

# **Behaviour of the invasive lionfish *Pterois miles* in the Aegean Sea**

**Vandenberghe Wout**

Bachelor's thesis submitted to obtain the degree of  
Bachelor of Agro- and Biotechnology: Animal Care

Promotor : Nikolaas Colpaert

Year 2025-2026  
First exam attempt, second exam period

©, Wout Vandenberghe. No part of this publication may be reproduced, stored in a retrieval system, or published in any form or by any means, whether electronic, mechanical, photocopying, recording or otherwise, without the prior written permission of the author. The use or reproduction of certain information from this work is permitted solely for personal use and provided that the source is acknowledged. Any use for commercial or advertising purposes is prohibited.

This bachelor's thesis was written by Wout Vandenberghe, a student at HOGENT University of Applied Sciences, as part of the completion of the Bachelor's programme in Agro- and Biotechnology. The views expressed in this thesis are solely those of the individual author and do not necessarily reflect the views, official position or policy of HOGENT.



# **Behaviour of the invasive lionfish *Pterois miles* in the Aegean Sea**

**Vandenberghe Wout**

Bachelor's thesis submitted to obtain the degree of  
Bachelor of Agro- and Biotechnology: Animal Care

Promotor : Nikolaas Colpaert

Year 2025-2026  
First exam attempt, second exam period

©, Wout Vandenberghe. No part of this publication may be reproduced, stored in a retrieval system, or published in any form or by any means, whether electronic, mechanical, photocopying, recording or otherwise, without the prior written permission of the author. The use or reproduction of certain information from this work is permitted solely for personal use and provided that the source is acknowledged. Any use for commercial or advertising purposes is prohibited.

This bachelor's thesis was written by Wout Vandenberghe, a student at HOGENT University of Applied Sciences, as part of the completion of the Bachelor's programme in Agro- and Biotechnology. The views expressed in this thesis are solely those of the individual author and do not necessarily reflect the views, official position or policy of HOGENT.



By: Wout Vandenberghe

Supervisor: Emma P. Donald

# Preface

Finishing this thesis cost blood, sweat, tears and the loss of plenty of personal items. From the first article read and the countless surveys to hours spent behind four different laptops and eventually finishing the last pieces of this work, I could not have done any of it without the immense help of a lot of people.

I want to thank Anastasia Miliou and Thodoris Tsimpidis for giving me the opportunity to live an international experience in the field I have dreamed of since I was a kid, surrounded by so many amazing people and cultures. I also want to thank them for giving me the chance to continue working on Samos after finishing my degree. I want to thank my supervisor Nikolaas Colpaert for the constant support through the entire process. Arranging meetings despite his busy schedule to answer my many questions, coordinating his expectations with mine and Archipelagos' and being generally interested and enthusiastic about the project allowed me to create something I can be proud of.

I want to thank Silvia Giacomello and Emma Donald for introducing me to the marine ecology team and the incredible feat of teaching me from the ground up how to conduct research in such a short period of time. It amazes me how much I have learned and grown from these past couple of months under their guidance. I have to give a special thanks to Emma Donald, who was my direct supervisor regarding the lionfish project. Through Emma's unwavering enthusiasm, determination and support I was able to lift up the quality of my work every step of the way. Every contribution, comment, meeting or answered question was invaluable for both my personal development as well as this thesis in its totality. Emma kept motivating and pushing me to do better than the norm, challenging me to go above and beyond instead of taking the easy way out of "doing enough". I can't thank her enough for all the hours spent trying to figure out this project and the surveys, teaching a complete beginner how to code in R, helping me make a thesis representative of the effort put in and inspiring me on a personal and professional level. She always stayed positive and dragged me along in this positivity, energizing me to get back on track after every setback. I could not have done this without her and I feel privileged to have her as a friend and soon also as a colleague.

I want to thank Keira Cassidy for all the effort put into making this internship a reality and a pleasure, including but not limited to: arranging my admission to Archipelagos, all the grant paperwork I saddled her with, the arduous task of fixing my CV and last but not least offering me the unbelievably exciting opportunity to come back to Samos as part of the team.

I want to thank all my friends, at home and on Samos, who motivated and helped me overcome every bump in the road (they know there were a few) and inspired me every day to keep going, no matter how short-lived our meeting might have been. I can't wait to see what the future brings for all the bright, promising and kind people I met during this time and I am sure we will see each other again. Last but not least, I want to thank my parents for letting me feel their continuous support, even 2000 km away from home.

# Use of artificial intelligence

Declaration of Honour regarding the use of artificial intelligence in the preparation of the internship report.

The undersigned,

Student's name: Wout Vandenberghe

hereby declare on my honour that the internship report (tick the appropriate box):

☒ was written entirely independently, without the use of artificial intelligence (such as ChatGPT, Copilot, Gemini, etc.).

☐ was written with partial support from artificial intelligence.

If applicable, please provide a brief explanation below of the type of support (e.g. proofreading, structural suggestions, brainstorming, etc.):

.....

☐ was largely drafted with the help of artificial intelligence.

If applicable, please provide an explanation below regarding the nature and extent of its use:

.....

The student confirms that they remain responsible for the content, accuracy and originality of the report, regardless of the use of AI tools.

Date: 02/06/2026.....

Student's signature:



# Abstract

The Mediterranean Sea has been invaded by the lionfish *Pterois miles* through the Suez Canal. These visually distinct fish originating from the Red Sea have venomous spines serving as defence mechanism against predators. Combined with their reproductive abilities and generalist diet, they are highly successful invaders. Although direct socioeconomic impact and risks to human health are not deemed significant, their continuous spread through the entirety of the Mediterranean Sea threatens the local biodiversity, as they are capable of reducing populations of key native species. These factors motivate the creation of sustainable and effective measures supported by science-based arguments. Consequently, lionfish behaviour in the Aegean Sea was recorded and analysed during different times of day and night over a period of two and a half months. While snorkelling, data was collected during 10-minute observations, as well as different environmental factors possibly influencing behaviour. After statistical analysis, only survey time seemed to have a significant effect on lionfish behaviour. Lionfish were found significantly less likely to be hunting during the day compared to sunrise, sunset and during the night, similar to behaviour documented in their native range. Lionfish were significantly less likely to be out of hiding during the day compared to other times. General activity levels were lower than observed around Cyprus, where the lionfish invasion has been ongoing for a longer time. Specific behaviours were difficult to statistically analyse because of low recorded amounts. Notably, strikes were recorded at all times, even while inactive and the same key prey species (*Thalassamo pavo* and *Chromis chromis*) were targeted as seen around Cyprus. Considering the results of this study, it could be stated that lionfish control measures would be most effective during the night, sunrise and sunset. This study builds on current understanding of lionfish behaviour and trophic impact in an invaded range, providing valuable knowledge that can inform invasive species management and conservation measures to preserve native ecosystem balance in the Mediterranean Sea.

## Table of contents

|                                                       |     |
|-------------------------------------------------------|-----|
| Preface .....                                         | 5   |
| Use of artificial intelligence .....                  | 6   |
| Abstract.....                                         | 7   |
| List of figures .....                                 | 9   |
| List of tables.....                                   | 10  |
| 1 Introduction.....                                   | 11  |
| 1.1 Lionfish biology .....                            | 12  |
| 1.1.1 Morphology .....                                | 12  |
| 1.1.2 <i>P. miles</i> vs <i>P. volitans</i> .....     | 12  |
| 1.1.3 Reproductive biology.....                       | 14  |
| 1.2 Invasion topography .....                         | 15  |
| 1.2.1 Invasion of the Eastern Mediterranean Sea ..... | 15  |
| 1.2.2 Implications for the future .....               | 15  |
| 1.3 Impact of lionfish.....                           | 17  |
| 1.3.1 Socioeconomic.....                              | 17  |
| 1.3.2 Health and safety .....                         | 18  |
| 1.3.3 Interaction with native ecosystems.....         | 19  |
| 1.3.4 Prey species .....                              | 20  |
| 1.4 Behaviour .....                                   | 22  |
| 1.4.1 Hunting times .....                             | 22  |
| 1.4.2 Hunting behaviour .....                         | 22  |
| 1.4.3 Blowing .....                                   | 23  |
| 1.4.4 Research methods.....                           | 24  |
| 1.5 Research aims.....                                | 25  |
| 2 Materials and methods .....                         | 26  |
| 2.1 Study area .....                                  | 26  |
| 2.2 Equipment .....                                   | 28  |
| 2.3 Survey method .....                               | 29  |
| 2.4 Dissection .....                                  | 31  |
| 2.5 Statistical analysis .....                        | 31  |
| 3 Results.....                                        | 33  |
| 4 Discussion .....                                    | 35  |
| 4.1 Recommendations for future research .....         | 38  |
| 4.2 Limitations .....                                 | 39  |
| 5 Conclusion.....                                     | 41  |
| Reflection.....                                       | 42  |
| Bibliography .....                                    | 43  |
| Appendices .....                                      | I   |
| Appendix 1 .....                                      | I   |
| Appendix 2 .....                                      | II  |
| Appendix 3 .....                                      | III |
| Appendix 4 .....                                      | IV  |

# List of figures

|                                                                                                                                                                                                                                                                                                |    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 1: Meristic comparison of <i>P. miles</i> and <i>P. volitans</i> (Ronchi, 2017). .....                                                                                                                                                                                                  | 13 |
| Figure 2: Map of responses of dive centres regarding lionfish sightings during a study conducted by Philips and Kotrschal (2021), also highlighting the Suez Canal through which lionfish have invaded the Mediterranean range (taken from Phillips & Kotrschal, 2021). .....                  | 14 |
| Figure 3: Visualization of dive centres responses: (A) shows whether the dive centres contacted in 2023 responded; (B) shows the response from the dive centres contacted that responded in 2023; (C) shows the responses to the survey in 2021 (taken from Bottacini et al., 2024). .....     | 17 |
| Figure 4: (A-C) Three cases of lionfish envenomation (from Resiere et al., 2016). .....                                                                                                                                                                                                        | 19 |
| Figure 5: <i>C. chromis</i> individual (top) and remains of an unidentified fish (bottom) found by members of the Archipelagos Institute of Marine Conservation in the stomach of a <i>P. miles</i> individual from the port of Kokkari, Samos in 2026.....                                    | 21 |
| Figure 6: Partially digested <i>Thalassoma pavo</i> individual (left) found by a member of Archipelagos Institute of Marine Conservation in the stomach of a <i>P. miles</i> individual (right) from the port of Kokkari, Samos in 2024. ....                                                  | 22 |
| Figure 7: Blowing behaviour in a controlled environment, visualized with a droplet of food dye between the lionfish and a potential prey (from Albins & Lyons, 2012). .....                                                                                                                    | 24 |
| Figure 8: Map of the Aegean Sea, Greece and Turkey, with Samos island and Lipsi island highlighted. ....                                                                                                                                                                                       | 26 |
| Figure 9: Map of the east of the Aegean Sea, highlighting Samos island, Lipsi island, the two survey sites on Samos (West Beach and East Beach) and the one survey site on Lipsi (Vroulia) where lionfish behavioural data was collected. ....                                                 | 27 |
| Figure 10: Maps of survey sites, the red arrows indicating the path followed by snorkellers collecting lionfish behavioural data, with (A) East Beach in Agios Konstantinos, Samos, (B) West Beach in Agios Konstantinos, Samos and (C) Vroulia bay, Lipsi. ....                               | 28 |
| Figure 11: Metal frame with HOBO logger attached on top used during lionfish surveys to measure water temperature and light intensity. ....                                                                                                                                                    | 29 |
| Figure 12: The probability (%) of lionfish exhibiting hunting behaviour per survey time where A = immediately after sunrise, B = during the day, C = right before sunset and D = during the night; the bars around each point show the standard deviation of probability per survey time. .... | 33 |
| Figure 13: The probability (%) of lionfish being out of hiding per survey time, where A = immediately after sunrise, B = during the day, C = right before sunset, D = during the night; the bars around each point show the standard deviation of probability per survey time. ....            | 34 |
| Figure 14: <i>P. miles</i> individual from Vroulia Bay, Lipsi, with unidentified ectoparasite on its caudal fin. ....                                                                                                                                                                          | 37 |

## List of tables

|                                                                                                                                                                                                                                                                                                                                            |     |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Table 1: Ethogram of timed behaviours documented during observations of <i>P. miles</i> in the eastern Aegean Sea. ....                                                                                                                                                                                                                    | 30  |
| Table 2: Ethogram of counted behaviours documented during observations of <i>P. miles</i> in the eastern Aegean Sea. ....                                                                                                                                                                                                                  | 31  |
| Table 3: Summary of generalised linear model, where probability of lionfish to exhibit hunting behaviour was the response variable and survey times are the predictor variables. ....                                                                                                                                                      | I   |
| Table 4: Variance inflection factors of the maximal model with binomial distribution, where the response variable is probability of lionfish to exhibit hunting behaviour and the predictor variables are survey time, total length (cm), current, visibility(m), depth (m), weather, sea state, pedestrians, water temperature (°C). .... | II  |
| Table 5: Summary of generalised linear model, where probability of lionfish to be out of hiding was the response variable and survey times are the predictor variables. ....                                                                                                                                                               | III |
| Table 6: Variance inflection factors of the maximal model with binomial distribution, where the response variable is probability of lionfish to be out of hiding and the predictor variables are survey time, total length (cm), current, visibility(m), depth (m), weather, sea state, pedestrians, water temperature (°C). ....          | IV  |

# 1 Introduction

Over recent decades, the Mediterranean Sea has been invaded by a vast number of invasive species due to the opening and widening of the Suez Canal, warming waters, industrial shipping and the aquarium trade (Katsanevakis et al., 2014). One of the recent invasive species in the Mediterranean Sea is the lionfish (Bernardi et al., 2024). Lionfish are successful invaders because of their unique biological and morphological features: intricate patterns and elongated pectoral fins used for herding fish, as well as venomous anal, dorsal and pelvic spines and high reproductive proficiency (Cohen & Olek, 1989; Morris, 2009). The establishment of invasive lionfish populations has been found to have various negative consequences for local marine ecosystems, including reducing native fish populations, reducing biodiversity and decreasing the abundance of species vital to these ecosystems (Albins & Hixon, 2008; D'Agostino et al., 2020).

In the Mediterranean Sea, the common name 'lionfish' collectively refers to the species *Pterois miles* and *Pterois volitans*, but invasive lionfish in this area are generally *P. miles* individuals (Bernardi et al., 2024). *P. miles*, along with many other invasive species in the Mediterranean Sea, migrated through the Suez Canal to reach the eastern Mediterranean Sea, making it a Lessepsian migrant (Bariche et al., 2017). Lionfish were confirmed established around Cyprus in 2015 (Kletou et al., 2016). Since then, they have spread further to the west and north of the Mediterranean Sea (Bottacini et al., 2024). Invasive species can have severe socioeconomic impacts in their introduced regions, as well as endanger human health and safety and destabilize local ecosystems (Reaser et al., 2007). The extremity of their negative effect on these ecosystems is largely due to native prey naivety, minimal predation by natural predators and their generalist diet (Hackerott et al., 2013; Paolucci et al., 2013; D'Agostino et al., 2020).

Through predation, they endanger key species populations (Albins & Hixon, 2008; Galanidi et al., 2018; Pinnegar, 2018; D'Agostino et al., 2020). Lionfish behaviour is an important aspect in monitoring the invasion process as it informs efficient management and control measures. Documenting habitat preference, hunting behaviour, and specific hunting techniques like blowing, is vital to understand the impact of lionfish in their invaded range, as well as aid in protecting local biodiversity. This creates the need for reliable and standardised research methods, allowing researchers to compare behavioural patterns.

The conducted research focuses on activity patterns, hunting behaviour, trophic impact and habitat preferences of *P. miles* in the eastern Aegean Sea, an area where lionfish populations are now beginning to become established (Phillips & Kotrschal, 2021). The aim is to document at what times lionfish are active, hunting and out of hiding, as well as what kind of prey selection lionfish have in this region of the Mediterranean Sea. Furthermore, by analysing how environmental factors could

influence these factors, activity patterns could be predicted using environmental data. Ultimately, this study will contribute to building a crucial knowledge base for establishing efficient and sustainable management of invasive lionfish in the Aegean Sea.

## **1.1 Lionfish biology**

### **1.1.1 Morphology**

*P. miles* is a predatory fish from the Indian Ocean known for its impact on invaded ranges. Lionfish are easily recognizable by their striking appearance with intricate patterns, long fins and venomous dorsal, pelvic and anal spines, making them popular fish in the aquarium trade (Lyons et al., 2019). A study by Savva et al. (2020) in Cyprus found that individuals grow faster and larger there than in their native range. Lionfish have dorsal, pelvic and anal spines containing high concentrations of acetylcholine, which possibly works as a catalyser for the neurotoxin also found in these spines (Cohen & Olek, 1989). After a spine penetrates tissue, the membrane covering the spines tears and the venomous contents are released, causing symptoms ranging from swelling to extreme pain and paralysis, possibly even death (Cohen & Olek, 1989). Lionfish having venomous spines increases handling time and risk of injury for fishermen, as well as reduces their appeal as a commercial fish species.

### **1.1.2 *P. miles* vs *P. volitans***

While *P. miles* and *P. volitans* are both referred to as lionfish and are morphologically similar, the two are genetically distinct (Freshwater et al., 2009). *P. miles* and *P. volitans* have the following small meristic and morphological differences: typically, *P. volitans* individuals have 11 dorsal and 7 anal fin rays, while *P. miles* individuals only have 10 dorsal and 6 anal fin rays (Schultz, 1986). Furthermore, *P. volitans* individuals have considerably larger pectoral fins than *P. miles* individuals (Schultz, 1986). White spots on the soft vertical fins grow bigger in accordance with total individual length in both species, but with *P. volitans*, this growth is more evident, resulting in generally larger spots seen on *P. volitans* compared to *P. miles* (Figure

## Meristic Differences



|                   | Indian: <i>Pterois miles</i> | Pacific: <i>Pterois volitans</i> |
|-------------------|------------------------------|----------------------------------|
| Dorsal Fin Rays   | 10                           | 11                               |
| Anal Fin Rays     | 6                            | 7                                |
| Pectoral Fin Size | Shorter                      | Longer                           |
| Spot Size         | Smaller                      | Larger                           |
| Geographic Range  | Africa – Indonesia           | Indonesia – W Pacific            |

Figure 1: Meristic comparison of *P. miles* and *P. volitans* (Ronchi, 2017).

*P. miles* and *P. volitans* have some spatial overlap in their native range, but are mainly found in different regions (Kulbicki et al., 2012). *P. miles* is found in the Indian Ocean from South Africa to the Red Sea and going as far east as Sumatra, while *P. volitans* mainly inhabits the Pacific Ocean, they are seen from Australia to Japan and as far east as Oeno Island (Schultz, 1986). In the invaded Atlantic range, *P. volitans* is way more abundant than *P. miles*, making up 93% of the found lionfish population taken off the coast of North Carolina, Florida and New York (Hamner et al., 2007).

In the invaded Atlantic range, a strong founder effect was confirmed, meaning low genetic diversity between individuals caused by a population stemming from a low number of “founder” parent animals (Hamner et al., 2007). Because lionfish migrated through the Suez Canal (Figure 2) to reach the Mediterranean Sea instead of stemming from occasional aquarium releases, these populations have a higher genetic diversity, possibly making them more resilient than their Atlantic counterparts (Bariche et al., 2017; Bernardi et al., 2024). The only native waters *P. miles* and *P. volitans* share is a small not yet defined range around Indonesia (Kulbicki et al., 2012). Through genetic sequencing, Bernardi et al. (2024) found all examined lionfish specimens in the Mediterranean Sea, as well as in the native Red Sea, to be *P. miles* individuals. In essence, lionfish is a common name referring to the morphologically similar, but genetically distinct species *P. miles* and *P. volitans*, with the Mediterranean lionfish invasion solely pertaining *P. miles* populations.

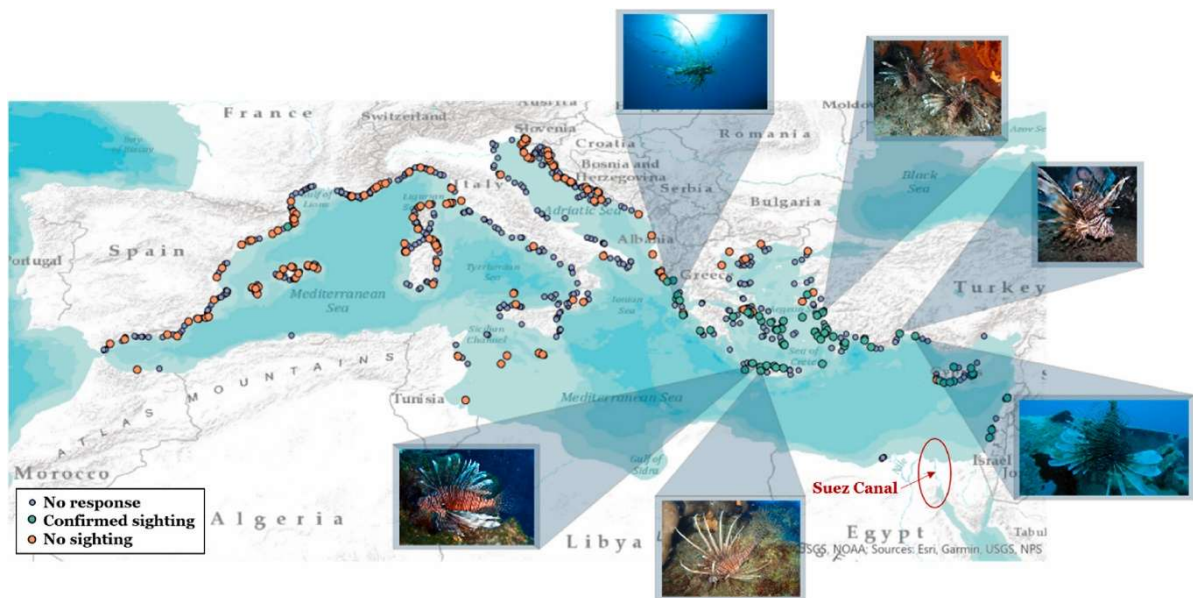


Figure 2: Map of responses of dive centres regarding lionfish sightings during a study conducted by Philips and Kotrschal (2021), also highlighting the Suez Canal through which lionfish have invaded the Mediterranean range (taken from Phillips & Kotrschal, 2021).

### 1.1.3 Reproductive biology

Lionfish reproductive abilities play an important part in their invasive success. Morris et al. (2011) found no differences in ovarian morphology or in the observed oocyte diameters between *P. miles* and *P. volitans*, so data gathered on *P. volitans* can be used to discuss *P. miles* reproduction and possible linked effects to the Mediterranean invasion. Lionfish develop to sexual maturity at the age of one year old (Morris, 2009). After reaching maturity, lionfish release 1800-41945 eggs at a time, being able to do so as frequently as every two to three days (Gardner et al., 2015). Eggs are released in the form of two buoyant gelatinous masses that are fertilized by males close to the surface (Fishelson, 1975). Later, these masses dissolve and the larvae become free floating (Fishelson, 1975).

In their native range, as well as the invaded Caribbean Sea, they are fertile throughout the entire year, with increased spawning frequency in the summer (Morris, 2009; Gardner et al., 2015). Around Cyprus in 2017 - 2018, Savva et al. (2020) found that there were more female than male lionfish individuals, which could have led to male lionfish being able to mate with multiple females, increasing their population growth. In the Mediterranean Sea, Mouchlianitis et al. (2022) recorded actively spawning females only in June and July. Spawning-capable females were also recorded in August and November, but these individuals were not in the actively spawning subphase (Mouchlianitis et al., 2022). It could be argued that this absence of year-round spawning could be attributed to considerably lower water temperatures in winter documented in this area and time period by Savva et al. (2020). Considering these findings, rising water temperatures caused by climate change might be

facilitating longer spawning periods and in turn increasing population growth of these invasive fish. The connection between lionfish reproduction and water temperatures could play a crucial role in their invasion success now and in the future, and should therefore be further defined and monitored. In essence, lionfish invasion success is partially dependent on their reproductive abilities, rendering it an important topic for research and control.

## **1.2 Invasion topography**

### **1.2.1 Invasion of the Eastern Mediterranean Sea**

Lionfish invaded the Mediterranean Sea through the Suez Canal and have since continuously spread across the Eastern Mediterranean Sea. In the invaded Mediterranean habitat, lionfish are mainly found in rocky reefs, after that in underwater caves, rock slits and less frequently by muddy and sandy bottoms, seaweed beds, algal patches and coralligenous formations (Dimitriadis et al., 2020). Lionfish from the Red Sea invaded the Mediterranean Sea through the Suez Canal (Bariche et al., 2017). *P. miles* was first recorded in 1991 off the coast of Israel (Golani & Sonin, 1992). Two specimens were captured off the coast of Lebanon in 2012 (Bariche et al., 2013). In 2014, another specimen was sighted in Turkey and in 2015 two more were recorded, one in Cyprus and another sighted in Rhodes in Greece, showing an evolution more east- and northward over the years (Turan et al., 2014; Crocetta et al., 2015; Iglésias and Frotté, 2015; Oray et al., 2015). A successful invasion and distribution (i.e., establishment of a self-sustaining population) in the Mediterranean Sea was initially deemed questionable because of unfavourable conditions for long-distance dispersion of lionfish larvae (Johnston & Purkis, 2014). Lionfish sightings off the coast of Cyprus contradict this argument as Kletou et al. (2016) provided conclusive evidence of breeding pairs. Furthermore, questioned dive centres in the Mediterranean Sea reported lionfish off the coast of Crete in 2016 and off the coast of Greece in the Ionian Sea in 2018 (Phillips & Kotrschal, 2021). The northernmost sightings in the Ionian and Aegean Sea were in the years 2019 and 2020, respectively (Phillips & Kotrschal, 2021). Following the trends of the sightings, it could be argued that there is a continuously increasing distribution to colder waters, more distant from the native Indian Ocean. Combining this recorded proof of individuals and reported sightings, it can be concluded that lionfish have invaded the Eastern Mediterranean Sea, establishing breeding populations in Greek waters, with evidence pointing to further spread of these populations.

### **1.2.2 Implications for the future**

Citizen science is an effective tool to monitor the evolution of lionfish sightings and thus the scale of their invasion. Data collection involving non-scientist volunteers with an appropriate amount of knowledge and expertise, or citizen science, is a valuable method to collect vast amounts of data across large geographical areas with minimal efforts (López-Gómez et al., 2014). Phillips et al. (2021) applied this methodology by questioning a large number of dive centres around the Mediterranean Sea about

lionfish encounters while on dives. 1131 dive centres were contacted, from which 216 responses were received timely enough for analysis: 75 reported lionfish sightings and 141 reported no lionfish sightings (Phillips & Kotrschal, 2021). Bottacini et al. (2024) repeated this study to compare sightings from more recent years with the findings up until 2021. Contacting 996 dive centres, 326 responded: 82 reported lionfish sightings and 244 reported no lionfish sightings (Bottacini et al., 2024). Combining the data from these studies gives a representation of the spread of lionfish distribution over the last years. The sightings have continuously spread both to the north and west (Figure 3), appearing as far as Sicily, Sardinia, Croatia and Malta in the most recent reported year range (Bottacini et al., 2024). Considering there have been no earlier sightings in these areas, Bottacini et al. (2024) insinuated that these are results of lionfish migrating through the Mediterranean Sea and not aquarium releases, although conclusive evidence is necessary to support this claim. The northernmost report of lionfish is that of a single individual found near the island of Vis, Croatia (Dragičević et al. 2021), while the westernmost lionfish was a single individual sighted in the Alboran Sea, Spain, even further west than the furthest sightings reported by Bottacini et al. (2024) (Fortič et al. 2023). Altogether, this database of lionfish sightings puts forth a strong argument that the lionfish invasion is passively continuing to spread throughout the Mediterranean basin, highlighting the importance of understanding lionfish behaviour in order to establish effective management plans.

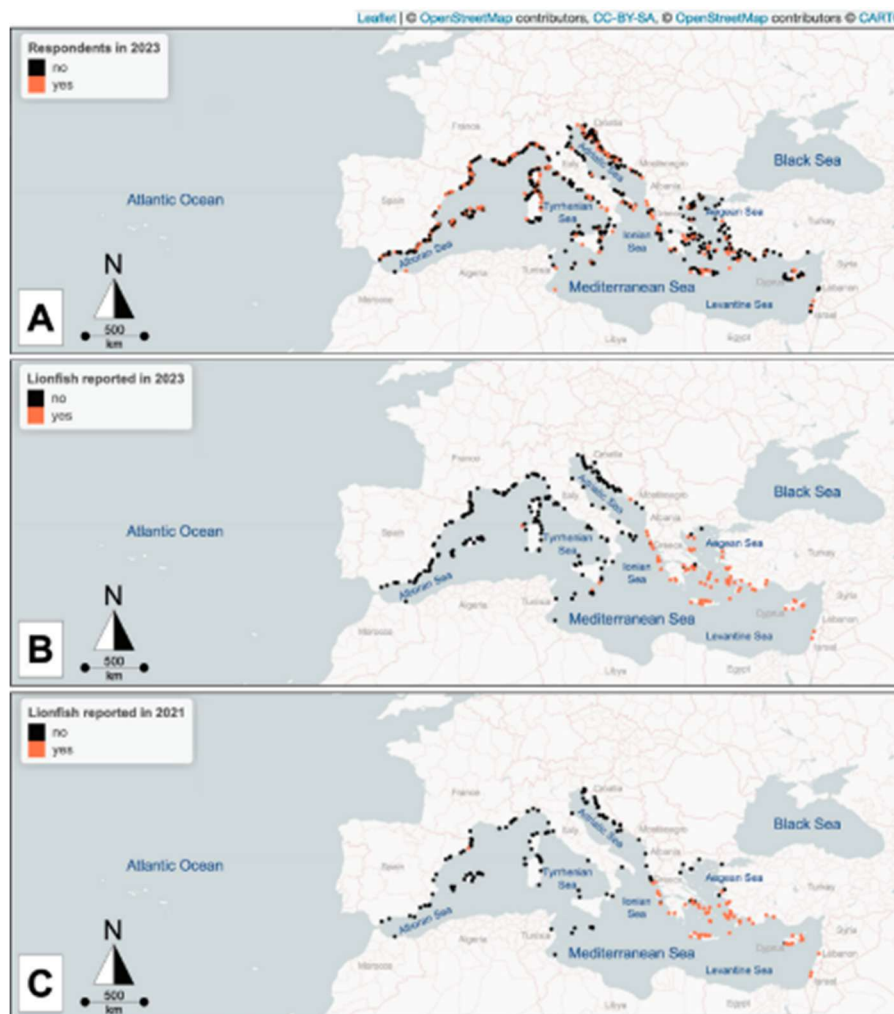


Figure 3: Visualization of dive centres responses: (A) shows whether the dive centres contacted in 2023 responded; (B) shows the response from the dive centres contacted that responded in 2023; (C) shows the responses to the survey in 2021 (taken from Bottacini et al., 2024).

## 1.3 Impact of lionfish

### 1.3.1 Socioeconomic

Invasive species can have an impact on a range of socioeconomic factors including population decrease or extinction of economically important species, disturbance of economic or personal activities and damage to infrastructure or equipment. The World Health Organization (2015) warned about the significant impacts of the increasing numbers of invasive species on human health and welfare. For example, invasive *P. volitans* in the Bahamas have had a negative effect on the spiny lobster fishery due to increased handling times caused by encountering lionfish (Henderson, 2012). However, this is not necessarily representative of the effect of *P. miles* on fisheries in the Mediterranean since it is a different species of lionfish in a different environment.

Invasive species can negatively impact a variety of socioeconomic factors when introduced to island ecosystems, such as fisheries, agriculture, ornamentals,

infrastructure, tourism, human health, animal health, governance and costs, with prevention and reactive management often costing millions of dollars (Reaser et al., 2007). Lionfish ecological impact is mainly dictated by their high predation pressure, allowing them to decrease prey populations and destabilize ecosystems (Albins & Hixon, 2008). Savva et al. (2020) documented lionfish preying on commercially important species, potentially reducing their abundance. The greater predation pressure on native fish populations could be the biggest contributor to lionfish economic impact, potentially decreasing fisheries success and generating high management costs. Galanidi et al. (2018) estimated the direct socioeconomic concern of a lionfish invasion in the Mediterranean as 'minor' based on earlier research done on *P. volitans*, but there is limited literature on the direct effect of *P. miles* on fisheries in the Mediterranean.

Kleitou et al. (2022) reviewed Cypriot national fishery data and interviewed fishermen on the impact of different invasive species on their fishing success. Fishers reported no direct financial impact of lionfish on in-net predation of other catch or damage to equipment, but stated that to achieve their income, they needed to fish more due to non-indigenous bycatch like lionfish (Kleitou et al., 2022). Importantly, these interviews don't reflect the effect of lionfish on economically important species' abundance, as this is extremely difficult for fishermen to determine. Although socioeconomic concern for the lionfish invasion in the Mediterranean Sea is estimated as minor and local fishermen report no direct financial impact, reduced fish populations, destabilized ecosystems and bycatch of lionfish could have negative economic impacts for long-term fisheries success and management costs.

### **1.3.2 Health and safety**

Dangers of lionfish to human health exist and can be severe, but they are not considered the main problem. Even though the fish have venomous spines that can cause symptoms ranging from swelling (Figure 4) to extreme pain and paralysis and in exceptional cases even death, lionfish are not naturally aggressive towards humans (Resiere et al., 2016). Interviewed fishermen around Cyprus reported no incidents of injury, except for one fisherman who reported getting stung three times in the same year (Kleitou et al., 2022). Harm done to humans is generally a result of careless handling, accidental treading on the fish or improper preparation of the fish as food and can easily be avoided with the right safety precautions (Resiere et al., 2016). Seeing as lionfish stings can easily be avoided with proper precautions, educating the public and fishermen on these precautions is essential with increasing spread and abundance of lionfish in the Mediterranean Sea.



Figure 4: (A-C) Three cases of lionfish envenomation (from Resiere et al., 2016).

### 1.3.3 Interaction with native ecosystems

#### 1.3.3.1 Prey naivety

Invasive predators oftentimes profit greatly from prey naivety, which stems from inexperience of the native prey species with the invading species. Insufficient experience with exotic predators results in a lack of predator recognition, avoidance and countermeasures in native prey species (Sih et al., 2010; Paolucci et al., 2013). A study by D'Agostino et al. (2020) analysed *P. miles* feeding ecology and hunting behaviour off the coast of Cyprus and observed native prey species swimming significantly closer to lionfish than invasive prey species, pointing to a lower level of predator awareness compared to Lessepsian migrants. Albins and Hixon (2008) researched the effect of lionfish individuals on recruitment of fish on reefs. Recruitment refers to the hatching and developing of juvenile fish, highlighting the amount of new juveniles and thus potential future adults in a population (Albins & Hixon, 2008). The reefs with a lionfish present showed a staggering average reduction in net recruitment of 79% compared to reefs with no lionfish present (Albins & Hixon, 2008). This significant contrast in recruitment between reefs was attributed to lionfish predation after examining lionfish stomach contents (Albins & Hixon, 2008). Mediterranean prey species targeted by lionfish have a proven decrease in predator awareness compared to invasive prey species towards lionfish, allowing these predators to significantly reduce the abundance of juvenile native fish and thus threatening the balance of the ecosystem.

#### 1.3.3.2 Biotic resistance

Biotic resistance describes the ability of native ecosystems with high biodiversity to reduce invasion success of invasive species. Ecosystems with higher biodiversity have greater resistance to introduction and success of invasive species (Stachowicz et al., 1999). Native predators can decrease invasive species success through predation (deRivera et al., 2005). Native predators that have not developed ways to

hunt and eat these invasive species are not able to utilize them as food, which would contribute to controlling the invasive populations. *P. volitans* was recorded being predated on by two species of grouper in the Bahamas, *Mycteroperca tigris* and *Epinephelus striatus* (Maljković et al., 2008). Further research in the Caribbean showed that there is no direct relation between native predator biomass density and lionfish biomass density, proving that the native top-predators are not successfully controlling the invasive population (Hackerott et al., 2013). It must be mentioned that these results were obtained from different habitats, a different species of lionfish and different top predators, so it cannot be assumed the same is true in the Mediterranean. Comparable research in the Mediterranean Sea is necessary to determine whether top predators like large groupers could combat the lionfish invasion. In the Mediterranean Sea, *P. miles* remains have been found in the stomachs of *Epinephelus marginatus*, *Epinephelus aeneus*, *Octopus vulgaris* and *Lagocephalus sceleratus* (Ulman et al., 2021). The impact of these predators on lionfish populations has yet to be determined. *Fistularia commersonii*, another invasive predator in the Mediterranean Sea has also been recorded consuming lionfish in its native range (Bernadsky & Goulet, 1991; Azzurro et al., 2013). *F. commersonii* feeding on lionfish has not yet been recorded in the invaded Mediterranean Sea, but it could be valuable to research this in the future.

Native parasites could decrease growth, natality and survivability of invasive species (Torchin et al., 2002). Ectoparasites have been recorded on Mediterranean lionfish before (Yanar et al., 2022). Parasite abundance on invasive lionfish in the Atlantic range is significantly lower compared to native species in the same area (Tuttle et al., 2017). Should this also be the case for lionfish in the Mediterranean Sea, this is another factor potentially increasing invasion success, but no data is currently available on parasite abundance on Mediterranean lionfish. However, in essence, invasive species like the lionfish have easy access to food sources with no evidence pointing to native top predators successfully competing with lionfish or decreasing the population through predation. Neither is there any proof of native parasites negatively impacting lionfish.

### **1.3.4 Prey species**

#### **1.3.4.1 *Chromis chromis***

In the Mediterranean Sea, lionfish are generalist predators with a preference for some specific species. Damselfish (*Chromis chromis*), and species within the Labridae family are frequently eaten by lionfish (Figure 5) (D'Agostino et al., 2020). Schools of damselfish close to rocky shores in the Mediterranean Sea play a crucial part in the food chain in a nutrient-scarce sea (Pinnegar, 2018). Predatory birds, as well as marine predators, feed on these fish (Pinnegar, 2018). Simultaneously, these fish consume zooplankton and transfer carbon, nitrogen and phosphorus from the pelagic system to the rocky substrate in the form of solid and liquid waste (Pinnegar & Polunin, 2006; Pinnegar, 2018). These known trophic links emphasise the potential impacts, direct and indirect, that invasive lionfish can have if they are able to establish

populations and destabilize the ecosystem, such as indirect effects on nutrient cycling and the balance of the native food web.



Figure 5: *C. chromis* individual (top) and remains of an unidentified fish (bottom) found by members of the Archipelagos Institute of Marine Conservation in the stomach of a *P. miles* individual from the port of Kokkari, Samos in 2026.

#### 1.3.4.2 *Thalassoma pavo*

Lionfish show a preference for *Thalassoma pavo* juveniles, which threatens their valuable role in a functional ecosystem as, among other things, a key species for biological control of overgrazing sea urchins. The frequency of predation on Labridae species is disproportionate to the abundance of these fish recorded around the lionfish, showing a preference for these species, mainly for juvenile *T. pavo* individuals (Figure 6) (D'Agostino et al., 2020). *T. pavo* assist the starfish *Marthasterias glacialis* with hunting the sea urchins *Paracentrotus lividus* and *Arbacia lixula* (Galasso et al., 2015). When *M. glacialis* is detected by sea urchins, the threatened sea urchins extend their tube feet to escape predation. *T. pavo* individuals capitalize on this opportunity by biting and eating the tube feet without the danger of the defensive spikes of the sea urchins (Galasso et al., 2015). Thereafter, *M. glacialis* can eat the immobilised sea urchins more easily. The increased predation decreases overgrazing by the sea urchins, which allows healthy algal forests to exist (Galasso et al., 2015). Seeing as algal forests in the Mediterranean Sea are already under pressure from other invasive species (mainly *Siganus rivulatus* and *S. luridus*), *T. pavo* play a vital role in the conservation and natural recovery of these areas (Sala et al., 2011). Increased predation of *T. pavo* by invasive lionfish could lead to reduced *M. glacialis* hunting efficiency, resulting in a higher abundance of overgrazing sea urchins, creating urchin barrens in the coastal habitats of the Mediterranean Sea.



Figure 6: Partially digested *Thalassoma pavo* individual (left) found by a member of Archipelagos Institute of Marine Conservation in the stomach of a *P. miles* individual (right) from the port of Kokkari, Samos in 2024.

## 1.4 Behaviour

### 1.4.1 Hunting times

Lionfish hunt mainly during sunrise and sunset, but have also been documented hunting during the night. Mediterranean lionfish in shallow habitats have been found to spend the most time hunting at sunrise and sunset, known as the crepuscular period, and spend majority of their time resting at noon (D'Agostino et al., 2020). During hunting, they mainly hunt small benthic or benthic-pelagic species (D'Agostino et al., 2020). This reflects their light-based feeding behaviour in their native habitat, where they also have crepuscular hunting habits (McTee & Grubich, 2014). This crepuscular hunting behaviour could be linked to higher hunting success, related to lower light intensity and a greater abundance of prey (Green et al., 2011). Lionfish have also been documented hunting at night in their native habitats (McTee & Grubich, 2014). Lionfish have recently been recorded hunting during daytime hours by Archipelagos Institute of Marine Conservation, which sparked the question whether there could be a shift in feeding behaviour.

### 1.4.2 Hunting behaviour

Lionfish hunt slowly, utilising their oversized fins and quick strikes to swallow prey whole. While hunting, lionfish often swim slowly, focus on a singular prey animal and utilise their oversized pectoral fins to herd prey into corners or against surfaces until they are in striking range (Côté & Maljkovic, 2010). When in striking range, lionfish

consume prey whole, using a combination of rapidly opening the mouth and thus creating suction, and moving forward suddenly and quickly (Van Leeuwen & Muller, 1984). This causes a flow of water into the gaping mouth, resulting in capture and ingestion of prey (Van Leeuwen & Muller, 1984). This unique hunting strategy might play a role in prey naivety and high hunting success of lionfish in invaded ranges.

### **1.4.3 Blowing**

Lionfish might resort less to specialized hunting behaviour that increases hunting efficiency in the invaded range compared to the native range because of prey naivety in the invaded range. Lionfish sometimes blow jets of water out of their mouth toward prey (Figure 7), the reason for which has not yet been proven (Albins & Lyons, 2012). Albins and Lyons (2012) speculated that blowing behaviour is aimed to disorient or stun prey species, increasing the strike success rate. Bathow (n.d.) hypothesized that blowing is used to reorient fish too large to swallow sideways, which was supported by an increase of headfirst kills observed when lionfish blow during hunting in their study, as well as the study conducted by Albins and Lyons (2012). This is theorized to be caused by instinctive orientation toward the generated current, as this is useful to reduce energy consumption while retaining position in a current (Bathow, n.d.). This was supported by recording a significantly longer successful striking distance caused by the prey fish orientating and moving towards the blowing predator (Bathow, n.d.). Additionally, an increase of blowing behaviour seen in smaller lionfish also support this hypothesis (Bathow, n.d.). Positioning prey fish headfirst is advantageous for the lionfish, as it allows them to consume larger fish, as well as avoid potential barbs or spines that point towards the back of the prey's body (Albins & Lyons, 2012). This behaviour is seen 3 times more often in the native range compared to the invaded range (Albins & Lyons, 2012). It could be argued that because of prey naivety lionfish have less reliance on these hunting techniques in their invaded ranges, resulting in them being observed less frequently.

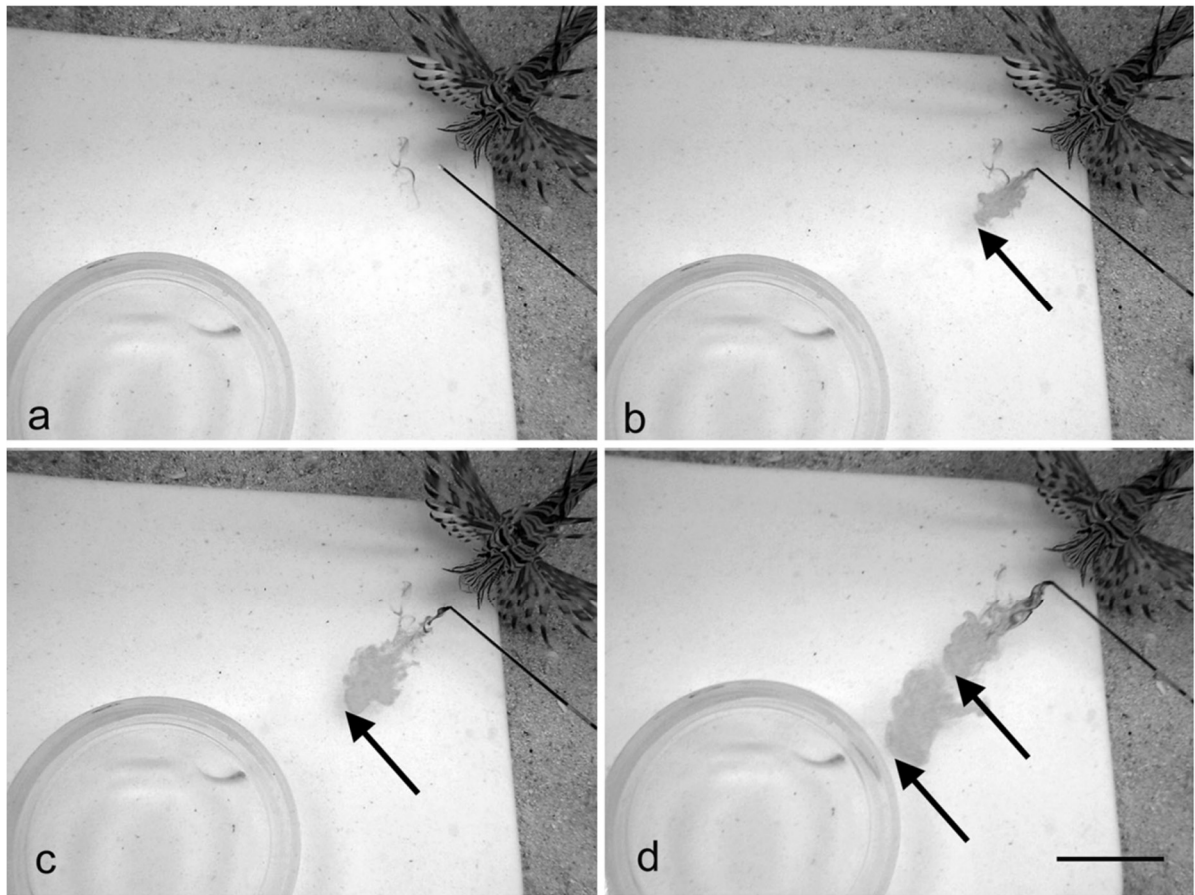


Figure 7: Blowing behaviour in a controlled environment, visualized with a droplet of food dye between the lionfish and a potential prey (from Albins & Lyons, 2012).

#### 1.4.4 Research methods

Effectively combatting biological invasions requires extensive knowledge, creating the need for standardized research methods. Two key studies have previously focused on lionfish behaviour: Cure et al. (2012) and D'Agostino et al. (2020), the methods from which this paper are based on. In both studies, observations were conducted between sunrise and sunset. In the study by Cure et al. (2012), 10-minute long visual observations were employed using SCUBA and snorkelling gear and in the study by D'Agostino et al. (2020), five-minute video recordings of every lionfish were employed using SCUBA gear. In both studies, researchers stayed at least three meters away from the lionfish and only in the study by D'Agostino et al. (2020), lionfish were given three minutes to acclimatise to the presence of divers. Care was taken to observe every individual only once per day and every observation was treated as independent (Cure et al., 2012; D'Agostino et al., 2020). Observations took place with varying time, location and environmental conditions for both studies (Cure et al., 2012; D'Agostino et al., 2020). Four categories of behaviour were scored every 10 seconds per observation in the study by D'Agostino et al. (2020): inactive, hovering, transiting and hunting, while in the study by Cure et al. (2012), behaviours were either counted as proportions of time: inactive, minimal activity, active and hunting or they were counted as events: aggressive interactions, strikes, kills and blows. Behaviours were either counted or measured as proportions of time.

Environmental data was collected in each study, as well as data on potential prey targeted by lionfish: estimated length, identification to the lowest possible taxonomical level and, only in the study by D'Agostino et al. (2020), proximity of the target to the lionfish (Cure et al., 2012; D'Agostino et al., 2020). Additionally to these methods, individual lionfish identification based on the patterns of the lionfish is possible, because these patterns are unique for each individual (Phillips et al., 2024). Utilizing this, behaviour can be attributed to different lionfish individuals. This method has been tested and utilized for *P. volitans* as well as *P. miles* (Chaves et al., 2016; Phillips et al., 2024). Seeing as this kind of research has not yet been done in shallow coastal areas of the Aegean Sea where lionfish populations are now increasing, it could be valuable to replicate in this area. The results could prove useful for the management and control measures of the lionfish invasion in this region.

## **1.5 Research aims**

The aim of this project was to document *P. miles* hunting behaviour and general activity patterns in the eastern Aegean Sea. To address this, research was structured around the following questions:

- (i) What times of day are lionfish hunting and/or out of hiding?
- (ii) What environmental factors influence lionfish behaviour in the Aegean Sea?
- (iii) What prey species do lionfish target in the Aegean Sea?
- (iv) How does recorded lionfish behaviour in the Aegean Sea compare to behaviour documented in the native range?

## 2 Materials and methods

### 2.1 Study area

Lionfish behaviour surveys were carried out at three different sites in Greece (Figure 8), over a period of two and a half months, from 03/03/2026 until 20/05/2026. Two sites are located on Samos island, the third on Lipsi island (Figure 9). Preliminary surveys found populations of lionfish around the end of the pier at Paralia Agios Konstantinos (East Beach), primarily along the part to the northwest of the pier. They were found between 1 – 6 m depth exclusively on rocky substrate and boulders. Further east, lionfish were also occasionally found between 3 – 10 m depth. Similar preliminary surveys found populations of lionfish around the Agios Konstantinos Marina (West Beach), with most sightings located along the artificial rock wall of the marina, between 2 - 7 m depth. In Vroulia bay, Lipsi (Vroulia), preliminary surveys found lionfish in and around caves around the bay. They were found between 1 - 3 m depth, close to the edges of the bay where the habitat consists of rock walls and deep caves. Considering these observations, these three sites were chosen to collect behavioural data.



Figure 8: Map of the Aegean Sea, Greece and Turkey, with Samos island and Lipsi island highlighted.



Figure 9: Map of the east of the Aegean Sea, highlighting Samos island, Lipsi island, the two survey sites on Samos (West Beach and East Beach) and the one survey site on Lipsi (Vroulia) where lionfish behavioural data was collected.

The coastline of East Beach consists of large boulders and rocky substrate, increasing in depth until around seven meters deep. After that, the sea floor transitions into a sandy slope. To the east of the pier, there is a mix of rocky and sandy substrate as well as some seagrass patches, with a limited large boulder habitat. This area has a more gradual slope than the steep rocky boulders around the East Beach pier. Surveys at East Beach were conducted around the end of the pier, scanning the area from the pier itself down to the sandy bottom. The area east of the pier was also searched, swimming parallel to the coastline until the end of the tree line on the coast (Figure 10a).

West Beach surveys took place parallel to the coastline close to the rock wall of the marina (Figure 10b). This rock wall is made of artificial concrete blocks designed to dissipate wave energy, piled on top of the natural rocky sea floor. The sea floor slopes down away from the rock wall, starting from a depth of 2 m and transitioning to large, naturally occurring rocks, caves and overhangs, with a steeper slope until reaching around 7 meters depth, where the rocky substrate transitions to a sandy sea floor. The full length of the artificial rock wall was searched, as well as around the eastern end of the pier, on the edge of the marina.

Surveys at the Vroulia site took place along the shallow sides of the bay, focussing on the locations where lionfish were sighted most frequently in preliminary surveys (Figure 10c): on the northeast side of the bay in a series of open caves with a max depth of 1,5 m and on the west side at a large rift in the rock wall containing a cave, as well as multiple caves found along the edges of the bay on either side of this rift between 1,5 – 4 m depth. These areas consist of rocky/sandy bottoms, many caves and overhanging rocks. The searched area varies from one to five meters depth, but lionfish individuals here were almost exclusively found at depths of 2,5 meters or shallower.

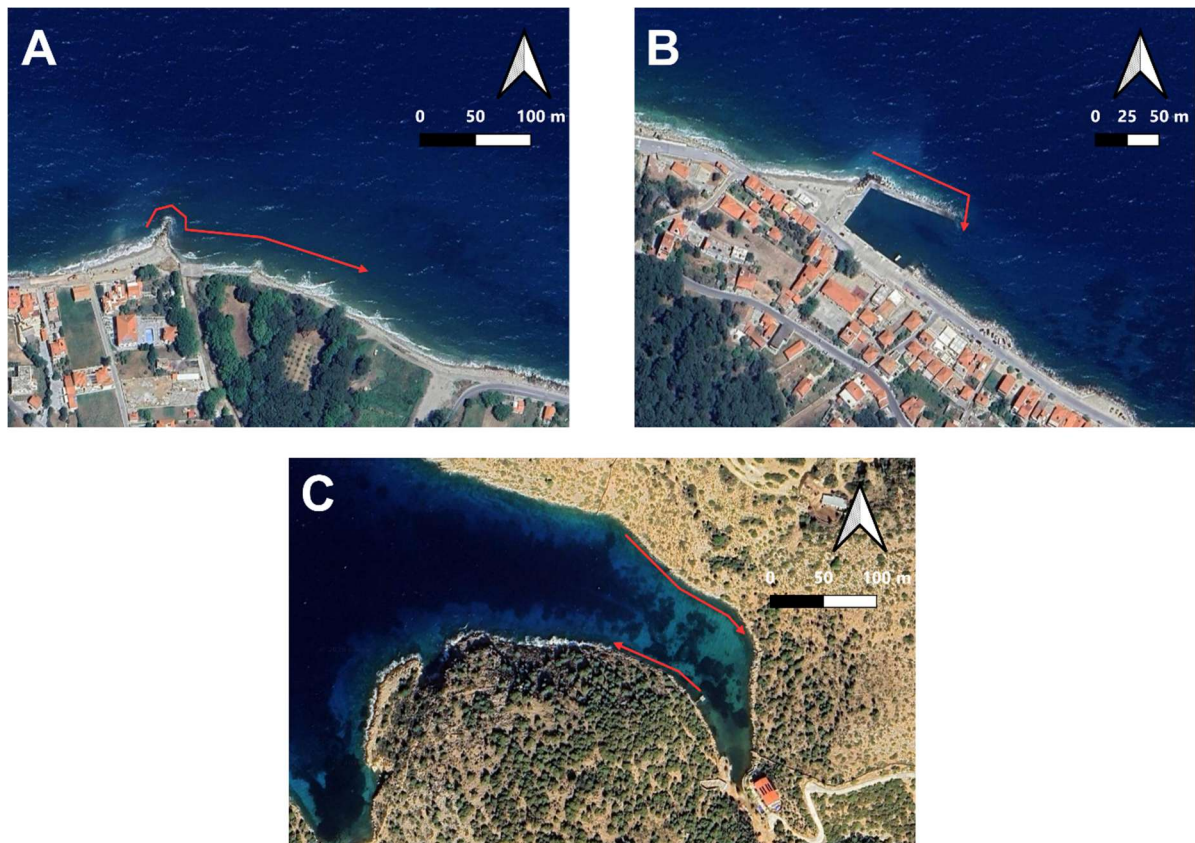


Figure 10: Maps of survey sites, the red arrows indicating the path followed by snorkellers collecting lionfish behavioural data, with (A) East Beach in Agios Konstantinos, Samos, (B) West Beach in Agios Konstantinos, Samos and (C) Vroulia bay, Lipsi.

## 2.2 Equipment

Materials used included a surface marker buoy, one underwater camera per person (in this case an Insta360 and a GoPro Hero 9), snorkelling equipment, a diving watch, slates and pencils to take notes, dive torches, a measuring tape to measure lionfish sizes, a waterproof watch with GPS recording capabilities (in this case a Garmin Instinct 2 Solar) and a HOBO logger attached to a stable frame to record water temperature and light intensity (Figure 11).



Figure 11: Metal frame with HOBOT logger attached on top used during lionfish surveys to measure water temperature and light intensity.

## 2.3 Survey method

Following a method based on similar studies by Cure et al. (2012) and D'Agostino et al. (2020), lionfish behaviour was visually observed at different times of day through implementing underwater visual census (UVC) technique in snorkelling surveys. Observation periods for each individual lionfish were 10 minutes, similar to the study by Cure et al. (2012), after which the observer went closer to capture a picture of the pattern on each side of the lionfish for post-survey photo ID, and to measure the total length (TL) to the closest cm with the aid of a measuring tape. To minimize interference with natural behaviour during the 10-minute observation period, the observer stayed a minimum of three meters away from the lionfish. In addition, after a lionfish was sighted and before the 10 minute observation window began, there was a three-minute break to allow the lionfish to return to its original behaviour, as done by D'Agostino et al. (2020) in a similar study. Photo-ID data was collected, but the processing was done in a different project by another member of Archipelagos Institute of Marine Conservation. Photo-ID data was not yet available at the time of data analysis, so this was not used for this thesis.

During the 10-minute observation period, behaviour was quantified in two ways: (1) the proportion of time exhibiting either inactive (resting in contact with substrate with spines folded along the body) or hunting (moving around, looking for prey with flared pectoral fins) and (2) the counted behaviours: aggressive behaviour (chasing a specific animal with pectoral fins completely flared), strikes (all attempts, successful or not, to consume another animal), kills (strikes that were visually confirmed to be successful), misses (strikes that were confirmed to be unsuccessful or strikes with uncertainty of success) and blows (moving water in the direction of another animal

using the mouth). In addition, when a lionfish was observed hunting, the prey species (whether it was successfully killed or not) was identified to the lowest taxonomic level possible. Lionfish were also documented being either out in the open or in an enclosed space under/between rocks or in a cave. This data could prove useful for this research, as determining what factors cause lionfish to come out of hiding is very important information for divers culling these fish.

During pilot surveys, lionfish were initially filmed using underwater cameras for the duration of the 10 minute observation period. However, this footage was often unclear and thus less valuable and harder to interpret than live observations. In order to ensure reliability of the data, video analysis was not used for behavioural observation and instead all observations were recorded *in situ* on a slate by the observer.

The behaviours recorded were analysed based on the description made in the reference study by Cure et al. (2012) on *P. volitans* as shown in Table 1 and Table 2. In their study, the behaviours ‘minimal activity’ and ‘active’ were differentiated based on a short or a long distance covered, which is debatably too subjective to usefully implement in this study. Afterwards, less than 10% of the recorded time was labelled as one of these behaviours, so these were not used in the statistical analysis (Cure et al., 2012). To prevent prematurely eliminating useful data while keeping every observation objective and significant, this study initially recorded the behaviour ‘active’, but not ‘minimal activity’. After pilot surveys, the lionfish were either seen actively seeking out prey or being almost entirely stationary. To prevent ambiguity, the lionfish were recorded being either inactive or hunting.

Table 1: Ethogram of timed behaviours documented during observations of *P. miles* in the eastern Aegean Sea.

| Behaviour | Description                                                                                                           |
|-----------|-----------------------------------------------------------------------------------------------------------------------|
| Inactive  | In contact with the substrate/rocks/reef with dorsal spines folded across the dorsal midline while stationary         |
| Hunting   | Looking for prey, moving around with completely flared pectoral fins (= fins spread out, pointing away from the body) |

Table 2: Ethogram of counted behaviours documented during observations of *P. miles* in the eastern Aegean Sea.

| Behaviour              | Description                                                                                   |
|------------------------|-----------------------------------------------------------------------------------------------|
| Aggressive interaction | Chasing other lionfish or other species                                                       |
| Strike                 | Attack on potential prey (successful and unsuccessful)                                        |
| Kill                   | Successful attack and consumption of prey                                                     |
| Blow                   | Water current directed at prey generated with mouth                                           |
| Unsuccessful attack    | Strike where prey was not consumed or it could not be clearly determined if prey was consumed |

Recorded environmental factors were the microhabitat the observation was taking place in (rocks and/or sand and/or seagrass habitat), the date, the start and end time of the survey, the cloud cover (<25% = clear; 25-75% = partly cloudy; >75% = cloudy), current strength (researcher barely kicking to maintain position = weak; periodic kicking to maintain position = medium; constant kicking to maintain position = strong), coordinates of lionfish individuals using a GPS dive watch, sea state (wave height  $\leq 0 = 0$ ,  $< 0.1$  m = 1,  $0.1 - 0.5$  m = 2,  $0.5 - 1.25$  m = 3,  $1.25 - 2.5$  m = 4), water visibility (m), depth (m) of the observed lionfish, the presence of people in the water other than the researchers, water temperature (°C) and light intensity (lux). All this data was recorded on a slate while surveying and input into a database using Microsoft Excel.

## 2.4 Dissection

A range of dissections were done by members of the Archipelagos Institute of Marine Conservation. Data on lionfish diet was gathered through dissection and analysis of stomach contents from the *P. miles* individuals killed during this dive. The author of this paper assisted with the dissection and recorded the data during some of these procedures. A range of factors were recorded, the relevant data for this study being the stomach contents. This additional data reflects which fish were confirmed to be eaten by lionfish and helps in estimating lionfish prey choice and potential trophic impact.

## 2.5 Statistical analysis

A total of 90 observations were usable for data analysis. However, only 60 had all variables recorded. For statistical analysis on lionfish behaviour, only the 60 observations with all variables measured were used. The extra 30 observations were only used for analysis of prey species and the counted hunting behaviours (aggressive interactions, strikes, kills, misses and blows). Analyses were carried out

in R Studio (version 4.6.0) largely based on the statistical analysis done by D'Agostino et al. (2020) on behavioural patterns of Mediterranean lionfish. Habitat was excluded from data analysis because it was always rocky except for only 2 instances (<3% of all observations), where lionfish were found on rocky/sandy substrate. Similarly to D'Agostino et al. (2020), activity was transformed from proportions of time to a binary value: lionfish were either labelled 'inactive' or 'hunting', based on the majority of time spent being inactive or hunting.

To test for the effects of time of day, total length, current, visibility, depth, sea state, pedestrians, weather, light intensity and temperature on the probability of lionfish exhibiting hunting behaviour (i.e. showing hunting behaviour the majority of the observed time), a generalised linear model (GLM) with a binomial distribution and logit link was used to fit the maximal model. The residual deviance was approximately equal to the residual degrees of freedom, so no overdispersion was present in the model and variance inflation factors were low (<5) for all predictor variables, so the assumptions of multicollinearity and binomial distributions were satisfied (Appendix A, Appendix B).

Through backwards selection using p-values, non-significant predictor variables were removed from the maximal model, leaving only water temperature and survey times. Water temperature was a significant predictor of probability of lionfish exhibiting hunting behaviour, but when, using a likelihood ratio test based on the Chi-squared distribution, the model including water temperature was compared to a simpler model only including survey times, the p-value was significant and thus the simpler model was better suited for statistical analysis. D'Agostino et al. (2020) removed temperature from the equation, considering the water temperature stayed within normal diel temperature changes, which was also the case for the water temperature in this study (17-21°C). Combining these two factors, the omission of water temperature from the model was justified.

Using the same predictor variables, a second GLM model was constructed to investigate whether any of the measured environmental variables were significant predictors of the probability of lionfish being out of hiding. The model was not overdispersed and predictors showed no multicollinearity, so the assumptions of multicollinearity and binomial distributions were satisfied (Appendix C, Appendix D). The maximal model was simplified using backwards selection using p-values, again leaving survey time as the only significant predictors of probability of lionfish being out of hiding.

### 3 Results

During this research project, data was collected from 90 observations (sunrise  $n = 25$ , daytime  $n = 22$ , sunset  $n = 16$  and night  $n = 27$ ; East Beach/Forest  $n = 45$ , West Beach  $n = 22$ , Vroulia  $n = 23$ ) taken during 43 surveys. The number of lionfish observations per survey varied, with at least one observation for every single survey. East Beach surveys yielded a maximum of nine observations, West Beach a maximum of four observations and Vroulia a maximum of two. All of these maxima were observed at nighttime, while the minimal number of observations per survey site were all documented during daytime surveys. Average observations per sunrise survey were 2,55, per day survey 1,63, per sunset survey 2,78 and per night survey 4,88.

Analysing the probability (%) of lionfish exhibiting hunting behaviour and comparing the different times surveys were conducted, the probability of lionfish exhibiting hunting behaviour was significantly less during the daytime than during other survey times (Appendix A). (Figure 12).

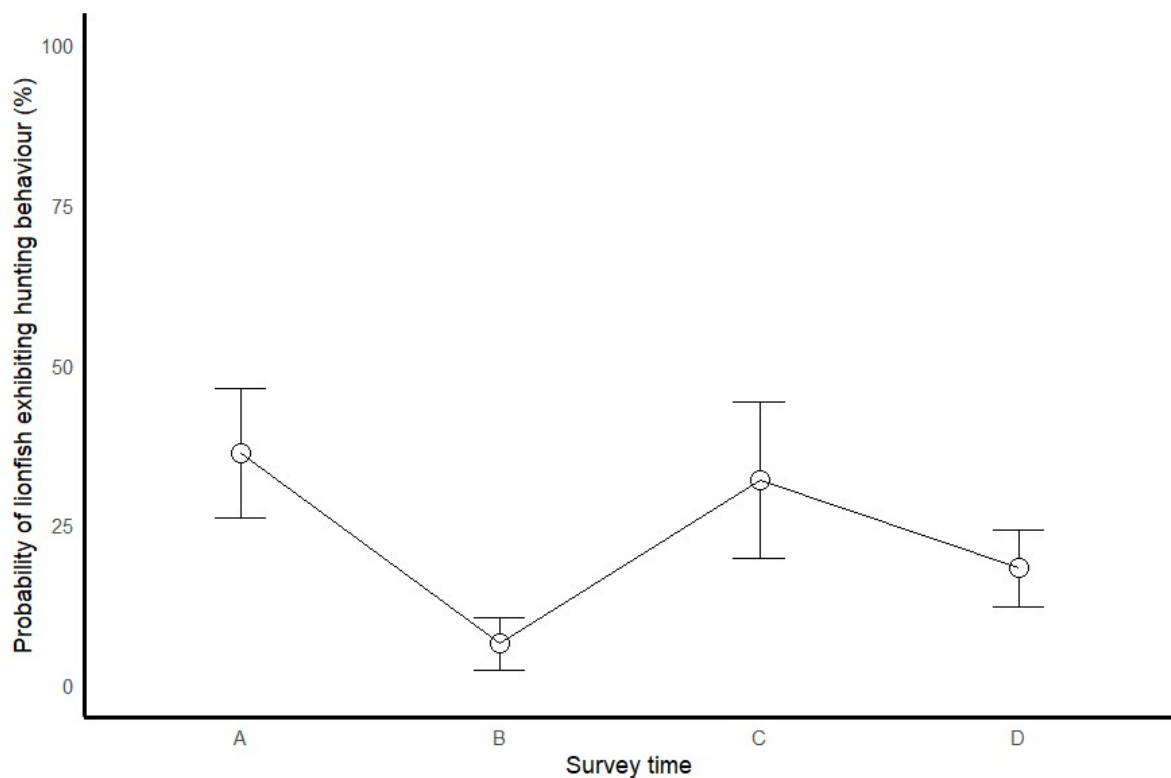


Figure 12: The probability (%) of lionfish exhibiting hunting behaviour per survey time where A = immediately after sunrise, B = during the day, C = right before sunset and D = during the night; the bars around each point show the standard deviation of probability per survey time.

Analysing the probability (%) of lionfish being out of hiding and comparing the different times surveys were conducted, the probability of lionfish being out of hiding was significantly less during the daytime than during other survey times (Appendix C). (Figure 13).

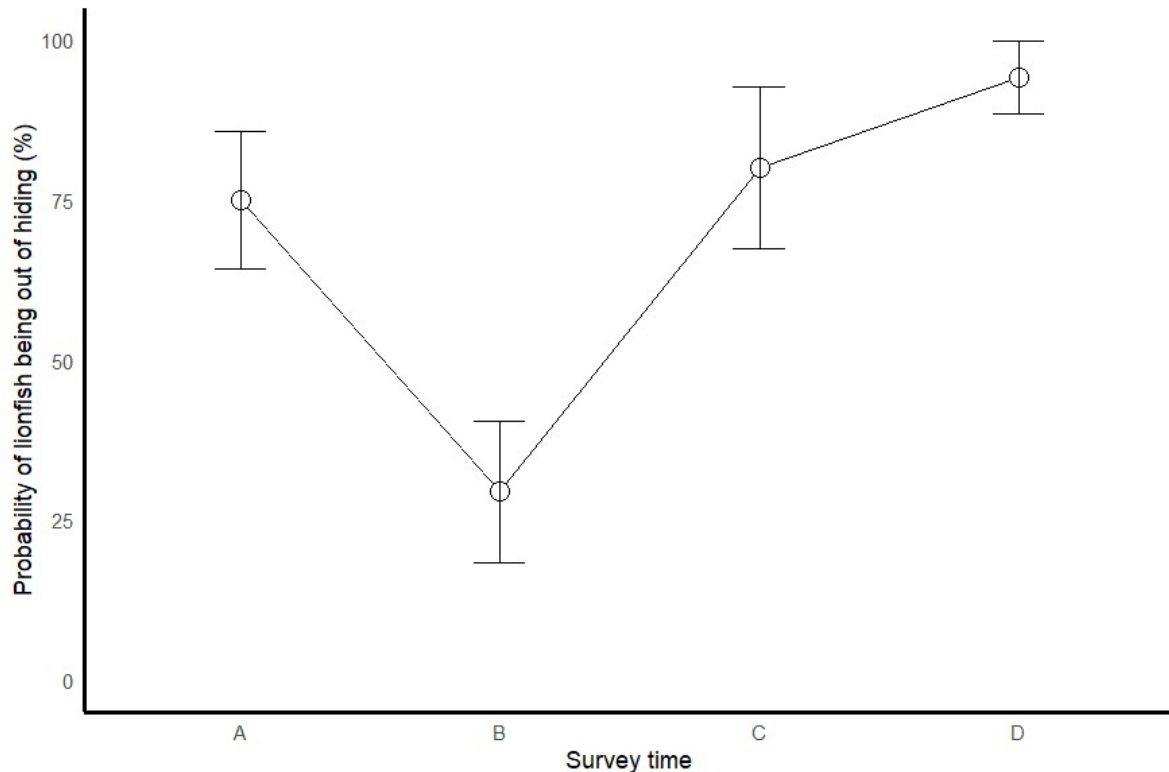


Figure 13: The probability (%) of lionfish being out of hiding per survey time, where A = immediately after sunrise, B = during the day, C = right before sunset, D = during the night; the bars around each point show the standard deviation of probability per survey time.

26 aggressive interactions were recorded, 34,62% which were recorded during sunrise, 15,38% during the day, 38,46% during sunset and 11,54% during the night. Prey species targeted by lionfish during aggressive interactions were *Chromis chromis* and *Spicara smaris* (identified twice each as targeted species), *G. couchi*, *Thalassoma pavo*, *Sargocentron rubrum*, *Atherina boyeri*, *Sepia officinalis* and an unidentified member of the *Gobiidae* family (identified once each). Remaining observed targeted prey species were unidentified fish species. Seven strikes were recorded, with only one prey species being identified as *Atherina boyeri*, which was missed. Two strikes were recorded at sunrise, two were recorded at sunset, two were recorded at night and one was recorded during the day. A successful kill was observed only once, on an unidentified fish species, the remaining six strikes were misses. Blowing behaviour was observed only once, targeted towards a *G. couchi* individual. Lionfish dissected by members of the Archipelagos Institute of Marine Conservation had *T. pavo*, as well as *C. chromis* individuals in their stomachs.

## 4 Discussion

*P. miles* in the eastern Aegean Sea show similar hunting behaviour in this invaded range compared to their native range, other invaded areas in the Mediterranean Sea and to *P. volitans* in their native and invaded range, supporting the findings of Cure et al. (2012) and D'Agostino et al. (2020). Results showed that the probability of lionfish hunting was significantly lower during the daytime than during other survey times. This reflects their crepuscular hunting activity during periods of low light intensity and nocturnal activity in their native habitats, as well as in other locations in the Mediterranean Sea and the invaded Atlantic range (Fishelson, 1975; Green et al., 2011; Cure et al., 2012; D'Agostino et al., 2020). This crepuscular hunting behaviour could be linked to invasion success, because of increased hunting success and a greater array of prey (Green et al., 2011). However, an interesting difference found between the lionfish observed in this study and the results of the similar study by D'Agostino et al. (2020) further south in Cyprus, is that the estimated probabilities of lionfish to be hunting were much less (around 15 – 20% lower) in this study. This lower hunting activity could potentially be attributed to the lower population sizes present in this study area, due to the relatively recent establishment of invasive lionfish around Samos and Lipsi islands compared to further south around Cyprus, where lionfish have been established for longer (Kletou et al., 2016; D'Agostino et al., 2020).

Knowing where lionfish reside and at what times they are out of hiding could prove crucial to effectively targeting and controlling their populations in invaded ranges. Lionfish were always found in rocky habitats in this study. Lionfish were significantly less probable to be out of hiding during the daytime compared to other survey times. During nighttime, lionfish had a high estimated probability of being out of hiding (94,12%). Even though there was no significant difference found in probability of lionfish being out of hiding between nighttime and sunrise and sunset, when regarding the standard deviations, there is very little overlap with these other times. This could motivate a repeat of this study with a higher sample size to determine whether lionfish could really have a significantly higher probability of being out of hiding during the night than during other times of increased hunting activity. Their tendency to be out of hiding presents an opportunity for divers to target these fish effectively during spearfishing culls, which is a common control measure employed globally for invasive lionfish (Kleitou et al., 2021).

Strikes were rarely recorded, with only seven counts from all 90 observations and kills were hard to confirm, rendering this data unusable for statistical analysis. Remarkably though, strikes were recorded during every survey time, including during the day. Lionfish were also observed striking while inactive, which was also recorded in *P. volitans* in invaded Bahamian coral reefs by Côté and Maljkovic (2010). Because of the small sample size of strikes, no conclusions could be made from these observations, but it could prove interesting to monitor in the future whether lionfish

are still hunting and feeding during periods of reduced hunting activity during the day. Feeding while inactive could potentially explain why lionfish were generally observed to be less active here than in the comparable study by D'Agostino et al. (2020). This low activity feeding behaviour also lines up with proven native prey species naivety to these novel predators (D'Agostino et al., 2020). Prey species approaching lionfish closer because of inexperience with these fish increases hunting success and could reduce the energy spent on hunting. Strike success was impossible to confidently determine, because small fish were hard to track and identify using snorkel equipment. Since strikes were labelled as misses if the result could not be clearly determined, all strikes but one were labelled as misses.

Aggressive interactions (e.g. lionfish chasing after other animals) were aimed towards a range of species, some to individuals far too big to be seen as prey (two *Sepia officinalis* and a *Muraena helena* individual more than three times the total length of the lionfish), but the majority were directed towards small fish, of which the majority were unidentified. Mediterranean lionfish have been documented to have a preference for juvenile *T. pavo* and *C. chromis* (D'Agostino et al., 2020). These species were both recorded being hunted by lionfish during this study, combined with both being found in lionfish stomachs during dissections by members of the Archipelagos Institute of Marine Conservation. These dissected fish originated from the Kokkari harbour on the north of Samos. Henceforth, this study supports previous literature and confirms these species as prey of invasive lionfish in the eastern Aegean Sea. These species have been documented as important species for the local ecosystems, so reduced populations of these species caused by increased predation pressure could negatively impact the ecosystem and food web (Pinnegar & Polunin, 2006; Galasso et al., 2015; Pinnegar, 2018). Possible negative impacts of reduced *T. pavo* and *C. chromis* populations are a disturbance in nutrient distribution and increased abundance of sea urchin-mediated barrens on the sea floor (Galasso et al., 2015; Pinnegar, 2018).

Blowing behaviour was recorded only once in this study. The purpose of blowing behaviour is not yet confidently determined, but one of the hypotheses is that it is used to reorient prey too big to swallow sideways, so the lionfish can swallow them head-first (Bathow, n.d.). The one documented instance of blowing behaviour during this study was targeted towards a *G. couchi* individual clearly too big in relation to the lionfish's mouth to swallow sideways, supporting this hypothesis. Albins and Lyons (2012) recorded blowing behaviour three times more frequently in the native range than in the invaded range, which may explain the very limited amount of recorded blows this study in the Aegean Sea. These findings could indicate that lionfish are already highly successful in hunting in invaded habitats, so much so that they are able to without having to rely on specialised hunting techniques as they do in their native range.

Biotic resistance is an important natural defence of native ecosystems to invasive species. According to data from this site in 2025, there used to be at least five

individuals in the bay. However, since April 2025, a maximum of two individuals was sighted during all surveys in the bay. Whether this decrease can be attributed to migration, predation or any other cause is hard to determine and needs further investigation. Photo-ID collected during this project could prove useful to determine the actual population size. Notably, a group of at least 16 individuals of large *Fistularia commersonii* individuals was regularly sighted in Vroulia, which have been documented to prey on lionfish in their native range (Bernadsky & Goulet, 1991). Multiple *E. marginatus* individuals also inhabit the same caves in Vroulia lionfish are often sighted in, with one large individual frequently sighted together with one of the lionfish. *E. marginatus* are one of the few natural predators known to hunt lionfish in the Mediterranean Sea (Ulman et al., 2021). Determining if lionfish are under predation pressure in Vroulia could prove difficult, but biotic resistance to these invasive species may be a valuable area to explore further.

Biotic resistance can manifest through native predators reducing invasive species population sizes, but native parasites can also affect and reduce the success of established invasive species. One lionfish individual in Vroulia had an ectoparasite attached to its caudal fin (Figure 14). Parasites might negatively impact lionfish health and reduce their invasion success, but there has not been clear proof of the presence of parasites reducing lionfish success, neither has there been any sign of parasites negatively impacting *P. volitans* individuals in the invaded Atlantic range (Tuttle et al., 2017). Further research is necessary to quantify the effect of parasites on lionfish in the Mediterranean and whether the presence of ectoparasites on lionfish might point to increased biotic resistance in this region.



Figure 14: *P. miles* individual from Vroulia Bay, Lipsi, with unidentified ectoparasite on its caudal fin.

A large lionfish individual found at West Beach had scarring on its flanks, the most likely cause being a failed spearfishing attempt. When approached, the lionfish always fled more quickly and at a higher speed than seen with any other lionfish encounter. Phillips et al. (2024) discovered that lionfish in the Mediterranean Sea

have more subtle behavioural changes when exposed to culling compared to lionfish in the Caribbean Sea. This is highly relevant for informing potential population control efforts using spearfishing. The observed individual shows clear, consistent behavioural differences compared to other individuals in the same location. It is impossible to determine whether this behaviour was caused by a spearfishing incident, but scarring, combined with remarkable behaviour, points in the direction of learned caution towards approaching divers caused by some form of trauma. Learned evasive behaviour towards divers could make it incredibly hard to cull lionfish in the future, so future research into this phenomenon could add valuable knowledge to informing management practises.

## **4.1 Recommendations for future research**

During statistical analysis in this study, light intensity was not found to have a significant effect on hunting behaviour or whether lionfish were out of hiding or not. Seeing as other studies have proven a meaningful effect of time of day and cloud cover on lionfish activity (Côté & Maljkovic, 2010; D'Agostino et al., 2020), light intensity seems to be a logical predictor variable to measure, but the method or sample size of this study may not have been enough to identify a significant trend. Despite this, light intensity is recommended for future monitoring, potentially utilising different collection methods.

Identifying lionfish individuals using photo-ID could be incredibly valuable in future behavioural research. Attributing behaviour to different individuals could highlight individual preferences and differences. Furthermore, population size, recruitment and local movement dynamics can be more confidently determined using this method. After determining total population size, percentages of the total population showing different behaviours (i.e., being out of hiding or hunting) can be calculated, creating a broader view of behaviour on a population level. This is important for understanding population dynamics and the best approach to controlling them in invaded ranges.

Longer observation times per observation could also be valuable to implement in future. Ten minutes often proved too short and so it could be useful to observe individuals for extended periods of time, maybe with the aid of remote underwater video (RUV) surveys. This way, activity patterns and hunting behaviour can be more confidently documented and impact of human disturbance can also be decreased.

Seeing as lionfish populations have only recently been established in the Mediterranean Sea and are still actively expanding their range and population sizes, it is necessary to continue monitoring them across the entire Mediterranean Sea. Abundance, distribution and behaviour are important factors in understanding the scale of their impact, as well as key points of information needed to combat their invasion. Comparing populations in places with different habitats, water temperatures and native species could also be valuable in estimating future success and spread of lionfish.

Understanding the effect of lionfish removals on individual behavioural changes is vital to justify culling as an effective long-term solution to the lionfish invasion. As removal efforts increase, lionfish may learn to evade divers, rendering this method useless in the future and impeding on future solutions to the problematic situation. Similarly, behavioural changes caused by employed research methods (e.g. shining a torch close to the fish, approaching the fish) need to be considered further to ensure gathered data is valid and can be used as the basis for conservation efforts. Comparative research with red light torches is recommended.

The decreased populations of lionfish in Vroulia bay demonstrate the importance of long-term monitoring and highlights the need for investigation into the cause of this. If biotic resistance is present and can in any way be supported, this should be applied and supported as a viable contribution to protecting native ecosystems.

## **4.2 Limitations**

This research project encountered a series of limitations. The main one was the usage of snorkel surveys instead of SCUBA surveys. Less qualifications, logistical and organizational effort are needed to conduct snorkel surveys, but successful gathering of data was oftentimes disturbed because of issues inherent to using this technique. Bad water visibility often made it very challenging to find and observe lionfish, increasing survey time and reducing data collected per completed survey. This was an unpredictable factor and very hard to mitigate. At the eastern side of the pier of East Beach, a stream continuously delivered cold, fresh water from the mountains to the sea, generating a layer of halocline with varying thickness and area coverage, ranging from the eastern side of the pier to the water around the entire pier. This 'blurry' layer of water up to 30 cm thick at the surface made it practically impossible at times to efficiently search the area and observe behaviour. Because of these visibility issues, in combination with lionfish inhabiting deeper parts, it was difficult to identify prey species and determine whether strikes were successful or not. During observations, lionfish would sometimes go into hiding, which led to incomplete data for behaviour, size and photo-ID, since they could not be followed long into underwater caves or under overhangs. To mitigate this, photo-ID data was taken before the three-minute break before the observation, which could have had an impact on the behavioural data. Another limitation of snorkelling instead of SCUBA diving was the depth, as individuals below 5 m depth were not able to be included in this study for safety reasons.

Because of the intensity, inconvenient times and high frequency of surveys, data was often recorded by different interns of the Archipelagos Institute of Marine Conservation to distribute the workload. These interns were sometimes inexperienced with any marine fieldwork or snorkelling in general before participating in surveys, which introduced bias caused by the variety of observers. This reduces

the credibility of more subjective and ambiguous parts of the survey, for example the exact transition time between inactive and hunting behaviour.

Approaching the lionfish for photo-ID, as well as shining a torch too close to the animals, clearly changed the lionfish behaviour. A three-minute break was used to let the lionfish go back to its original behaviour, but it was unclear whether this was enough. Furthermore, after approaching, some individuals would go straight into hiding, resulting in a sighting with no behavioural data collected.

## 5 Conclusion

Invasive lionfish could have significant effects on the biodiversity of the Aegean Sea, creating the need for effective control measures. These generalist predators from the Indian Ocean are capable of significantly reducing native populations in invaded ranges. Understanding behavioural patterns is a key factor in informing and implementing population control measures. During this study, survey time proved to be the only variable significantly influencing lionfish hunting behaviour. Lionfish in the invaded Aegean Sea target the same key fish species as documented around Cyprus, but any preferences, strike success or overall prey variety were difficult to determine due to limited visibility inherent to using snorkelling equipment instead of SCUBA gear and low amounts of recorded strikes. Invasive lionfish in the Aegean Sea show similar hunting activity as in their native range, being less likely to show hunting activity during the day and more likely during sunrise, sunset and nighttime. The predatory fish are out of hiding most frequently during the night, sunrise and sunset, and less during the day. Nighttime, sunrise and sunset removal efforts seem the most promising for future population control measures, as lionfish are more probable to be out of hiding at these times. Overall this study provides vital supporting information to organise effective population control of invasive lionfish, while providing insights for future research into lionfish behaviour.

# Reflection

The majority of the work contributing to this thesis was new to me, which made it invaluable and incredibly educational. I learned the following practical skills while undertaking this research: data collection by surveying and freediving with snorkel gear, completing and improving protocols to collect scientific data and using and taking care of equipment used in marine research. Furthermore, I developed certain soft skills: inputting and analysing data using Microsoft Excel and R Studio, critical thinking and problem solving skills in survey settings as well as in a broader way by helping to manage and maintain the base, planning and organizing equipment and time schedules for surveys, conducting independent and supervised research from start to finish, giving presentations about the team and the project and answering prepared and impromptu questions for a video documentary about the project.

The whole experience broadened my vision and image of research and conservation work, as well as my view on my place in this field. Closely living and working with people from different cultures and backgrounds was extremely enriching on a personal and professional level, granting new insights useful in every aspect of my future career and life. The thesis was an important part of this, challenging me to discover new skills and learn from other people's knowledge and experience to overcome any roadblocks.

# Bibliography

- Albins, M., & Hixon, M. (2008). Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. *Marine Ecology Progress Series*, 367, 233–238. <https://doi.org/10.3354/meps07620>
- Albins, M., & Lyons, P. (2012). Invasive red lionfish *Pterois volitans* blow directed jets of water at prey fish. *Marine Ecology Progress Series*, 448, 1–5. <https://doi.org/10.3354/meps09580>
- Azzurro, E., Soto, S., Garofalo, G., & Maynou, F. (2013). *Fistularia commersonii* in the Mediterranean Sea: Invasion history and distribution modeling based on presence-only records. *Biological Invasions*, 15(5), 977–990. <https://doi.org/10.1007/s10530-012-0344-4>
- Bariche, M., Kleitou, P., Kalogirou, S., & Bernardi, G. (2017). Genetics reveal the identity and origin of the lionfish invasion in the Mediterranean Sea. *Scientific Reports*, 7(1), 6782. <https://doi.org/10.1038/s41598-017-07326-1>
- Bariche, M., Torres, M., & Azzurro, E. (2013). The Presence of the invasive Lionfish *Pterois miles* in the Mediterranean Sea. *Mediterranean Marine Science*, 14(2), 292. <https://doi.org/10.12681/mms.428>
- Bathow, E. (n.d.). *Invasive lionfish Pterois miles blow water jets at Sparus aurata to reorientate them and strike from a greater distance*. [Master's thesis, Wageningen University & Research]. Wageningen University and Research eDepot.
- Bernadsky, G., & Goulet, D. (1991). A Natural Predator of the Lionfish, *Pterois miles*. *Copeia*, 1991(1), 230. <https://doi.org/10.2307/1446269>

- Bernardi, G., Azzurro, E., Bariche, M., Jimenez, C., Kalogirou, S., & Kleitou, P. (2024). Invasion genomics of lionfish in the Mediterranean Sea. *Ecology and Evolution*, 14(3), e11087. <https://doi.org/10.1002/ece3.11087>
- Bottacini, D., Pollux, B. J. A., Nijland, R., Jansen, P. A., Naguib, M., & Kotrschal, A. (2024). Lionfish (*Pterois miles*) in the Mediterranean Sea: A review of the available knowledge with an update on the invasion front. *NeoBiota*, 92, 233–257. <https://doi.org/10.3897/neobiota.92.110442>
- Chaves, L. C. T., Hall, J., Feitosa, J. L. L., & Côté, I. M. (2016). Photo-identification as a simple tool for studying invasive lionfish *Pterois volitans* populations. *Journal of Fish Biology*, 88(2), 800–804. <https://doi.org/10.1111/jfb.12857>
- Cohen, A. S., & Olek, A. J. (1989). An extract of lionfish (*Pterois volitans*) spine tissue contains acetylcholine and a toxin that affects neuromuscular transmission. *Toxicon*, 27(12), 1367–1376. [https://doi.org/10.1016/0041-0101\(89\)90068-8](https://doi.org/10.1016/0041-0101(89)90068-8)
- World Health Organization (2015). Biodiversity and human health linkages: concepts, determinants, drivers of change and approaches to integration. *Connecting global priorities: Biodiversity and human health: a state of knowledge review*, 11.
- Côté, I., & Maljkovic, A. (2010). Predation rates of Indo-Pacific lionfish on Bahamian coral reefs. *Marine Ecology Progress Series*, 404, 219–225. <https://doi.org/10.3354/meps08458>
- Crocetta, F., Agius, D., Balistreri, P., Bariche, M., Bayhan, Y. K., Çakir, M., Ciriaco, S., Corsini-Foka, M., Deidun, A., El Zrelli, R., Ergüden, D., Evans, J., Ghelia, M., Giavasi, M., Kleitou, P., Kondylatos, G., Lipej, L., Mifsud, C., Özvarol, Y., ... Zenetos, A. (2015). New Mediterranean Biodiversity Records (October 2015). *Mediterranean Marine Science*, 16(3), 682-702. <https://doi.org/10.12681/mms.1477>

- Cure, K., Benkwitt, C., Kindinger, T., Pickering, E., Pusack, T., McIlwain, J., & Hixon, M. (2012). Comparative behavior of red lionfish *Pterois volitans* on native Pacific versus invaded Atlantic coral reefs. *Marine Ecology Progress Series*, 467, 181–192. <https://doi.org/10.3354/meps09942>
- D’Agostino, D., Jimenez, C., Reader, T., Hadjioannou, L., Heyworth, S., Aplikioti, M., Argyrou, M., & Feary, D. (2020). Behavioural traits and feeding ecology of Mediterranean lionfish and naiveté of native species to lionfish predation. *Marine Ecology Progress Series*, 638, 123–135. <https://doi.org/10.3354/meps13256>
- deRivera, C. E., Ruiz, G. M., Hines, A. H., & Jivoff, P. (2005). Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. *Ecology*, 86(12), 3364–3376. <https://doi.org/10.1890/05-0479>
- Dimitriadis, C., Galanidi, M., Zenetos, A., Corsini-Foka, M., Giovos, I., Karachle, P. K., Fournari – Konstantinidou, I., Kytinou, E., Issaris, Y., Azzurro, E., Castriota, L., Falautano, M., Kalimeris, A., & Katsanevakis, S. (2020). Updating the occurrences of *Pterois miles* in the Mediterranean Sea, with considerations on thermal boundaries and future range expansion. *Mediterranean Marine Science*, 21(1), 62. <https://doi.org/10.12681/mms.21845>
- Freshwater, D. W., Hamner, R. M., Parham, S., & Wilbur, A. E. (2009). Molecular evidence that the lionfishes *Pterois miles* and *Pterois volitans* are distinct species. *Journal of the North Carolina Academy of Science*, 125(2), 39-46.
- Galanidi, M., Zenetos, A., & Bacher, S. (2018). Assessing the socio-economic impacts of priority marine invasive fishes in the Mediterranean with the newly proposed SEICAT methodology. *Mediterranean Marine Science*, 19(1), 107. <https://doi.org/10.12681/mms.15940>

- Galasso, N. M., Bonaviri, C., Trapani, F. D., Picciotto, M., Gianguzza, P., Agnetta, D., & Badalamenti, F. (2015). Fish-seastar facilitation leads to algal forest restoration on protected rocky reefs. *Scientific Reports*, 5(12409). <https://doi.org/10.1038/srep12409>
- Gardner, P. G., Frazer, T. K., Jacoby, C. A., & Yanong, R. P. E. (2015). Reproductive biology of invasive lionfish (*Pterois* spp.). *Frontiers in Marine Science*, 2. <https://doi.org/10.3389/fmars.2015.00007>
- Golani, D., & Sonin, O. (1992). New records of the Red Sea fishes, *Pterois miles* (Scorpaenidae) and *Pteragogus pelycus* (Labridae) from the eastern Mediterranean Sea. *Japanese Journal of Ichthyology*, 39(2), 167–169. <https://doi.org/10.1007/BF02906001>
- Green, S., Akins, J., & Côté, I. (2011). Foraging behaviour and prey consumption in the Indo-Pacific lionfish on Bahamian coral reefs. *Marine Ecology Progress Series*, 433, 159–167. <https://doi.org/10.3354/meps09208>
- Hackerott, S., Valdivia, A., Green, S. J., Côté, I. M., Cox, C. E., Akins, L., Layman, C. A., Precht, W. F., & Bruno, J. F. (2013). Native Predators Do Not Influence Invasion Success of Pacific Lionfish on Caribbean Reefs. *PLoS ONE*, 8(7), e68259. <https://doi.org/10.1371/journal.pone.0068259>
- Hamner, R. M., Freshwater, D. W., & Whitfield, P. E. (2007). Mitochondrial cytochrome *b* analysis reveals two invasive lionfish species with strong founder effects in the western Atlantic. *Journal of Fish Biology*, 71, 214–222. <https://doi.org/10.1111/j.1095-8649.2007.01575.x>
- Henderson, E. B. (2012). *Economic and Ecological Implications of Interactions Between Lobsters and Invasive Lionfish in the Bahamas*. [Master's thesis, Simon Fraser University]. SFU Library Catalogue.

- Iglésias, S., & Frotté, L. (2015). Alien marine fishes in Cyprus: Update and new records. *Aquatic Invasions*, 10(4), 425–438.  
<https://doi.org/10.3391/ai.2015.10.4.06>
- Johnston, M. W., & Purkis, S. J. (2014). Are lionfish set for a Mediterranean invasion? Modelling explains why this is unlikely to occur. *Marine Pollution Bulletin*, 88(1–2), 138–147. <https://doi.org/10.1016/j.marpolbul.2014.09.013>
- Katsanevakis, S., Coll, M., Piroddi, C., Steenbeek, J., Ben Rais Lasram, F., Zenetos, A., & Cardoso, A. C. (2014). Invading the Mediterranean Sea: Biodiversity patterns shaped by human activities. *Frontiers in Marine Science*, 1. <https://doi.org/10.3389/fmars.2014.00032>
- Kleitou, P., Moutopoulos, D. K., Giovos, I., Kletou, D., Savva, I., Cai, L. L., Hall-Spencer, J. M., Charitou, A., Elia, M., Katselis, G., & Rees, S. (2022). Conflicting interests and growing importance of non-indigenous species in commercial and recreational fisheries of the Mediterranean Sea. *Fisheries Management and Ecology*, 29(2), 169–182.  
<https://doi.org/10.1111/fme.12531>
- Kleitou, P., Rees, S., Cecconi, F., Kletou, D., Savva, I., Cai, L. L., & Hall-Spencer, J. M. (2021). Regular monitoring and targeted removals can control lionfish in Mediterranean Marine Protected Areas. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 31(10), 2870–2882.  
<https://doi.org/10.1002/aqc.3669>
- Kletou, D., Hall-Spencer, J. M., & Kleitou, P. (2016). A lionfish (*Pterois miles*) invasion has begun in the Mediterranean Sea. *Marine Biodiversity Records*, 9(1), 46.  
<https://doi.org/10.1186/s41200-016-0065-y>
- Kulbicki, M., Beets, J., Chabanet, P., Cure, K., Darling, E., Floeter, S., Galzin, R., Green, A., Harmelin-Vivien, M., Hixon, M., Letourneur, Y., De Loma, T.,

- McClanahan, T., McIlwain, J., MouTham, G., Myers, R., O’Leary, J., Planes, S., Vigliola, L., & Wantiez, L. (2012). Distributions of Indo-Pacific lionfishes *Pterois* spp. in their native ranges: Implications for the Atlantic invasion. *Marine Ecology Progress Series*, 446, 189–205. <https://doi.org/10.3354/meps09442>
- López-Gómez, M. J., Aguilar-Perera, A., & Perera-Chan, L. (2014). Mayan divers-fishers as citizen scientists: Detection and monitoring of the invasive red lionfish in the Parque Nacional Arrecife Alacranes, southern Gulf of Mexico. *Biological Invasions*, 16(7), 1351–1357. <https://doi.org/10.1007/s10530-013-0582-0>
- Lyons, T. J., Tuckett, Q. M., & Hill, J. E. (2019). Characterizing the US trade in lionfishes. *PLOS ONE*, 14(8), e0221272. <https://doi.org/10.1371/journal.pone.0221272>
- Maljković, A., Van Leeuwen, T. E., & Cove, S. N. (2008). Predation on the invasive red lionfish, *Pterois volitans* (Pisces: Scorpaenidae), by native groupers in the Bahamas. *Coral Reefs*, 27(3), 501–501. <https://doi.org/10.1007/s00338-008-0372-9>
- McTee, S., & Grubich, J. (2014). Native densities, distribution, and diurnal activity of Red Sea lionfishes (Scorpaenidae). *Marine Ecology Progress Series*, 508, 223–232. <https://doi.org/10.3354/meps10847>
- Morris, J. A., & Akins, J. L. (2009). Feeding ecology of invasive lionfish (*Pterois volitans*) in the Bahamian archipelago. *Environmental Biology of Fishes*, 86(3), 389–398. <https://doi.org/10.1007/s10641-009-9538-8>

- Morris, Jr, J. A., Sullivan, C. V., & Govoni, J. J. (2011). Oogenesis and spawn formation in the invasive lionfish, *Pterois miles* and *Pterois volitans*. *Scientia Marina*, 75(1), 147–154. <https://doi.org/10.3989/scimar.2011.75n1147>
- Mouchlianitis, F. A., Kalaitzi, G., Kleitou, P., Savva, I., Kletou, D., & Gantias, K. (2022). Reproductive dynamics of the invasive lionfish ( *Pterois miles* ) in the Eastern Mediterranean Sea. *Journal of Fish Biology*, 100(2), 574–581. <https://doi.org/10.1111/jfb.14971>
- Oray, I. K., Sinay, E., Saadet Karakulak, F., & Yıldız, T. (2015). An expected marine alien fish caught at the coast of Northern Cyprus: *Pterois miles* (Bennett, 1828). *Journal of Applied Ichthyology*, 31(4), 733–735. <https://doi.org/10.1111/jai.12857>
- Paolucci, E. M., MacIsaac, H. J., & Ricciardi, A. (2013). Origin matters: Alien consumers inflict greater damage on prey populations than do native consumers. *Diversity and Distributions*, 19(8), 988–995. <https://doi.org/10.1111/ddi.12073>
- Phillips, E. W., Bottacini, D., Schoonhoven, A. N. M., Kamstra, Y. J. J., De Waele, H., Jimenez, C., Hadjioannou, L., & Kotrschal, A. (2024). Limited effects of culling on the behavior of invasive lionfish ( *PTEROIS MILES* ) in the Mediterranean. *Journal of Fish Biology*, 104(5), 1401–1410. <https://doi.org/10.1111/jfb.15686>
- Phillips, E. W., & Kotrschal, A. (2021). Where are they now? Tracking the Mediterranean lionfish invasion via local dive centers. *Journal of Environmental Management*, 298, 113354. <https://doi.org/10.1016/j.jenvman.2021.113354>
- Pinnegar, J. K. (2018). Why the damselfish *CHROMIS CHROMIS* is a key species in the Mediterranean rocky littoral – a quantitative perspective. *Journal of Fish Biology*, 92(3), 851–872. <https://doi.org/10.1111/jfb.13551>

- Pinnegar, J. K., & Polunin, N. V. C. (2006). Planktivorous damselfish support significant nitrogen and phosphorus fluxes to Mediterranean reefs. *Marine Biology*, 148(5), 1089–1099. <https://doi.org/10.1007/s00227-005-0141-z>
- Reaser, J. K., Meyerson, L. A., Cronk, Q., De Poorter, M., Eldrege, L. G., Green, E., Kairo, M., Latasi, P., Mack, R. N., Mauremootoo, J., O'Dowd, D., Orapa, W., Sastroutomo, S., Saunders, A., Shine, C., Thrainsson, S., & Vaiutu, L. (2007). Ecological and socioeconomic impacts of invasive alien species in island ecosystems. *Environmental Conservation*, 34(2), 98–111. <https://doi.org/10.1017/S0376892907003815>
- Resiere, D., Cerland, L., De Haro, L., Valentino, R., Criquet-Hayot, A., Chabartier, C., Kaidomar, S., Brouste, Y., Mégarbane, B., & Mehdaoui, H. (2016). Envenomation by the invasive *Pterois volitans* species (lionfish) in the French West Indies – a two-year prospective study in Martinique. *Clinical Toxicology*, 54(4), 313–318. <https://doi.org/10.3109/15563650.2016.1143100>
- Sala, E., Kizilkaya, Z., Yildirim, D., & Ballesteros, E. (2011). Alien Marine Fishes Deplete Algal Biomass in the Eastern Mediterranean. *PLoS ONE*, 6(2), e17356. <https://doi.org/10.1371/journal.pone.0017356>
- Savva, I., Chartosia, N., Antoniou, C., Kleitou, P., Georgiou, A., Stern, N., Hadjioannou, L., Jimenez, C., Andreou, V., Hall-Spencer, J. M., & Kletou, D. (2020). They are here to stay: The biology and ecology of lionfish ( *Pterois miles* ) in the Mediterranean Sea. *Journal of Fish Biology*, 97(1), 148–162. <https://doi.org/10.1111/jfb.14340>
- Schultz, E. T. (1986). *Pterois volitans* and *Pterois miles*: Two Valid Species. *Copeia*, 1986(3), 686. <https://doi.org/10.2307/1444950>
- Sih, A., Bolnick, D. I., Luttbeg, B., Orrock, J. L., Peacor, S. D., Pintor, L. M., Preisser, E., Rehage, J. S., & Vonesh, J. R. (2010). Predator–prey naïveté, antipredator

- behavior, and the ecology of predator invasions. *Oikos*, 119(4), 610–621.  
<https://doi.org/10.1111/j.1600-0706.2009.18039.x>
- Stachowicz, J. J., Whitlatch, R. B., & Osman, R. W. (1999). Species Diversity and Invasion Resistance in a Marine Ecosystem. *Science*, 286(5444), 1577–1579.  
<https://doi.org/10.1126/science.286.5444.1577>
- Torchin, M. E., Lafferty, K. D., & Kuris, A. M. (2002). Parasites and marine invasions. *Parasitology*, 124(7), 137–151. <https://doi.org/10.1017/S0031182002001506>
- Turan, C., Ergüden, D., Gürlek, M., Yağlıoğlu, D., Uyan, A., & Uygur, N. (2014). First record of the Indo-Pacific lionfish *Pterois miles* (Bennett, 1828) (*Osteichthyes: Scorpaenidae*) for the Turkish marine waters. *Journal of Black Sea Mediterranean Environment*, 20(2), 158-163. <https://izlik.org/JA77KC22PC>
- Tuttle, L. J., Sikkel, P. C., Cure, K., & Hixon, M. A. (2017). Parasite-mediated enemy release and low biotic resistance may facilitate invasion of Atlantic coral reefs by Pacific red lionfish (*Pterois volitans*). *Biological Invasions*, 19(2), 563–575.  
<https://doi.org/10.1007/s10530-016-1342-8>
- Ulman, A., Harris, H. E., Doumpas, N., Deniz Akbora, H., Al Mabruk, S. A. A., Azzurro, E., Bariche, M., Çiçek, B. A., Deidun, A., Demirel, N., Fogg, A. Q., Katsavenakis, S., Kletou, D., Kleitou, P., Papadopoulou, A., Ben Souissi, J., Hall-Spencer, J. M., Tiralongo, F., & Yildiz, T. (2021). Low Pufferfish and Lionfish Predation in Their Native and Invaded Ranges Suggests Human Control Mechanisms May Be Necessary to Control Their Mediterranean Abundances. *Frontiers in Marine Science*, 8, 670413.  
<https://doi.org/10.3389/fmars.2021.670413>
- Van Leeuwen, J. L., & Muller, M. (1984). Optimum sucking techniques for predatory fish. *The Transactions of the Zoological Society of London*, 37(2), 137–169.  
<https://doi.org/10.1111/j.1096-3642.1984.tb00069.x>

Yanar, A., Turan, C., & Dođdu, S. A. (2022). Report of *Nerocila bivittata* (Risso, 1816) (*Isopoda: Cymothoidae*) Parasitic on Alien Fish, *Pterois miles* (Bennett, 1828) from the Aegean and Mediterranean Sea. *Natural and Engineering Sciences*, 7(2), 169–181. <https://doi.org/10.28978/nesciences.1159261>

# Appendices

## Appendix 1

Table 3: Summary of generalised linear model, where probability of lionfish to exhibit hunting behaviour was the response variable and survey times are the predictor variables.

| Coefficients              | Estimate | Std. Error | <i>t</i> -value | <i>p</i> -value |
|---------------------------|----------|------------|-----------------|-----------------|
| <b>Intercept</b>          | -0,2513  | 0,5040     | -0,499          | 0,618           |
| <b>Survey time B</b>      | -2,5213  | 1,1474     | -2,197          | 0,028           |
| <b>Survey time C</b>      | -1,1350  | 0,9375     | -1,211          | 0,226           |
| <b>Survey time D</b>      | -1,2891  | 0,8116     | -1,588          | 0,112           |
| <b>Residual deviance</b>  | 55,388   |            |                 |                 |
| <b>Degrees of freedom</b> | 56       |            |                 |                 |

## Appendix 2

Table 4: Variance inflation factors of the maximal model with binomial distribution, where the response variable is probability of lionfish to exhibit hunting behaviour and the predictor variables are survey time, total length (cm), current, visibility(m), depth (m), weather, sea state, pedestrians, water temperature (°C).

| Variable          | Vif      |
|-------------------|----------|
| Survey time       | 1,780420 |
| Total length      | 1,095140 |
| Current           | 1,016618 |
| Visibility        | 2,173984 |
| Depth             | 1,930577 |
| Weather           | 1,73984  |
| Sea state         | 3,023603 |
| Pedestrians       | 1,366910 |
| Water temperature | 2,848942 |

## Appendix 3

Table 5: Summary of generalised linear model, where probability of lionfish to be out of hiding was the response variable and survey times are the predictor variables.

| Coefficients       | Estimate | Std. Error | t-value | p-value |
|--------------------|----------|------------|---------|---------|
| Intercept          | -1,0986  | 0,5774     | -1,903  | 0,0571  |
| Survey time B      | 1,9741   | 0,7853     | 2,514   | 0,0119  |
| Survey time C      | -0,2877  | 0,9789     | -0,294  | 0,7689  |
| Survey time D      | -1,6740  | 1,1814     | -1,417  | 0,1565  |
| Residual deviance  | 56,206   |            |         |         |
| Degrees of freedom | 56       |            |         |         |

## Appendix 4

Table 6: Variance inflection factors of the maximal model with binomial distribution, where the response variable is probability of lionfish to be out of hiding and the predictor variables are survey time, total length (cm), current, visibility(m), depth (m), weather, sea state, pedestrians, water temperature (°C).

| Variable          | Vif      |
|-------------------|----------|
| Survey time       | 1,583157 |
| Total length      | 1,312220 |
| Current           | 1,276187 |
| Visibility        | 2,306745 |
| Depth             | 1,756586 |
| Weather           | 1,424452 |
| Sea state         | 1,939904 |
| Pedestrians       | 1,694654 |
| Water temperature | 2,058757 |