapter. My daily supervisor (AK) helped me improve the draft, after which I sent it to the rest of co-authors (LvdG, DvdA, SN, LdJ, GS, YJJK, HCOW, EV, TD, PAJ, BJAP, RN), who commented on it. I implemented their input and prepared the draft for submission to the peer-reviewed journal.

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Chapter 4 *Camouflage in a moving predator: testing for background choice in stalking lionfish (Pterois miles).* I conceptualised the experiment with my daily supervisor AK. I

developed the methods with AK and CN. CN and VR collected the data. I analysed the data and wrote the first draft of the chapter. My daily supervisor AK helped me improve the draft, after which I sent it to the rest of co-authors (CN, VR, GS, TD, MM, SC), who commented on it. I implemented their input and prepared the draft for submission to the peer-reviewed journal.

Davide Bottacini, Carien Noordman, Victor Renaud, Grigorios Skouradakis, Thanos Dailianis, Maria Mastoraki, Stavros Chatzifotis, Alexander Kotrschal

Chapter 5 *Lionfish (Pterois miles) blow directed jets of water to manipulate prey behavior*. I conceptualised the experiment with my daily supervisor AK. I developed the methods with AK and MR. MR and EB collected the data. I analysed the data and wrote the first draft of the chapter. My daily supervisor AK helped me improve the draft, after which I sent it to the rest of co-authors (MR, EB, GS, TD, MM, SC), who commented on it. I implemented their input and prepared the draft for submission to the peer-reviewed journal.

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Chapter 6 *General discussion*. I wrote this chapter independently. My supervisor Alexander Kotrschal and Patrick Jansen read a first draft and shared their thoughts on its general coherence and logical structure. Where relevant, I incorporated their input in preparation of the final version.

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And last, to the ***Mare Nostrum***: forgive us humans for being so selfish, so careless, so short-sighted. May your blue keep inspiring me forever. That blue, so intense that it feels like home every time I lose myself in it. I call it Mediterranean blue.

About the author





I am a fish enthusiast with an intrinsic sense of curiosity towards nature. I am following my dream of pushing the boundaries of human knowledge so we can better understand and protect the natural world. I wish I could do this for the rest of my life through the study of fishes, in all their wonderful colours, shapes and behaviours.

I was born in Verona on 7th January 1996 and grew up in San Giovanni Lupatoto, in the North-East of Italy. After completing a bachelor in agricultural technologies in Bologna, I moved to Wageningen, where I did two masters: Plant Sciences and Biology. By the end of my studies, Wageningen was the place where I had felt most at home as an adult, so I decided to stick around for a PhD at the Kotrschal lab (Behavioural Ecology group). The goal was never to write a thesis or gain another title. Rather, I wanted this to be the first step of a journey towards a career in science.

During my PhD, I had the privilege of waking up every day inspired by my job. In total, I spent more than a whole year in the field (Crete, Greece and elsewhere) to study lionfish in multiple aspects of their ecology and behaviour. It was awesome to be able to focus on these beautiful animals for four years. I am looking forward to applying my skills and learn a lot more during the next steps in my scientific journey.

WIAS training and education statement

Description	Year
The Basic Package	
WIAS Introduction Day	2023
WGS Scientific Integrity course	2023
WGS Ethics in Animal Sciences course	2025
Introduction course on Personal Effectiveness (recommended)	2023
Disciplinary Competences	
Project proposal	2022
Generalised Linear Models	2024
Mixed Linear Models	2024
Scientific diving course - theory and practice	2023
Advanced Molecular Ecology	2023
Professional Competences	
WGS Project and Time Management	2024
Research Data Management	2024
WEES board membership	2023-2026
Start to teach	2025
Societal Relevance	
Making an Impact: Increasing the relevance of research through science-society interaction	2026
Presentation Skills	
NVG 2025, Egmond aan Zee, the Netherlands, poster presentation	2025
Behaviour 2023, Bielefeld, Germany, oral presentation	2023
NVG 2024, Egmond aan Zee, the Netherlands, oral presentation	2024
WIAS Science Day, Ede, the Netherlands, oral presentation	2025
Teaching Competences	
Supervision of MSc students during their theses	2023-2024
Education and Training Total: 31.3 ECTS*	

**One ECTS credit equals a study load of approximately 28 hours*

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