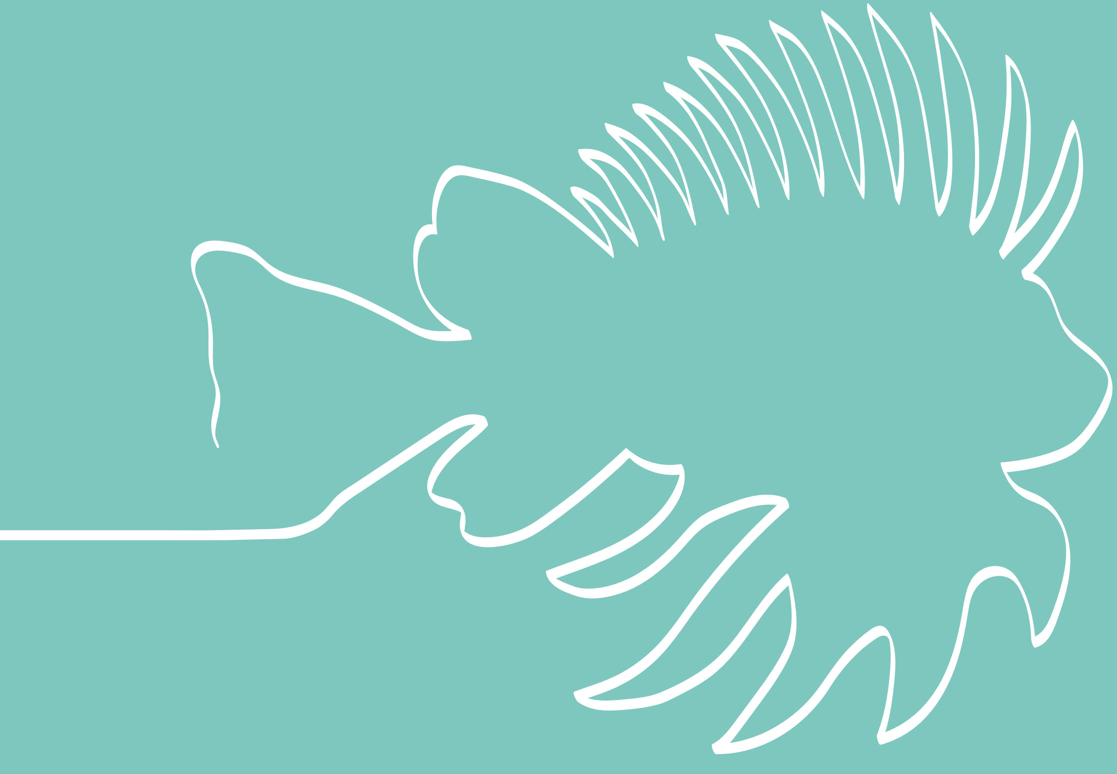
What Makes Lionfish Succesful Invaders?

Exploring Lionfish Behavior and Cognition



Elizabeth Phillips

Propositions

1. Invasive Lionfish are a biodiversity threat in the Mediterranean environment. (this thesis)
2. Future research must continue to investigate invasive species' cognitive abilities in order to discover what makes successful invaders.
(this thesis)
3. Scientists must modernize how they communicate their findings to the general public.
4. Academics need to be more welcoming and forgiving of new researchers that are still learning how to become experts in their field.
5. Productive work can only be achieved with a proper work-life balance.
6. In one's career, it's more valuable to be a jack-of-all-trades, rather than an expert on one topic.

Propositions belonging to the thesis, entitled

What Makes Lionfish Successful Invaders? Exploring Lionfish Behavior and Cognition

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Wageningen, 7 May 2024

What Makes Lionfish Successful Invaders?

Exploring Lionfish Behavior and Cognition

Elizabeth Phillips

Thesis Committee

Promotor
Prof. Dr Marc Naguib
Professor of Behavioural Ecology
Wageningen University & Research

Co-promotor
Dr Alexander Kotrschal
Assistant Professor, Behavioural Ecology Group
Wageningen University & Research

Other members
Prof. Dr Florian Muijres, Wageningen Unviersity & Research
Dr Josefin Sundin, Swedish University of Agricultural Sciences, Uppsala, Sweden
Dr Marion Nicolaus, University of Groningen
Dr James Herbert-Read, University of Cambridge, United Kingdom

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Wageningen Institute of Animal Sciences

What Makes Lionfish Successful Invaders?

Exploring Lionfish Behavior and Cognition

Elizabeth Phillips

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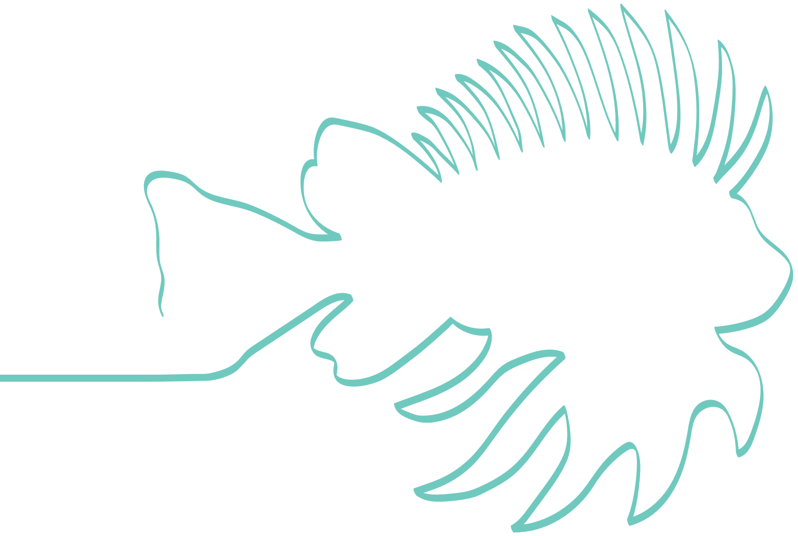
Chapter 1

General Introduction



Worldwide, introduced and invasive species are on the rise, due to increased human activity (Vitousek et al., 1997). This has become a concern for society and many environment management agencies as invasive species can cause harm to native ecosystems or result in loss of biodiversity in natural habitats (Pimentel et al., 2000; Vitousek et al., 1997). Therefore, one of the most commonly asked questions in research on species invasions is: what makes a species invasive? Understanding what factors lead to invasions and in general what makes a species a successful invader is key to understanding how to protect native ecosystems from the harmful effects of invasions, and thus remains of high concern in studies of invasive ecology (Kolar & Lodge, 2001; Mack et al., 2000). First though, I discuss what an invasion is and the known consequences of current invasions.

A Brief History of Invasive Ecology - Natural vs. Human-Caused Introductions

In the 1950s, invasive ecology emerged as a new field of research focused on understanding the impacts and causes of invasions by non-native plants and animals. While research on succession ecology and natural colonizations had been well-established, invasions were considered a unique phenomenon different from natural occurrences due to humans being the main cause of invasions, (J. H. Brown & Sax, 2004; Davis et al., 2001; Elton, 2020; Vitousek et al., 1997). Recently however, researchers have argued to close that gap between traditional ecology and invasive ecology. These researchers argue that, practically, natural colonizations and invasions share many of the same mechanisms during introduction to non-native habitats, so research on these mechanisms should not be impacted by whether the invaders are naturally spreading or introduced by humans (J. H. Brown & Sax, 2004; Davis et al., 2001; Hoffmann & Courchamp, 2016; Thompson et al., 1995). Furthermore, these proponents argue that this dichotomy between natural and human-influenced invasions in fact hurts advancement in the field of invasive ecology, as much of the insights and knowledge gained through traditional ecology is not built upon, resulting in invasive ecologists having to research and establish already known principles and theories (Catford et al., 2009; Davis et al., 2001; Hoffmann & Courchamp, 2016).

However, from a policy and management perspective, differences between natural colonizations and human-caused invasions might have a substantial impact (Cassey et al., 2005a; Hoffmann & Courchamp, 2016). While the mechanisms for dispersal and introduction might be the same between natural colonizations and human-influenced invasions, the frequency, speed, and scale of introductions can make human-influenced invasions more likely to succeed and therefore a larger threat to native ecosystems (Hoffmann & Courchamp, 2016). For example, the invasive lizard species (Anolis sagrei) is native to the Caribbean but has reached new environments worldwide, including just north in Florida, USA (Kolbe et al., 2004). While their original introduction during the late 1800's to the southern part of Florida remained stable and grew little from its initial introduction point, starting in the 1940's this invasive lizard has shown rapid expansion and started to invade further north, expanding now through almost the entire state of Florida. This more recent expansion is thought to be due in part to an increased number of unintentional introduction events during the 1940's via cargo ship and pet releases (Kolbe et al., 2004; Lee, 1985). This is not always true of human-caused invasions, however, as zebra mussels (Dreissena polymorpha) in contrast are an invasive species in North America that show that even after only one introduction event, it is possible to for invasive species to expand quickly and cause significant damage to the native environment (L. E. Johnson & Padilla, 1996; May et al., 2006; Vitousek et al., 1997).

Additionally, it is argued that the range of dispersal is greatly increased by humans (Cassey et al., 2005a). For example, species of pine trees are almost exclusively native to the Northern hemisphere but were introduced into parts of Southern hemisphere for economic and restoration reasons (Buckley et al., 2005; Richardson et al., 1994). However, after these introductions, pine species have shown rapid growth in the last 100 years to areas outside the original introduction zone, threatening natural grasslands and shrublands, areas pine species would most likely have never reached and spread into on their own. Similarly, in Antarctica, most of the current biodiversity has been introduced in the last two centuries, most likely caused by human activity on the previously isolated continent (Frenot et al., 2005). Therefore, having a clear distinction between human-mediated invasions and natural colonizations in research could be helpful in identifying potentially problematic species expansion and focusing research on how to prevent and manage these species.

Impacts of Invasions on Native Ecosystems

A question that commonly arises when talking about invasive species is: are invasive species harmful in the first place? While out of control invasions can outcompete native species and create imbalance in these ecosystems, many invasions start as a deliberate introduction by humans to help improve the native ecosystem. Similar to the case of pine species introduction to the Southern hemisphere, another plant species of lupin (Lupinus nootkatensis) was introduced to Iceland in 1945 to aid in revegetation of the land that had been damaged by centuries of over farming. This Alaska lupin was specifically chosen for its ability to thrive in similarly barren environments in Alaska, and for its ability to add more nitrogen to the ground that native Icelandic plants could use to regrow and recolonize the area (Benediktsson, 2015; Kuprian, 2018).

Species have not only been introduced for environmental, but also for economic reasons. For example, in the agriculture industry, damage caused by invasive pests are often controlled by introducing predators from their original native range in order to naturally balance invasive predators and prey in the new ecosystem (Messing & Wright, 2006). Hawaii has successfully used this biological control of invasive species throughout its agricultural history, with over 200 predatory species successfully introduced between 1890 and 1985 to control invasive pests (Funasaki et al., 1988). Even as far back as colonial times, European colonists would import familiar farm animals and plants from their home country to the New World, such as cattle to South America and apple trees to North America, to use as food and income sources there (Dolan, 2009; McTavish et al., 2013; Rouse, 1977).

However, invasive species can also cause damage to native ecosystem if left unchecked once they have been introduced. For instance, when pet owners release unwanted pets into non-native environments, many of these species are able to successfully survive and establish populations in the new environment (Lockwood et al., 2019). This is the case with many aquatic species of fish, such as the goldfish (Carassius auratus) that is now one of the most widespread invasive species around the world (Global Invasive Species Database, 2023), and even larger reptiles, such as the Burmese python (Python bivittatus), that has become an invasive species in areas such as the Everglades National Park in the Southeastern United States after to unregulated pet releases (Willson et al., 2011). Invasive pet releases can cause profound negative impacts on native species in the area for a number of reasons. First, invaders can become new predators to the area, like the Burmese python, and consume an large number of prey species due to “prey naivety”, or the tendency for native prey species to not recognize a new invasive predator when first introduced, resulting higher predation success by the invader (Dorcas et al., 2012; Dove et al., 2011). Alternatively, invasive species introduced via pet release can also generally impact native community structures by creating imbalance in the ecosystem, like what happened after an aquarium release of guppies (Poecilia reticulata) in Brazil (Cunico et al., 2009; Patoka et al., 2018).

Humans also move species unintentionally, meaning that some invasive species are able to establish and make an impact on native ecosystems before humans ever realize or have a chance to control the invading population. A fairly common occurrence of this is when cargo ships unintentionally take up larvae or adults of an aquatic species as part of the ballast water and transport the species to a new location along its trade route. This is how round gobies (Neogobius melanostomus) became invasive in the Great Lakes in North America (Kornis et al., 2012). This species has caused numerous damaging consequences to the regions where it is now invasive, including contributing to the local extinction of a native species of mottled sculpins via predation (Janssen & Jude, 2001), as well as outcompeting native fish in the Great Lakes, leading to their decline (Balshine et al., 2005).

Even species that were introduced with the intention to benefit native ecosystems may often become a nuisance in their new environment. One such case is that of the cane toad (Rhinella marina) that was introduced to Australia in 1935 as a form of biocontrol for agricultural pests, but has instead lead to declines in native predator populations, predicted to be due to them predating the toxic cane toad (Beckmann & Shine, 2009; B. L. Phillips et al., 2007). Similarly, while many of the species introduced to Hawaii as a means of biocontrol are successful in predating the target invasive pest, almost 14% of the introduced predators end up also or exclusively attacking native species, half of which are beneficial to Hawaii’s native ecosystem (Funasaki et al., 1988).

With this mix of consequences invasive species have on native environments, it can become unclear when a species goes from a neutral “introduced” species to a problematic “invasive” one. The debate on when a species can be considered invasive is ongoing in research today, with no clear consensus on a definition yet. Many factors fuel this discussion, such as researchers across fields using variations of the word "invasive" such as "alien", "exotic", or "introduced" without clarifying which criteria they used to define each of these terms (Davis & Thompson, 2000; Pereyra, 2016). Even when terms are defined, however, some papers will use definitions that emphasize the negative impact a species must have to be considered invasive (NOAA, 2020), others define any invader as a species that is non-native, regardless of its consequences on the native environment (Crowder & Snyder, 2010; Kolar & Lodge, 2001; Richardson et al., 2000), and even others define invasive species as those introduced by humans, regardless of their consequences, leading to even more debates in the field on what even is an invasive species (J. H. Brown & Sax, 2004; Cassey et al., 2005a).

Things get even more complicated when we try to define introduced species that have both positive and negative consequences on native environments. For example, previously mentioned was the Alaska lupin (Lupinus nootkatensis), a species of lupin introduced to Iceland in an effort to revegetate the land and help native plants thrive again. At the start of its introduction, the Alaska lupin was celebrated for its ability to enrich soil and aid native plants in reforesting Iceland, and even for its beauty on the Icelandic landscape (Benediktsson, 2015; Kuprian, 2018; Mossy Earth, 2023). However, as the lupin has continued to spread around the island, worry has grown about on if it will outcompete other native vegetation, changing the public's opinion on this alien species with calls to control and even eradicate the lupin completely. In this case, it depends on who you ask if lupin is a harmful invasive species or a beneficial introduced one, depending on how they might view lupin's impacts on the Icelandic environment.

While the field is still in debate, I turn my attention to another relevant question in invasive ecology: How do species become invasive? And what makes one species more invasive than another?

Factors that Contribute to Invasive Success

Research is ongoing into the factors and characteristics that make a species more or less likely to succeed as an invader. While the answer may seem simple, research has

found contradictory results, with little consensus across species about what makes a good invader. One of the reasons that's been proposed is the complex invasion pathway species must take in order to become established in a new environment. To become invasive, species have to survive a series of unique invasion stages, generally considered to be 1) transport, 2) introduction, 3) establishment, and 4) spread (Blackburn et al., 2011, 2015). Each stage of the invasive process contains unique challenges that species must overcome, and various stages may even select for different or contradicting traits (Blackburn et al., 2011; Chapple et al., 2012; Williamson, 2006). Thus, often invasive individuals have to strike a perfect balance of traits in order to successfully invade and establish in a new environment, leading to relatively low rates of successful invasions (Williamson, 2006).

In addition, species must possess traits that also are beneficial to the new specific environments they are invading, meaning that since successful characteristics of invasive species are often environment-dependent, many researchers struggle to link more general attributes of invaders to invasive success (Lodge, 1993; Thompson et al., 1995). For example, climate or habitat match between the native and invasive environment of an invasive species has been found to be one of the strongest predictors of if the invader will be successful (Hayes & Barry, 2008; Moyle & Marchetti, 2006). However, some environmental features have been found to make a native community more likely to be invaded, regardless of the specific environmental conditions. For instance, if the native community is relatively isolated and less species-dense, they are predicted to be more likely to be successfully invaded than more dense native communities, since invaders can more easily find and occupy unfilled niches in the existing community structure (Lodge, 1993; Vermeij, 1991). Similarly, communities that have been more recently disturbed are predicted to be more likely to be invaded, though it remains unclear if this is always the case (Lodge, 1993; Niemelä & Spence, 1991).

Next, I further discuss general traits that have been proposed to be related to invasive success in invasive species, starting with life history characteristics, moving then to how behavior and cognition may also play a role.

Life History Traits

Much of the early research into characteristics related to species' invasion potential were on life history traits, as a successful invasion hinges on a species' ability to not only survive but reproduce in the new environment (Sol & Maspons, 2016). For instance, in a study conducted on invasive and native fish species found in the Iberian Peninsula, fecundity and age at maturity were found to be the most likely traits that determine invasive ability (Vila-Gispert et al., 2005). Similarly, in invasive plants, individuals found in invasive ranges were found to grow larger, and therefore have greater fecundity than those found in the specie's native range (Jelbert et al., 2015). Indeed, size and growth rate are thought to have an impact on invasive potential, as many invasive species are shown to grow larger and faster than their native counter parts (Burns, 2006; Grotkopp et al., 2002; Jelbert et al., 2015). However, this is not always the case: while in some cases it might be beneficial for an invasive species possess fast life history characteristics that allow for quick population expansion once introduced, many other cases show that prioritizing future reproductive events over current ones can also be advantageous as it can lead to increased resist to population crashes during establishment (Allen et al., 2017; Sol et al., 2012).

Dispersal ability has also been proposed as an important factor in invasive success, as the ability to spread quickly and establish larger or multiple populations could defend against the negative consequences of potential population bottle necks. In pine species, the most successful invasive species were found to have traits associated with longer dispersal distances (Richardson et al., 1994), while in invasive butterflies and crickets, it has been shown that wing size, or the number of individuals possessing wings, is increased in populations on the invasion front compared to those in non-expanding ranges (Hughes et al., 2007; Simmons & Thomas, 2004). Similarly, round gobies (Neogobius melanostomus) on the invasive front were shown to have higher dispersal potential than those in non-expanding regions (Myles-Gonzalez et al., 2015a).

Sensory Systems

The sensory systems of invading species has been less well-studied in the field of invasion ecology, but still remains a potential factor in species' invasive success. For example, in aquatic environments, habitat degradation can result in decreased visual ability that can negatively affect species that reside in these environments (Casatti et al., 2006; Pratchett et al., 2011). However, it has been proposed that in freshwater fish species, invaders may be less likely to rely on visual hunting strategies than other species, increasing their ability to adapt to degradation in habitats and ultimately increase their invasive success (Abrahams et al., 2017; Rowe, 2007). Indeed, in one study, invasive Western Mosquitofish (Gambusia affinis) showed higher success in feeding in turbid water conditions when compared to the native Inanga (Galaxias maculatus). Two other studies have also investigated the sensory systems of arthropod invaders (Letoglossus spp. and Harmonia axyridis) and proposed that their sensory capabilities may be linked to their invasive success (Sevarika et al., 2021; Taszakowski et al., 2023). Outside of these studies, however, little attempts have been made to connect sensory ecology of species to invasive success.

Behavior

Investigations into the behavioral traits associated with invasion success has become a growing area of research in invasive ecology. Changes in behavior can occur rapidly, while changes in life history traits typically occur more slowly and often take several generations to appear in a population. Hence, adapting individual behavior in response to the novel environment during an invasion is a fast way of increasing the likelihood that populations can survive and establish themselves. For example, being an ecological generalist is predicted to improve invasive success, as these species are better able to adapt to new environments and challenges at the initial stages of introduction (Romanuk et al., 2009). Indeed, high tolerances to ecological conditions, such as temperature or salinity, has been found to be an important factor in predicting a species' invasive success (Côté & Reynolds, 2002; Moyle & Marchetti, 2006). More generally, global climate change has been shown to impact species invasive success by selecting for certain species that are more adaptable to the variable environments (Bellard et al., 2013; Dukes & Mooney, 1999; Finch et al., 2021; Root et al., 2005). Diet generalists in particular have been well studied and tend to have higher invasive success, as well as predators that are rarely predated on themselves (Crowder & Snyder, 2010; Tonella et al., 2018).

Recent studies have also started to investigate how personality traits of individuals in a species may influence invasive success. For instance, in the study examining dispersal potential in round gobies, they also found that bolder personalities were found in individuals from actively-expanding regions in invasive populations compared to non-expanding ones (Myles-Gonzalez et al., 2015a) In addition, common myna (Acridotheres tristis) on invasive fronts have been shown to have decreased neophobia when compared to individuals from noninvasive or native ranges (Cohen et al., 2020). Differences in exploration, activity, and boldness were found in populations of an invasive lizard (Lampropholis delicata) at various stages of invasion, suggesting that the invasion process can actually select for certain personality traits (Chapple et al., 2022). However, others have found other prominent invaders, such as the Trinidadian guppy (Poecilia reticulata), show similar personalities regardless of if they are on the invasive front or in source populations, mostly likely due to heavy fluctuations they experience even in their native environment (Lukas et al., 2021).

Cognition and Behavioral Flexibility

Cognition, or the perception, processing, storage, and retrieval of information, is thought to determine how animals behave in novel environments, so is hypothesized to impact invasive success (Shettleworth, 2010; Szabo et al., 2020). While not much is known yet, a few studies have started to investigate the link between cognition and invasion success.

Behavioral flexibility has been implicated as an important factor in invasive success, and some studies have started to investigate this link in a range of species, including birds, mammals, and reptiles (Wright et al., 2010). In all three of these taxa, brain size has been correlated with invasion success, as species with increased brain size are hypothesized to have increased behavioral flexibility, leading to better survival in novel environments (Amiel et al., 2011; Sol et al., 2002, 2005, 2008; Sol & Lefebvre, 2000). Similarly, in the common myna, increased innovation has been found in invasive populations compared to native ones (Cohen et al., 2020).

Learning ability has also been studied in invasive species, as increased learning ability may allow invasive individuals to more rapidly learn and adapt to their new environment once introduced, leading to increased invasive success. For example, in one study, invasive green crabs (Carcinus maenas) were able to more quickly learn the location of a prey item than their native counterpart, the blue crab (Callinectes sapidus) (Roudez et al., 2008). However, in another common invader, the Australian delicate skink (Lamprohpolis delicata) no difference was found in learning ability between them and a closely-related non-invader, the common garden skink (Lampropholis guichenoti) when tested on their ability to learn the location of a food source in a Y-maze (Bezzina et al., 2014). However, as no evidence of learning was found in either of the two groups in this study, it still remains unclear how learning maybe linked to invasive success.

Overall, researchers have had difficulty in pinpointing general factors that impact invasive success. Instead, the most success has come from studying individual species to determine what makes that particular species successful in their invasive environment. While this may hinder our understanding of invasive species as a whole, and how we can

preventatively determine and prevent future invasions, this approach can help in developing invasive management strategies that target individual invaders, or areas that are vulnerable to invasions.

Study Species: Lionfish

The Indo-Pacific lionfish (Pterois spp.) is a marine invader native to the Indo-Pacific Ocean and the Red Sea. Lionfish (Pterois volitans) first became invasive in the Atlantic Ocean and Caribbean Sea in the 1980's after aquarium releases mostly likely originating from Florida, USA (Côté et al., 2013). More recently, lionfish from the Red Sea (Pterois miles) have also traversed the Suez Canal and are now considered invasive in the eastern Mediterranean (Azzurro et al., 2017). Lionfish invaders in both of these regions have the potential to cause considerable negative impact on native ecosystems in invasive ranges, due in part to the larger sizes and higher densities lionfish are found in within invasive ranges (Darling et al., 2011a; Green & Côté, 2009; McTee & Grubich, 2014; Savva et al., 2020). In addition, lionfish are voracious predators and have a broad diet, feeding on a wide variety of fish and crustaceans, leading to the decline of many native fish species (Albins, 2013; Eddy

***Figure 1.1** Image of lionfish (Pterois miles). Photo taken by Kiwi Dive Center.*

et al., 2016; Morris Jr. & Akins, 2009). Studies have found that lionfish can reduce the abundance of native prey fish species by as much as 2.5 times more than that of native predators in areas of the Caribbean, with some of these species being of commercial and ecological importance in the area, such as the bluehead wrasse (Thalassoma bifasciatum) and Atlantic creolefish (Paranthias furcifer) (Albins, 2013; Eddy et al., 2016). Similarly, lionfish also have the potential to outcompete other native fish for food and habitat. In one study, researchers found that one of the predators native to the Caribbean, the Nassau grouper (Epinephelus striatus), chose shelter locations more remote and further way from lionfish when put into an arena together, indicating lionfish can affect, in potentially disadvantageous ways, other predatory fish distributions in native ecosystems (Raymond et al., 2015).

Lionfish in general are regarded as one of the most successful marine invaders, and their high invasion rates are generally attributed to a few key life history and morphological characteristics of the species (Côté et al., 2013). Lionfish have venomous spines along their dorsal ventral sides, making them unattractive to predators, thus it is generally assumed the lionfish has no natural predators in either their invasive or native ranges (Bos et al., 2018; Galloway & Porter, 2019). Lionfish also are known for their high reproductive rate, as females can produce up to 40,000 eggs every 4 days during favorable conditions, meaning population growth after introduction can be rapid (Côté et al., 2013). In addition, lionfish also have a piscivorous generalist diet and tend to eat what is most available to them, while their unique morphology and distinctive hunting techniques also make them a novel predator to native fish in invasive regions and therefore native prey more susceptible to being successfully hunted by lionfish (Cure et al., 2012; D'Agostino et al., 2020; Morris Jr. & Akins, 2009; Peake et al., 2018; Rojas-Vélez et al., 2019; Sancho et al., 2018). While one study has investigated the influence of behavior on learning ability in lionfish, much about the cognition of lionfish remains largely unknown (Deroy et al., 2020).

Thesis Overview

In my thesis, I investigate how lionfish behavior and cognition may impact their invasive success, looking at a range of traits from species-level adaptations, to changes individuals can make to better adjust to novel environments.

In **Chapter 2** I first turn my attention towards the lionfish invasion in the Mediterranean and use a citizen science survey of dive centers to investigate the spread of lionfish in this area. By adding more sighting to the current published data available, we can gain a better understanding of the how lionfish have spread, where potentially active fronts may be, and how this may impact their invasive potential.

Next, I dive into species-level adaptations of lionfish that may impact invasive success and in **Chapter 3** I investigate the color vision capabilities of lionfish (Pterois volitans) using a combination of both behavioral and genetic methods. Though lionfish tend to be crepuscular hunters, they have been shown to hunt diurnally as well in invasive ranges (Côté & Maljković, 2010; Morris Jr. & Akins, 2009), thus having color vision may benefit lionfish, as it would allow them more flexibility in when they hunt. Following up on this, in **Chapter 4** I further explore lionfish vision and investigate the potential for UV detection in lionfish. UV vision is common in fish, but mostly in small prey fish as larger fish predators often do not possess this capability (Losey et al., 1999; Siebeck & Marshall, 2000). If a predatory

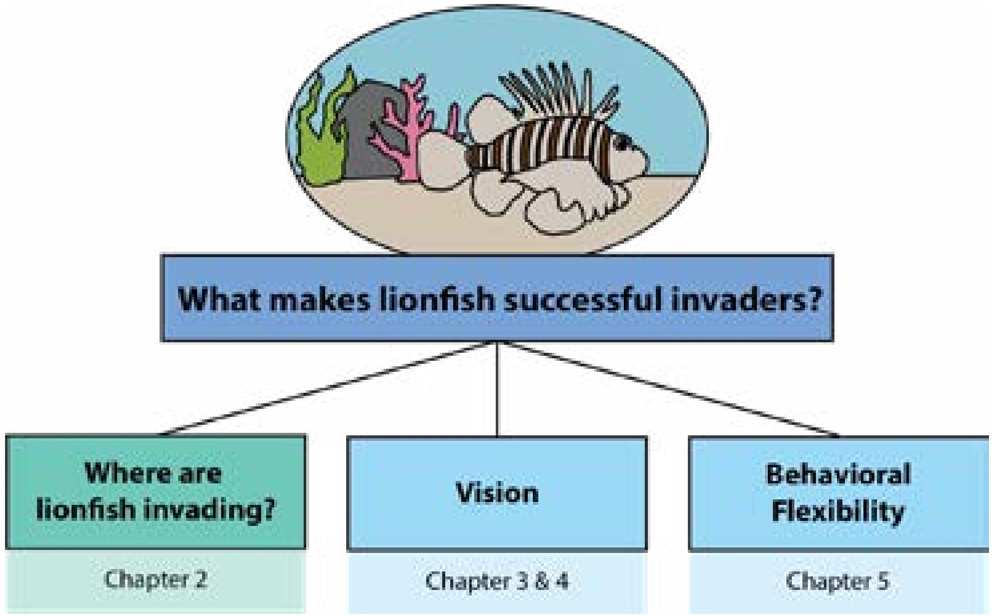


Figure 1.2 *Thesis Overview*

fish, such as the lionfish, could detect UV light like prey fish, it could potentially make detection of prey easier, as prey fish often use UV as a means of communication that is invisible to larger predators (Cummings et al., 2003; Losey, 2003; Siebeck, 2004). This might in turn increase invasive success of lionfish, through increased hunting ability as a new novel predator in native environments.

Finally, in **Chapter 5** I investigate the impact of individual behavior on invasive success, specifically focusing on the impact of long-term management efforts, such as repeated culling, on lionfish behavior in the Mediterranean. Lionfish in this region seem to show less long-term behavioral changes to culling than other fish species, and even compared to other invasive lionfish in the Caribbean (Beukema, 1970; Côté et al., 2014; Triki & Bshary, 2020). While this may partially be due to environmental differences, I argue this also represents lionfish's behavioral flexibility, a key trait predicted for invasive success.

In **Chapter 6**, I summarize my findings and discuss the implications my research will have on future research on lionfish invasive success and invasive ecology in general.

References

Abrahams, M. V., Bassett, D. K., & Montgomery, J. C. (2017). Sensory biology as a risk factor for invasion success and native fish decline. Transactions of the American Fisheries Society, 146(6), 1238–1244. https://doi.org/10.1080/00028487.2017.1353545

Albins, M. A. (2013). Effects of invasive Pacific red lionfish Pterois volitans versus a native predator on Bahamian coral-reef fish communities. Biological Invasions, 15(1), 29–43. https://doi.org/10.1007/s10530-012-0266-1

Allen, W. L., Street, S. E., & Capellini, I. (2017). Fast life history traits promote invasion success in amphibians and reptiles. Ecology Letters, 20, 222–230. https://doi.org/10.1111/ele.12728

Amiel, J. J., Tingley, R., & Shine, R. (2011). Smart moves: Effects of relative Brain size on Establishment success of invasive amphibians and reptiles. PLoS ONE, 6(4), e18277. https://doi.org/10.1371/journal.pone.0018277

Azzurro, E., Stancanelli, B., Di Martino, V., & Bariche, M. (2017). Range expansion of the common lionfish Pterois miles (Bennett, 1828) in the Mediterranean Sea: an unwanted new guest for Italian waters. BioInvasions Records, 6(2), 95–98. https://doi.org/10.3391/bir.2017.6.2.01

Balshine, S., Verma, A., Chant, V., & Theysmeyer, T. (2005). Competitive Interactions between Round Gobies and Logperch. Journal of Great Lakes, 31(1), 68–77.

Beckmann, C., & Shine, R. (2009). Impact of invasive cane toads on Australian birds. Conservation Biology, 23(6), 1544–1549. https://doi.org/10.1111/j.1523-1739.2009.01261.x

Bellard, C., Thuiller, W., Leroy, B., Genovesi, P., Bakkenes, M., & Courchamp, F. (2013). Will climate change promote future invasions? Global Change Biology, 19(12), 3740–3748. https://doi.org/10.1111/gcb.12344

Benediktsson, K. (2015). Floral hazards: Nootka lupin in Iceland and the Complex politics of invasive life. Geografiska Annaler, Series B: Human Geography, 97(2), 139–154. https://doi.org/10.1111/geob.12070

Beukema, J. J. (1970). Angling experiments with carp (Cyprinus carpio L). Decreasing catchability through one-trial learning. Netherlands Journal of Zoology, 20(1), 81–92. https://doi.org/doi.org/10.1163/002829670X00088

Bezzina, C. N., Amiel, J. J., & Shine, R. (2014). Does invasion success reflect superior cognitive ability? A case study of two congeneric lizard species (Lampropholis, Scincidae). PLoS ONE, 9(1), e86271. https://doi.org/10.1371/

journal.pone.0086271

Blackburn, T. M., Lockwood, J. L., & Cassey, P. (2015). The influence of numbers on invasion success. Molecular Ecology, 24(9), 1942–1953. https://doi.org/10.1111/mec.13075

Blackburn, T. M., Pyšek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarošík, V., Wilson, J. R. U., & Richardson, D. M. (2011). A proposed unified framework for biological invasions. Trends in Ecology and Evolution, 26(7), 333–339. https://doi.org/10.1016/j.tree.2011.03.023

Bos, A. R., Grubich, J. R., & Sanad, A. M. (2018). Growth, site fidelity, and grouper interactions of the Red Sea lionfish Pterois miles (Scorpaenidae) in its native habitat. Marine Biology, 165(10), 1–10. https://doi.org/10.1007/s00227-018-3436-6

Brown, J. H., & Sax, D. F. (2004). An Essay on Some Topics Concerning Invasive Species. Austral Ecology, 29, 530–536.

Buckley, Y. M., Brockerhoff, E., Langer, L., Ledgard, N., North, H., & Rees, M. (2005). Slowing down a pine invasion despite uncertainty in demography and dispersal. Journal of Applied Ecology, 42(6), 1020–1030. https://doi.org/10.1111/j.1365-2664.2005.01100.x

Burns, J. H. (2006). Relatedness and environment affect traits associated with invasive and noninvasive introduced commelinaceae. Ecological Applications, 16(4), 1367–1376. https://doi.org/10.1890/1051-0761(2006)016[1367:RAEATA]2.0.CO;2

Casatti, L., Langeani, F., & Ferreira, C. P. (2006). Effects of physical habitat degradation on the stream fish assemblage structure in a pasture region. Environmental Management, 38(6), 974–982. https://doi.org/10.1007/s00267-005-0212-4

Cassey, P., Blackburn, T. M., Duncan, R. P., & Chown, S. L. (2005). Concerning invasive species: Reply to Brown and Sax. Austral Ecology, 30(4), 475–480. https://doi.org/10.1111/j.1442-9993.2005.01505.x

Catford, J. A., Jansson, R., & Nilsson, C. (2009). Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Diversity and Distributions, 15, 22–40. https://doi.org/10.1111/j.1472-4642.2008.00521.x

Chapple, D. G., Naimo, A. C., Brand, J. A., Michelangeli, M., Martin, J. M., Goulet, C. T., Brunton, D. H., Sih, A., & Wong, B. B. M. (2022). Biological invasions as a selective filter driving behavioral divergence. Nature Communications, 13, 5996. https://doi.org/10.1038/s41467-022-33755-2

Chapple, D. G., Simmonds, S. M., & Wong, B. B. M. (2012). Can behavioral and personality traits influence the success of unintentional species introductions? Trends in Ecology and Evolution, 27(1), 57–64. https://doi.org/10.1016/j.tree.2011.09.010

Cohen, T. M., Kumar, R. S., Nair, M., Hauber, M. E., & Dor, R. (2020). Innovation and decreased neophobia drive invasion success in a widespread avian invader. Animal Behaviour, 163, 61–72. https://doi.org/10.1016/j.anbehav.2020.02.012

Côté, I. M., Darling, E. S., Malpica-Cruz, L., Smith, N. S., Green, S. J., Curtis-Quick, J., & Layman, C. (2014). What Doesn’t Kill You Makes You Wary? Effect of Repeated Culling on the Behaviour of an Invasive Predator. PLoS ONE, 9(4), e94248. https://doi.org/10.1371/journal.pone.0094248

Côté, I. M., Green, S. J., & Hixon, M. A. (2013). Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. Biological Conservation, 164, 50–61. https://doi.org/10.1016/j.biocon.2013.04.014

Côté, I. M., & Maljković, 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

Côté, I. M., & Reynolds, J. D. (2002). Conservation biology: Predictive ecology to the rescue? Science, 298, 1181–1182. https://doi.org/10.1126/science.1079074

Crowder, D. W., & Snyder, W. E. (2010). Eating their way to the top? Mechanisms underlying the success of invasive insect generalist predators. Biological Invasions, 12(9), 2857–2876. https://doi.org/10.1007/s10530-010-9733-8

Cummings, M. E., Rosenthal, G. G., & Ryan, M. J. (2003). A private ultraviolet channel in visual communication. Proc. R. Soc. Lond. B, 270, 897–904. https://doi.org/10.1098/rspb.2003.2334

Cunico, A. M., da Graça, W. J., Agostinho, A. A., Domingues, W. M., & Latini, J. D. (2009). Fish, Maringá Urban Streams, Pirapó river drainage, upper Paraná river basin, Paraná State, Brazil. Check List, 5(2), 273–280. http://www.nupelia.uem.br/colecao.

Cure, K., Benkwitt, C. E., Kindinger, T. L., Pickering, E. A., Pusack, T. J., McIlwain, J. L., & Hixon, M. A. (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. A. (2020). Behavioural traits and feeding ecology

Mediterranean lionfish and naiveté native species lionfish predation. Marine Ecology Progress Series, 638, 123–135.

Darling, E. S., Green, S. J., O'Leary, J. K., & Côté, I. M. (2011). Indo-Pacific lionfish are larger and more abundant on invaded reefs: A comparison of Kenyan and Bahamian lionfish populations. Biological Invasions, 13(9), 2045–2051. https://doi.org/10.1007/s10530-011-0020-0

Davis, M. A., & Thompson, K. (2000). Eight Ways to Be a Colonizer: Two Ways to Be an Invader: A Proposed Nomenclature Scheme for Invasion Ecology. Bulletin of the Ecological Society of America, 81(3), 226–230.

Davis, M. A., Thompson, K., & Philip Grime, J. (2001). Charles S. Elton and the dissociation of invasion ecology from the rest of ecology. Diversity and Distributions, 7, 97–102. https://doi.org/10.1046/j.1472-4642.2001.00099.x

Deroy, E. M., Hussey, N. E., & Macisaac, H. J. (2020). Behaviourally-mediated learning ability in an invasive marine fish. Biological Invasions, 0123456789. https://doi.org/10.1007/s10530-020-02329-y

Dolan, S. A. (2009). Fruitful Legacy: A Historic Context of Orchards in the United States, with Technical Information for Registering Orchards in the National Register of Historic Places. National Park Service, Olmsted Center for Landscape Preservation, Pacific West Regional Office - Cultural Resources, Park Historic Structures and Cultural Landscapes Program.

Dorcas, M. E., Willson, J. D., Reed, R. N., Snow, R. W., Rochford, M. R., Miller, M. A., Meshaka, W. E., Andreadis, P. T., Mazzotti, F. J., Romagosa, C. M., & Hart, K. M. (2012). Severe mammal declines coincide with proliferation of invasive Burmese pythons in Everglades National Park. Proceedings of the National Academy of Sciences of the United States of America, 109(7), 2418–2422. https://doi.org/10.1073/pnas.1115226109

Dove, C. J., Snow, R. W., Rochford, M. R., & Mazzotti3, F. J. (2011). Bird Consumed by the Invasive Burmese Python (Python molurus bivittatus) in Everglades National Park, Florida, USA. The Wilson Journal of Ornithology, 123(1), 126–131. https://about.jstor.org/terms

Dukes, J. S., & Mooney, H. A. (1999). Does global change increase the success of biological invaders? Trends in Ecology & Evolution, 14(4), 135–139.

Eddy, C., Pitt, J., Morris, J. A., Smith, S., Goodbody-Gringley, G., & Bernal, D. (2016). Diet of invasive lionfish (Pterois volitans and P. miles) in Bermuda. Marine Ecology Progress Series, 558, 193–206. https://doi.org/10.3354/meps11838

Elton, C. S. (2020). The Ecology of Invasions by Animals and Plants (D. Simberloff & A. Ricciardi, Eds.; Second). Springer International Publishing. https://doi.org/10.1007/978-3-030-34721-5

Finch, D. M., Butler, J. L., Runyon, J. B., Fettig, C. J., Kilkenny, F. F., Jose, S., Frankel, S. J., Cushman, S. A., Cobb, R. C., Dukes, J. S., Hicke, J. A., & Amelon, S. K. (2021). Effects of Climate Change on Invasive Species. In T. M. Poland, T. Patel-Weynand, D. M. Finch, C. F. Miniat, D. C. Hayes, & V. M. Lopez (Eds.), Invasive Species in Forests and Rangelands of the United States: A Comprehensive Science Synthesis for the United States Forest Sector (pp. 57–84). Springer Nature Switzerland AG.

Frenot, Y., Chown, S. L., Whinam, J., Selkirk, P. M., Convey, P., Skotnicki, M., & Bergstrom, D. M. (2005). Biological invasions in the Antarctic: Extent, impacts and implications. Biol. Rev., 80, 45–72. https://doi.org/10.1017/S1464793104006542

Funasaki, G. Y., Lai, P.-Y., Nakahara, L. M., Beardsley, J. W., & Ota, A. K. (1988). A Review of Biological Control Introductions in Hawaii: 1890 to 1985. Hawaiian Entomological Society Proceedings, 28, 105–160.

Galloway, K. A., & Porter, M. E. (2019). Mechanical properties of the venomous spines of Pterois volitans and morphology among lionfish species. Journal of Experimental Biology, 222(6), jeb197905. https://doi.org/10.1242/jeb.197905

Global Invasive Species Database. (2023). Species profile: Carassius auratus. http://www.iucngisd.org/gisd/species.php?sc=368

Green, S. J., & Côté, I. M. (2009). Record densities of Indo-Pacific lionfish on Bahamian coral reefs. Coral Reefs, 28(107). https://doi.org/10.1007/s00338-008-0446-8

Grotkopp, E., Rejmánek, M., & Rost, T. L. (2002). Toward a Causal Explanation of Plant Invasiveness: Seedling Growth and Life-History Strategies of 29 Pine (Pinus) Species. The American Naturalist, 159(4), 396–419.

Hayes, K. R., & Barry, S. C. (2008). Are there any consistent predictors of invasion success? Biological Invasions, 10(4), 483–506. https://doi.org/10.1007/s10530-007-9146-5

Hoffmann, B. D., & Courchamp, F. (2016). Biological invasions and natural colonisations: Are they that different? NeoBiota, 29, 1–14. https://doi.org/10.3897/neobiota.29.6959

Hughes, C. L., Dytham, C., & Hill, J. K. (2007). Modelling and analysing evolution of dispersal in populations at expanding range boundaries. Ecological Entomology, 32(5), 437–445. https://doi.org/10.1111/j.1365-2311.2007.00890.x

Janssen, J., & Jude, D. J. (2001). Recruitment failure of mottled sculpin Cottus bairdi in Calumet Harbor, southern Lake Michigan, induced by the newly introduced round goby Neogobius melanostomus. Journal of Great Lakes Research, 27(3), 319–328. https://doi.org/10.1016/S0380-1330(01)70647-8

Jelbert, K., Stott, I., Mcdonald, R. A., & Hodgson, D. (2015). Invasiveness of plants is predicted by size and fecundity in the native range. Ecology and Evolution, 5(10), 1933–1943. https://doi.org/10.1002/ece3.1432

Johnson, L. E., & Padilla, D. K. (1996). Geographic spread of exotic species: Ecologial lessons and opportunities from the invasion of the zebra mussel (Dreissena polymorpha). Biological Conservation, 78, 23–33.

Kolar, C. S., & Lodge, D. M. (2001). Progress in invasion biology: predicting invaders. Ecology & Evolution, 16(4), 199–-204. https://doi.org/10.1016/S0169-5347(01)02101-2

Kolbe, J. J., Glor, R. E., Schettino, L. R., Lara, A. C., Larson, A., & Losos, J. B. (2004). Genetic variation increases during biological invasion by a Cuban lizard. Nature, 431(7005), 177–181. https://doi.org/10.1038/nature02807

Kornis, M. S., Mercado-Silva, N., & vander Zanden, M. J. (2012). Twenty years of invasion: A review of round goby Neogobius melanostomus biology, spread and ecological implications. Journal of Fish Biology, 80, 235–285. https://doi.org/10.1111/j.1095-8649.2011.03157.x

Kuprian, A. (2018). Negotiating contested landscapes: The lupin controversy in Iceland. Northern Studies, 48, 25–37.

Lee, J. C. (1985). Anolis sagrei in Florida: Phenetics of a Colonizing Species I. Meristic Characters. Copeia, 1985(1), 182–194. https://www.jstor.org/stable/1444808

Lockwood, J. L., Welbourne, D. J., Romagosa, C. M., Cassey, P., Mandrak, N. E., Strecker, A., Leung, B., Stringham, O. C., Udell, B., Episcopio-Sturgeon, D. J., Tlusty, M. F., Sinclair, J., Springborn, M. R., Pienaar, E. F., Rhyne, A. L., & Keller, R. (2019). When pets become pests: the role of the exotic pet trade in producing invasive vertebrate animals. Frontiers in Ecology and the Environment, 17(6), 323–330. https://doi.org/10.1002/fee.2059

Lodge, D. M. (1993). Biological Invasions: Lessons for Ecology. Trends in Ecology & Evolution, 8(4), 133–137.

Losey, G. S. (2003). Crypsis and communication functions of UV-visible coloration in two coral reef damselfish, Dascyllus aruanus and D. reticulatus. Animal Behaviour, 66(2), 299–307. https://doi.org/10.1006/anbe.2003.2214

Losey, G. S., Cronin, T. W., Goldsmith, T. H., Hyde, D., Marshall, N. J., & McFarland, W. N. (1999). The UV visual world of fishes: A review. Journal of Fish Biology, 54(5), 921–943. https://doi.org/10.1006/jfbi.1998.0919

Lukas, J., Kalinkat, G., Miesen, F. W., Landgraf, T., Krause, J., & Bierbach, D. (2021). Consistent Behavioral Syndrome Across Seasons in an Invasive Freshwater Fish. Frontiers in Ecology and Evolution, 8, 583670. https://doi.org/10.3389/fevo.2020.583670

Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M., & Bazzaz, F. A. (2000). Biotic Invasions: Causes, Epidemiology, Global Consequences, and Control. Ecological Applications, 10(3), 689–710. https://doi.org/10.1890/0012-9623(2008)89[341:iie]2.0.co;2

May, G. E., Gelembiuk, G. W., Panov, V. E., Orlova, M. I., & Lee, C. E. (2006). Molecular ecology of zebra mussel invasions. Molecular Ecology, 15(4), 1021–1031. https://doi.org/10.1111/j.1365-294X.2006.02814.x

McTavish, E. J., Decker, J. E., Schnabel, R. D., Taylor, J. F., & Hillis, D. M. (2013). New World cattle show ancestry from multiple independent domestication events. PNAS, 110(15), E1398–E1406. https://doi.org/10.5061/dryad.42tr0

McTee, S. A., & Grubich, J. R. (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

Messing, R. H., & Wright, M. G. (2006). Biological control of invasive species: solution or pollution? Frontiers in Ecology and the Environment, 4(3), 132–140. www.frontiersinecology.org

Morris Jr., J. A., & Akins, J. L. (2009). Feeding ecology of invasive lionfish (Pterois volitans) in the Bahamian archipelago. Environmental Biology of Fishes, 86, 389–398. https://doi.org/10.1007/s10641-009-9538-8

Mossy Earth. (2023). Reforesting Iceland. https://www.mossy.earth/projects/reforesting-iceland

Moyle, P. B., & Marchetti, M. P. (2006). Predicting Invasion Success: Freshwater Fishes in California as a Model. BioScience, 56(6), 515–524. www.biosciencemag.org

Myles-Gonzalez, E., Burness, G., Yavno, S., Rooke, A., & Fox, M. G. (2015). To boldly go where no goby has gone before: Boldness, dispersal tendency, and metabolism at the invasion front. Behavioral Ecology, 26(4), 1083–1090. https://doi.org/10.1093/beheco/arv050

Niemelä, J., & Spence, J. R. (1991). Distribution and Abundance of an Exotic

Ground-Beetle (Carabidae): A Test of Community Impact. Oikos, 62(3), 351–359. https://www.jstor.org/stable/3545500

NOAA. (2020). What is an invasive species? National Ocean Service Website. https://oceanservice.noaa.gov/facts/invasive.html#:~:text=Invasive%20species%20are%20animals%20or,and%20most%20often%2C%20by%20people.

Patoka, J., Magalhães, A. L. B., Kouba, A., Faulkes, Z., Jerikho, R., & Vitule, J. R. S. (2018). Invasive aquatic pets: Failed policies increase risks of harmful invasions. Biodiversity and Conservation, 27, 3037–3046. https://doi.org/10.1007/s10531-018-1581-3

Peake, J., Bogdanoff, A. K., Layman, C. A., Castillo, B., Jennifer, K. R., Kristen, C., William, D., Iii, F. P., Eddy, C., Ellis, R. D., Faletti, M., Higgs, N., Juan, S., Johnston, M. A., Mun, R. C., & Jr, J. A. M. (2018). Feeding ecology of invasive lionfish (Pterois volitans and Pterois miles) in the temperate and tropical western Atlantic. Bio Invasions, 20, 2567–2597. https://doi.org/10.1007/s10530-018-1720-5

Pereyra, P. J. (2016). Revisiting the use of the invasive species concept: An empirical approach. Austral Ecology, 41, 519–528. https://doi.org/10.1111/aec.12340

Phillips, B. L., Brown, G. P., Greenlees, M., Webb, J. K., & Shine, R. (2007). Rapid expansion of the cane toad (Bufo marinus) invasion front in tropical Australia. Austral Ecology, 32(2), 169–176. https://doi.org/10.1111/j.1442-9993.2007.01664.x

Pimentel, D., Lach, L., Zuniga, R., & Morrison, D. (2000). Environmental and Economic Costs of Nonindigenous Species in the United States. BioScience, 50(1), 53–65.

Pratchett, M. S., Bay, L. K., Gehrke, P. C., Koehn, J. D., Osborne, K., Pressey, R. L., Sweatman, H. P. A., & Wachenfeld, D. (2011). Contribution of climate change to degradation and loss of critical fish habitats in Australian marine and freshwater environments. Marine and Freshwater Research, 62(9), 1062–1081. https://doi.org/10.1071/MF10303

Raymond, W. W., Albins, M. A., & Pusack, T. J. (2015). Competitive interactions for shelter between invasive Pacific red lionfish and native Nassau grouper. Environmental Biology of Fishes, 98(1), 57–65. https://doi.org/10.1007/s10641-014-0236-9

Richardson, D. M., Pyšek, P., Rejmánek, M., Barbour, M. G., Dane Panetta, F., & West, C. J. (2000). Naturalization and invasion of alien plants: Concepts and definitions. Diversity and Distributions, 6(2), 93–107. https://doi.org/10.1046/j.1472-4642.2000.00083.x

Richardson, D. M., Williams, P. A., & Hobbs, R. J. (1994). Pine Invasions in the Southern Hemisphere: Determinants of Spread and Invadability. Source: Journal of Biogeography, 21(5), 511–527.

Rojas-Vélez, S., Tavera, J., & Acero, A. (2019). Unraveling lionfish invasion: Is Pterois volitans truly a morphologically novel predator in the Caribbean? Biological Invasions, 21(6), 1921–1931. https://doi.org/10.1007/s10530-019-01946-6

Romanuk, T. N., Zhou, Y., Brose, U., Berlow, E. L., Williams, R. J., & Martinez, N. D. (2009). Predicting invasion success in complex ecological networks. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1524), 1743–1754. https://doi.org/10.1098/rstb.2008.0286

Root, T. L., Macmynowski, D. P., Mastrandrea, M. D., & Schneider, S. H. (2005). Human-modified temperatures induce species changes: Joint attribution. PNAS, 102(21), 7465–7469. www.pnas.orgcgidoi10.1073pnas.0502286102

Roudez, R. J., Glover, T., & Weis, J. S. (2008). Learning in an invasive and a native predatory crab. Biological Invasions, 10, 1191–1196. https://doi.org/10.1007/s10530-007-9195-9

Rouse, J. (1977). The Criollo: Spanish cattle in the Americas. University of Oklahoma Press.

Rowe, D. K. (2007). Exotic fish introductions and the decline of water clarity in small North Island, New Zealand lakes: A multi-species problem. Hydrobiologia, 583(1), 345–358. https://doi.org/10.1007/s10750-007-0646-1

Sancho, G., Kingsley-Smith, P. R., Morris, J. A., Toline, C. A., McDonough, V., & Doty, S. M. (2018). Invasive Lionfish (Pterois volitans/miles) feeding ecology in Biscayne National Park, Florida, USA. Biological Invasions, 20(9), 2343–2361. https://doi.org/10.1007/s10530-018-1705-4

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, April. https://doi.org/10.1111/jfb.14340

Sevarika, M., Rondoni, G., Conti, E., & Romani, R. (2021). Antennal sensory organs and glands of the harlequin ladybird, Harmonia axyridis. Entomologia Experimentalis et Applicata, 169(1), 111–124. https://doi.org/10.1111/

eea.12948

Shettleworth, S. J. (2010). Cognition, Evolution, and Behavior (Second Edi). Oxford University Press.

Siebeck, U. E. (2004). Communication in coral reef fish: the role of ultraviolet colour patterns in damselfish territorial behaviour. Animal Behaviour, 68, 273–282. https://doi.org/10.1016/j.anbehav.2003.11.010

Siebeck, U. E., & Marshall, N. J. (2000). Transmission of ocular media in labrid fishes. Philosophical Transactions of the Royal Society B: Biological Sciences, 355(1401), 1257–1261. https://doi.org/10.1098/rstb.2000.0679

Simmons, A. D., & Thomas, C. D. (2004). Changes in dispersal during species' range expansions. American Naturalist, 164(3), 378–395. https://doi.org/10.1086/423430

Sol, D., Bacher, S., Reader, S. M., & Lefebvre, L. (2008). Brain size predicts the success of mammal species introduced into novel environments. American Naturalist, 172, S63–S71. https://doi.org/10.1086/588304

Sol, D., Duncan, R. P., Blackburn, T. M., Cassey, P., & Lefebvre, L. (2005). Big brains, enhanced cognition, and response of birds to novel environments. Proceedings of the National Academy of Sciences of the United States of America, 102(15), 5460–5465. https://doi.org/10.1073/pnas.0408145102

Sol, D., & Lefebvre, L. (2000). Behavioural flexibility predicts invasion success in birds introduced to New Zealand. Oikos, 90(3), 599–605. https://doi.org/10.1034/j.1600-0706.2000.900317.x

Sol, D., & Maspons, J. (2016). Life History, Behaviour and Invasion Success. In J. S. Weis & D. Sol (Eds.), Biological Invasions and Animal Behaviour. Cambridge University Press.

Sol, D., Maspons, J., Vall-llosera, M., Bartomeus, I., García-Peña, G. E., Piñol, J., & Freckleton, R. P. (2012). Unraveling the life history of successful invaders. Science, 337(6094), 580–583. https://doi.org/10.1126/science.1221523

Sol, D., Timmermans, S., & Lefebvre, L. (2002). Behavioural flexibility and invasion success in birds. 2000, 495–502. https://doi.org/10.1006/anbe.2001.1953

Szabo, B., Damas-moreira, I., & Whiting, M. J. (2020). Can Cognitive Ability Give Invasive Species the Means to Succeed ? A Review of the Evidence. Frontiers in Ecology and Evolution, 8(187), 1–13. https://doi.org/10.3389/fevo.2020.00187

Taszakowski, A., Masłowski, A., Daane, K. M., & Brożek, J. (2023). Closer view of antennal sensory organs of two Leptoglossus species (Insecta, Hemiptera,

Coreidae). Scientific Reports, 13, 617. https://doi.org/10.1038/s41598-023-27837-4

Thompson, K., Hodgson, J. G., & Rich, T. C. G. (1995). Native and Alien Invasive Plants: More of the Same? 18(4), 390–402. https://about.jstor.org/terms

Tonella, L. H., Fugi, R., Vitorino, O. B., Suzuki, H. I., Gomes, L. C., & Agostinho, A. A. (2018). Importance of feeding strategies on the long-term success of fish invasions. Hydrobiologia, 817(1), 239–252. https://doi.org/10.1007/s10750-017-3404-z

Triki, Z., & Bshary, R. (2020). Long-term memory retention in a wild fish species Labroides dimidiatus eleven months after an aversive event. Ethology, 126, 372–376. https://doi.org/10.1111/eth.12978

Vermeij, G. J. (1991). When Biotas Meet: Understanding Biotic Interchange. Science, 253(5024), 1099–1104.

Vila-Gispert, A., Alcaraz, C., & García-Berthou, E. (2005). Life-history traits of invasive fish in small Mediterranean streams. Biological Invasions, 7(1), 107–116. https://doi.org/10.1007/s10530-004-9640-y

Vitousek, P. M., D, C. M., Loope, L. L., Rejmánek, M., & Westbrooks, R. (1997). Introduced Species: A Significant Component of Human-Caused Global Change. In Source: New Zealand Journal of Ecology (Vol. 21, Issue 1). https://www.jstor.org/stable/24054520

Williamson, M. (2006). Explaining and predicting the success of invading species at different stages of invasion. Biological Invasions, 8(7), 1561–1568. https://doi.org/10.1007/s10530-005-5849-7

Willson, J. D., Dorcas, M. E., & Snow, R. W. (2011). Identifying plausible scenarios for the establishment of invasive Burmese pythons (Python molurus) in Southern Florida. Biological Invasions, 13(7), 1493–1504. https://doi.org/10.1007/s10530-010-9908-3

Wright, T. F., Eberhard, J. R., Hobson, E. A., Avery, M. L., & Russello, M. A. (2010). Behavioral flexibility and species invasions: the adaptive flexibility hypothesis: Ethology Ecology and Evolution, 22(4), 393–404. https://doi.org/10.1080/03949370.2010.505580

Chapter 2

Where are they now?

Tracking the Mediterranean lionfish invasion via local dive centers

Elizabeth W. Phillips, Alexander Kotrschal
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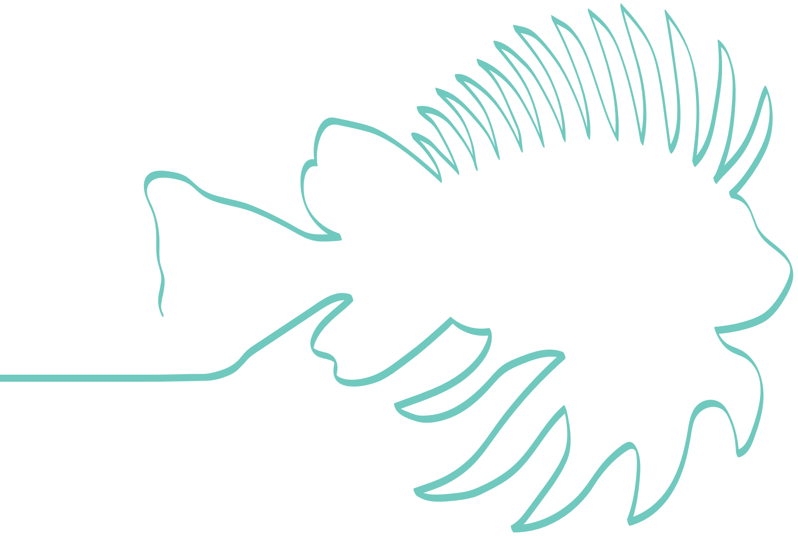


Abstract

Invasive species are globally on the rise due to human-induced environmental change and are often a source of harm to their new ecosystems. Tracking the spread of invaders is crucial to better manage invasive species, and citizen science is often used to collect sighting data. However, this can be unreliable due to the general public's limited expertise for accurate identification and a lack of clear absence data. Here, we introduce a refined method of citizen science by tracking the spread of the invasive lionfish (Pterois miles) in the Mediterranean Sea using dive centers' expertise on local marine wildlife. We contacted 1131 dive centers on the Mediterranean coast via email and received 216 responses reporting whether or not lionfish were present in their area and, if present, the year they were first sighted. Currently, lionfish sightings are observed in the eastern half of the Mediterranean, though the front is continuing to move west with the furthest sighting as far as Corfu, Greece (19.939423°E, 39.428017°N). In 2020, lionfish also expanded their invasive range north on the Turkish Aegean coast to Karaburun (26.520657°E, 38.637033°N), showing that the invasion is ongoing. We found that the invasive range is now exceeding previous invasion models, highlighting the need for additional research on lionfish biology to inform management efforts. Continuous monitoring of invasive fronts based on dive center reports and a better understanding of what makes lionfish so invasive is crucial to creating effective management strategies and mitigating their negative impact on native ecosystems.

Introduction

As humans continue to shape and modify their environment, animals often find themselves in novel situations to which they must react and adapt. One of these situations is when direct human transport or man-made constructions allow animals to move from one area to the other and become invasive. Globally, invasive species have been on the rise due to human-induced environmental change, and often cause ecological damage to the areas they invade (Cassey et al., 2005b; Simberloff et al., 2013). A crucial step to creating effective management strategies for invasive species is tracking their introduction and expansion into new regions. Often citizen science databases, like "Is it Alien to you? Share it!!!", Seawatchers or IUCN MedMIS, are used by researchers to gain insight into new invasive species or expansion on the front of existing invasive species (Dimitriadis et al., 2020; Katsanevakis et al., 2020). These databases offer a platform for the general public to report and collect data for professional scientists to use in a collaborative effort, often tracking species sightings across larger areas (Larson et al., 2020). Although these types of reports often allow researchers to gain larger quantities of data than what could be collected alone, citizen scientists may not have the expertise to reliably identify unfamiliar species and usually these reports only list confirmed sightings, so clear absence data is not obtainable (Crall et al., 2010; Larson et al., 2020; Sandahl & Tøttrup, 2020).

Species tracking using citizen science is a common method used to collect sighting data on invasive marine species in the Mediterranean Sea. In 1869 the Suez Canal opened up a new passageway for marine organisms to move between the Red Sea and the Mediterranean either by currents carrying pelagic larvae or by adult movement through the channel (B. S. Galil et al., 2014). Though the canal is open to movement from either direction, species mainly migrate from the Red Sea to the Mediterranean because of current direction and increased habitat flexibility and biodiversity in Red Sea–native organisms (Mavruk & Avsar, 2008). These "Lessepsian species" totaled 63 by 2008 and have been on the rise due to the recent expansion of the Suez Canal (B. S. Galil et al., 2015; Mavruk & Avsar, 2008).

One of these species, the lionfish (Pterois miles) was rarely sighted in the Mediterranean until 2012 when sightings began to rise and their invasion commenced (M Bariche, Torres, and Azzurro 2013; Jimenez et al. 2016; Golani and Sonin 1992; Kletou, Hall-Spencer, and Kleitou 2016). With their recent expansion into the Mediterranean, concern has risen about its ecological impacts based on the well-studied lionfish invasion in the Atlantic. Release of lionfish from the aquarium trade in Florida, USA has allowed them to spread throughout the Western Atlantic Ocean and Caribbean Sea with numerous detrimental effects on native communities, including decreases in biodiversity, reef deterioration, and economic loss in the fishing industry (Côté et al., 2013; Henderson, 2012; Lesser & Slattery, 2011). Their rapid expansion has been attributed to characteristics like their high fecundity, venomous spines, generalist diet, and habitat flexibility (Côté et al., 2013; Galloway & Porter, 2019; Peake et al., 2018; Rojas-Vélez et al., 2019). Studies on Mediterranean lionfish have revealed that similar to those in the Atlantic, these invasive fish are larger than their native counterparts, have high reproductive rates year-round, and prey on a large range of fish that consists of some that are economically important in the area (Savva et al., 2020). Genetic studies of lionfish from both invasive ranges suggests that only a few individuals were originally introduced and responsible for the subsequent establishment and invasion, suggesting that these fish are resilient even at low numbers (Bariche et al., 2017; Hamner et al., 2007; Stern et al., 2019). These characteristics are likely to facilitate the lionfish's invasion in the Mediterranean as well, with similar negative impacts on the Mediterranean ecosystem if allowed to spread (Galanidi et al., 2018; Kletou et al., 2016; Ulman et al., 2020). Even with these concerns, legislation is slow to take action and public awareness remains low (Kleitou et al., 2019; Kleitou, Hall-Spencer, et al., 2021).

Initial reports of the invasion showed lionfish spread and establishment around Cyprus and on the Levantine Coast located at the eastern border of the Mediterranean, but the most recent report documented sightings as far west as the Greek Ionian coast, Libya, as well as single sightings of lionfish in Italy and Tunisia (Azzurro et al., 2017; Dimitriadis et al., 2020). These reports come from small studies of individual countries, or use citizen science databases to track the spread throughout the entire Mediterranean Sea (Azzurro & Bariche, 2017; Dimitriadis et al., 2020; Katsanevakis et al., 2020; Kletou et al., 2016; Özbek et al., 2017). To create a more complete and reliable overview of the lionfish invasion in the Mediterranean, we need to utilize people that have enough local expertise to be able to reliably identify the invasive fish and can say with some confidence that there are no lionfish present if they do not see them. This type of local underwater expertise can be found at dive centers, and centers can be found all over the Mediterranean. Dive instructors have been found to have species knowledge equal with scientific divers and significantly higher than average for recreational divers, with particularly high scores for species recognition capabilities (Hermoso et al., 2021). In this way, the use of dive centers in citizen science could create a more refined method of citizen science with a smaller chance of false identification of species. We conducted a survey of dive centers around the Mediterranean and asked them to report if they have seen lionfish in their area, and if so, when they first appeared in order to create the most up-to-date report of the lionfish invasion in this area.

Methods

Dive Center Search

Google Maps was used to locate dive centers situated along the Mediterranean Sea using the search term "dive center". The search was started at the mouth of the Suez Canal and continued westward along the coast, circling back to the start point, and included all islands located in the sea. Care was taken to ensure all costal area was searched and that all relevant dive center results would be revealed in an area. Search results would be considered dive centers and contacted for the survey if their name in Google Maps contained the phrase "dive center" or an equivalent in any language using the Latin alphabet, appeared in the first 20 results listed for a given area, offered tours or courses in diving (to ensure active divers on staff), and listed a website with contact information listing at least one email.

When a dive center was found that met our criteria, their website was searched for all available email addresses related to diving, which were then recorded along with the geographic coordinates of the dive center. If multiple email addresses were found, emails were sent to the most relevant email address for dive instructors. This yielded 1131 emails to dive centers. The distribution of the dive centers contacted is shown in Figure 2.1.

Survey Distribution

We contacted dive centers found through Google Maps via email and asked two questions: 1) Have you seen lionfish in your area? and 2) If so, when did you first see them?. Email text was written in English and translated into 7 other languages (Arabic, French, Spanish, Italian, Turkish, Greek, and German) by native speakers when possible in order to make the survey more accessible to those who do not speak English fluently. If after 1 week no response was received from a dive center, a reminder email was sent in order to maximize the number of responses received.

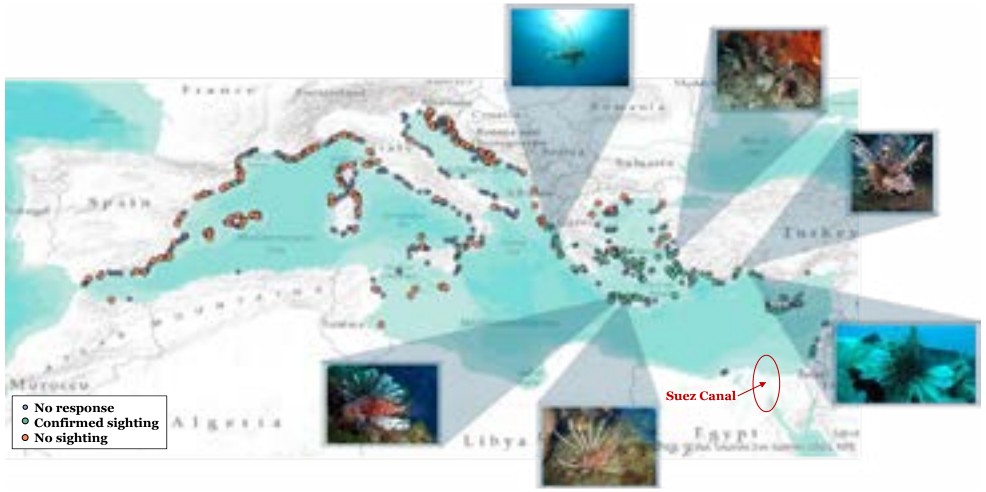


Figure 2.1 *Contacted dive centers and survey respondents. Each dot represents a dive center that was contacted during our survey: green dots represent survey respondents that reported sightings of lionfish, red dots are reported no-sightings, and gray dots are dive surveys that were contacted, but gave no response. Pictures of lionfish sighted in their area were provided by the following dive centers (starting at the top, moving clockwise): Fiskardo Divers (Greece), Kiwi Watersports & Dive Center (Turkey), Pearl Diving Center (Turkey), Scuba Side Diving Center (Turkey), Kalypso Dive Center & School (Crete), Paradise Dive Center (Crete).*

Data Analysis

Responses from individual dive centers and coordinates previously recorded during the search process were imported into ArcGIS Pro (version 2.5.1) to create maps illustrating lionfish sightings based on dive center coordinates (Esri, 2020). We chose to use dive center coordinates as locations of lionfish sightings since coastal dive centers typically use dive sites close to their dive center.

Results

After contacting 1131 dive centers, we received 216 responses. Of these responses, 75 reported sightings of lionfish in their local area and 141 reported that no lionfish were present (Figure 2.1). We found that lionfish had been sighted at least once in Israel, Lebanon, Cyprus, Turkey, Greece, and Spain, while dive centers in Tunisia, Morocco, France, Italy, Malta, Slovenia, Croatia, Montenegro, and Albania only reported no sightings. With the exception of one dive center on the south-west coast of Cyprus, dive centers from the east side of the Mediterranean up until the Turkish Aegean coast and mainland Greece unanimously reported lionfish sightings. Overall, the most north-western sighting of lionfish was a singular report on the Costa Brava in Spain (2.9030443°E, 41.7109527°N). In the eastern half of the Mediterranean where all other sightings were located, lionfish sightings were reported as far north as Karaburun, Turkey (26.5206573°E 38.6370333°N), and as far west as at Corfu Island, Greece (19.9394234°E 39.4280170°N).

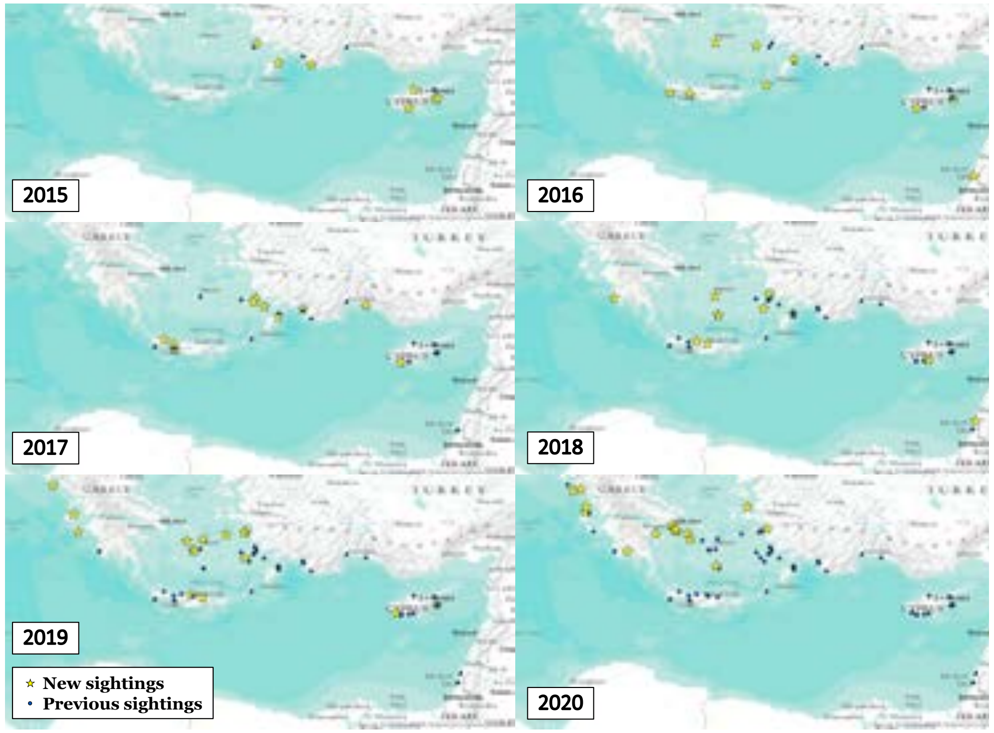


Figure 2.2 *Lionfish sightings by year. Yellow stars represent new sightings of lionfish fish in the given year and blue dots represent older sightings from previous years. Please note that the only sighting from the Western Mediterranean, from Tossa de mar, Spain (2.9030443°E, 41.7109527°N) discussed in the main text is not shown in this figure, but is important to note as a pre-2015 sighting.*

For reports of lionfish sighting, we also recorded the year that lionfish were first spotted in the area as reported by dive centers. We found that single lionfish sightings were observed in 1997 on a Greek island off the coast of Turkey (27.3126756°E 36.8851393°N), near Tossa de mar, Spain in 2002 (2.9030443°E, 41.7109527°N), Antalya, Turkey in 2005 (30.7054499°E 36.8767709°N), Ölüdeniz, Turkey in 2013 (29.1116429°E, 36.5499014°N) and Yeni Iskele, Cyprus in 2014 (33.949655°E, 35.312925°N). Establishment of lionfish occurred in Cyprus and Turkey by 2015, with the invasion front continuing to move west and north each year (Figure 2.2). In 2016, lionfish were first spotted near the island of Crete (24.3911364°E 35.1907813°N and 26.6803124°E 35.2259198°N), and in 2018 they were seen off of mainland Greece in the Ionian Sea (21.6503151°E 36.9944446°N). In the Aegean Sea and Ionian Sea, the furthest northern sightings as documented above were in the years 2020 and 2019, respectively.

Discussion

Using our novel methodology of tapping into the local expertise of 216 of professional divers, we found that lionfish were observed irregularly around Turkey from 1997-2014, but establishment was first seen in Cyprus and Turkey in 2015 with multiple sightings in these areas. We found a boom of sightings during the following years with a westward movement of the front along Turkey, Crete, mainland Greece, and the South Aegean Islands. Overall, this is consistent with the invasion spread reported using citizen science and local expertise at a smaller, national scale, with a few key differences (Azzurro et al., 2017; Dimitriadis et al., 2020). First, we found reports of lionfish sightings in Turkey prior to 2012 which is previously undocumented in the literature. Though lionfish have been previously reported in the Mediterranean as early as 1992, this earliest sighting is from Israel, closer to the mouth of the Suez Canal than our reported sightings in Turkey (Golani & Sonin, 1992). Second, unlike previous reports, we did not find sightings of lionfish in Italy or Tunisia, but did document the first reported sighting to our knowledge of lionfish in Spain in 2002 (Azzurro et al., 2017; Dailianis et al., 2016). All of these sightings were of single lionfish and represented rare events when compared to previous literature from citizen science reports (Dimitriadis et al., 2020). Our methodology may have missed very rare occurrences, such as in Italy and Tunisia, but did uncover an additional rare sighting previously undocumented in Spain. Due to the time and distance between the

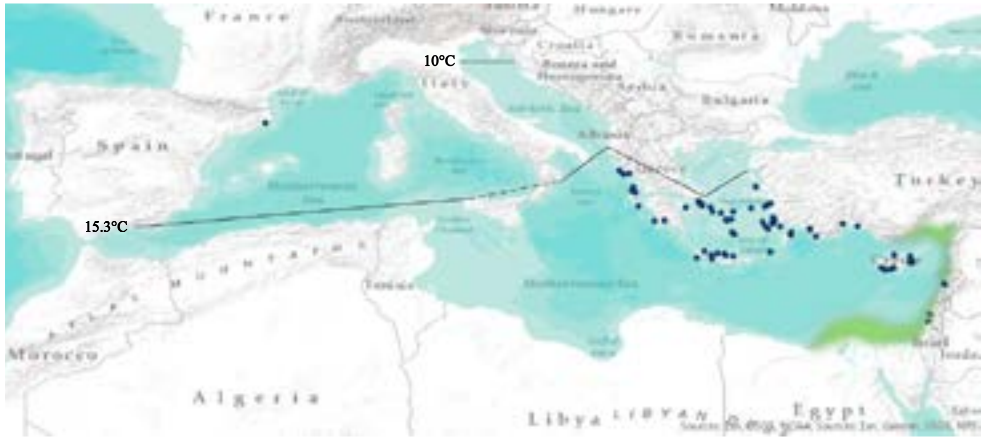


__Figure 2.3__ Thermal boundaries to invasion expansion. Black lines represent the thermal boundaries at 15.3°C and 10°C (Dimitriadis et al. 2020; Johnston and Purkis 2014) hypothesized to restrict lionfish expansion. The green shaded area represents the high pressure invasive range of lionfish via the Suez Canal as predicted by Johnston & Purkis (2014). Blue dots represent the lionfish sightings reported in our survey.

majority of sightings found in our study and that in Spain, we speculate that lionfish sighted here were mostly likely due to aquarium release without establishment prior to the ongoing current invasion via the Suez Canal. Singular, seemingly detached sightings like these are important to document, as releases from the aquarium trade in places like the Bay of Marseilles, France are predicted to result in high invasion pressure from lionfish (Johnston & Purkis, 2014). Together, the overall consistency, but small differences in findings between our methodology and traditional citizen science show that by using a combination of these two techniques as part of citizen science initiatives could increase the accuracy of invasive species detection through a refined method of citizen science.

Although we found no further establishment of lionfish west of Corfu, Greece (reported in 2019), we found an ongoing expansion north in 2020 to Karaburun, Turkey. This movement north in recent years is noteworthy, as a thermal barrier has long been considered a prominent hindrance to lionfish invasion across the entire Mediterranean Sea. In the Atlantic Ocean, lionfish only establish in locations with mean winter temperatures above 15.3°C, though lionfish have experimentally been shown to withstand temperatures as low as 10°C (Kimball et al., 2004; Whitfield et al., 2014). These isotherm boundaries are currently only present north of the lionfish sightings, though just barely, so it remains to be seen in the coming years if lionfish in the Mediterranean will be able to cross the thermal barrier and establish in more northern regions, or if they will be limited in their spread (Dimitriadis et al., 2020; Johnston & Purkis, 2014). In the Atlantic, lionfish are only seen north of this barrier during summer's high temperatures, but none have been able to establish year-round. In the next decades, climate change is also predicted to push the isotherms further north, likely allowing lionfish to invade the entire Mediterranean by 2100 (Dimitriadis et al., 2020; Turan, 2020). Importantly, several models based on other biogeographic barriers such as currents predict limited spread of lionfish in the Mediterranean past their present established territories (Johnston & Purkis, 2014; Poursanidis, 2015; Poursanidis et al., 2020). Current patterns in the Mediterranean Sea are thought to cause low connectivity between suitable habitats for lionfish, limiting potential larvae spread to the west (Johnston & Purkis, 2014). However, our data shows that lionfish have spread past these assumed invasion boundaries. What other factors facilitate this spread beyond previously suggested boundaries remains unknown. Here, we offer several non-mutually exclusive explanations.

Behavioral flexibility and/or better cognitive abilities may be key to lionfish invasive success. Behavioral adaptations have been shown to increase success of invasive species across a variety of taxa (Weis & Sol, 2016). For instance, in round gobies (Neogobius melanostomus), higher dispersal potential and bolder personalities are shown in individuals on the invasive front, while in the common myna (Acridotheres tristis) invasive populations have increased innovation and decreased neophobia compared to native populations (Magory et al., 2020; Myles-Gonzalez et al., 2015b). We predict that lionfish in invasive territories may similarly possess different behavioral and/or cognitive traits that are more advantageous to invasive ability compared to their native counterparts. For example, post-settlement invasive lionfish may find themselves in novel environments, so better operant learning or innovative ability might help these individuals adapt, survive, and establish in new areas (Weis & Sol, 2016). In this way, habitats or environments previously thought unsuitable for lionfish might become inhabited by invasive lionfish in the upcoming years. How invasive populations in the Atlantic and the Mediterranean differ in their behavior and cognition also has yet to be investigated, as this could cause differential expansion rate and invasive ability between the two populations.

Differences in predation level by filter feeders could also impact the precision of models in predicting invasive ability. The Enemy Release Hypothesis predicts that invaders escape population controls (predators, competitors, parasites, pathogens, etc.) present in their native range, causing them to thrive in novel invasive ecosystems (Mack et al., 2000; Torchin & Mitchell, 2004). There is conflicting evidence on the presence of natural predators to adult and juvenile lionfish in both their native and invasive ranges, as a few reports have been made of grouper and moray eel predation events, but there is no clear trend of population control due to predation (Bernadsky & Goulet, 1991; Bos et al., 2017; Côté et al., 2013; Hackerott et al., 2013a; Maljković et al., 2008; Mumby et al., 2011; N. S. Smith & Côté, 2021a). In contrast, larvae predation during the pelagic phase by filter feeders has yet to be explored. Filter feeders in the native range may have evolved with lionfish to be able to feed on them as larvae, but those in invasive ranges have not, causing the higher population density of invasive lionfish compared to native populations, and further range expansion of these species then previously predicted (Darling et al., 2011b). For example, a prevalent filter feeder in the Indo-Pacific, the Ascidian Herdmania momus, is also invasive in the Mediterranean via introduction through the Suez Canal (Rius & Shenkar, 2012). H. momus's invasive range does not expand as far north or west as the lionfish's however, suggesting that lionfish may have been able to spread far enough in the Mediterranean to escape the predation pressure of native filter feeders, allowing further expansion than predicted models (Çinar et al., 2006; Gewing et al., 2019). The absence of coral reefs in the Mediterranean Sea may also impact the predation pressure on lionfish larvae, as coral reef sponges are also known to decrease the abundance of pelagic larvae through filtration (Riisgård & Larsen, 2010). Finally, reduced parasite load may contribute to the rapid spread. Few studies have investigated the effect of parasite load between invasive and native lionfish, although one study has found that variation in parasite load in lionfish across the Caribbean has little effect on the health and fitness of the fish (Ramos-Ascherl et al., 2015; Sellers et al., 2015).

Another question that remains is why the Mediterranean invasion is starting now, rather than at the opening of the Suez Canal in 1869. One reason may be that the Suez Canal was recently expanded in 2015, allowing more opportunity for passage from native habitats in the Red Sea to the Mediterranean, but this may not be the only reason. For example, global warming in previous years may have led to an increased opportunity for lionfish to establish and thrive in the Mediterranean (Gattuso et al., 2015; Hoegh-Guldberg & Bruno, 2010). Lionfish may have also developed mutations causing better physiological cold resistance in invasive populations and the ability to reside closer to the temperature barrier. Additionally, adaptations that effect behavior could have made lionfish more able to adapt to Mediterranean conditions, similar to the blue tit innovation in the United Kingdom after urbanization in the 1940s. These birds learned to pierce the foil on the top of milk bottles as a novel way to obtain food in an increasingly urbanizing environment (Aplin et al., 2013). Similarly, lionfish may have learned and developed behavioral adaptations that have made them more likely to fit into an unoccupied ecological niche present in the Mediterranean, allowing them to thrive in the new niche (Poursanidis et al., 2020). These types of adaptations in invasive populations should be tested to better understand the invasive ability of various species.

Conclusions

Based on local dive center expertise, we found that lionfish established in the eastern part of the Mediterranean by 2015 and now have spread as far as the Greek Ionian coast, with clear evidence of ongoing expansion given by new sightings as recent as October 2020 (23.1537875°E, 37.6378821°N). This expansion into new invasive territory demonstrates the need for continuous monitoring of this species using local expertise and development of management methods to protect native biodiversity (B. Galil et al., 2017). For instance, careful documentation of lionfish distribution in the Caribbean has allowed for evaluation and implementation of management strategies to reduce the lionfish population in invasive areas (de León et al., 2013). Documentation of the invasive lionfish in Bonaire and Curaçao has revealed that the promotion of volunteer lionfish hunting programs has resulted in up to 4.14-fold reduction in lionfish in fished versus unfished areas. Similar efforts are being conducted in the Mediterranean with programs such as RELIONMED-LIFE; continuous monitoring of lionfish populations and expansion will be crucial to understanding the efforts of these efforts in the Mediterranean. We also argue that reliance on citizen may not tell the whole story, as it requires the general public to reliably identify invasive species, and for citizen science campaigns for certain species to be widely advertised in the area. For example, the sighting we found in Spain might have been unreported until this point because attention is not drawn to identifying and tracking lionfish there. Local experts, like those found at dive centers, are able to notice new species due to their familiarity with the area, though they are in low numbers compared to the general public and not as abundant in non-tourist destinations, such as the southern coast of the Mediterranean Sea (Figure 2.1). Ideally, these strategies should complement one another, combining the advantages of large data sets from general public citizen science, and increased accuracy in sightings from dive center reports. Finally, to create effective management strategies for lionfish populations in the Mediterranean, we need to intensify our research effort to know more about why this species is able to expand its territory so quickly, even under unfavorable conditions. Understanding the invasive potential of lionfish and other species will allow us to create more accurate invasive models and be more proactive in management strategies.

References

Aplin, L. M., Sheldon, B. C., & Morand-Ferron, J. (2013). Milk bottles revisited: Social learning and individual variation in the blue tit, Cyanistes caeruleus. Animal Behaviour, 85(6), 1225–1232. https://doi.org/10.1016/j.anbehav.2013.03.009

Azzurro, E., & Bariche, M. (2017). Local knowledge and awareness on the incipient lionfish invasion in the eastern Mediterranean Sea. Marine and Freshwater Research, 68(10), 1950–1954. https://doi.org/10.1071/MF16358

Azzurro, E., Stancanelli, B., Di Martino, V., & Bariche, M. (2017). Range expansion of the common lionfish Pterois miles (Bennett, 1828) in the Mediterranean Sea: an unwanted new guest for Italian waters. BioInvasions Records, 6(2), 95–98. https://doi.org/10.3391/bir.2017.6.2.01

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(6782), 1–6. 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), 292–294.

Bernadsky, G., & Goulet, D. (1991). A Natural Predator of the Lionfish , Pterois miles. American Society of Ichthyologists and Herpetologists, 1991(1), 230–231.

Bos, A. R., Sanad, A. M., & Elsayed, K. (2017). Gymnothorax spp. (Muraenidae) as natural predators of the lionfish Pterois miles in its native biogeographical range. Environmental Biology of Fishes, 100(6), 745–748. https://doi.org/10.1007/s10641-017-0600-7

Cassey, P., Blackburn, T. M., Duncan, R. P., & Chown, S. L. (2005). Concerning invasive species: Reply to Brown and Sax. Austral Ecology, 30(4), 475–480. https://doi.org/10.1111/j.1442-9993.2005.01505.x

Çinar, M. E., Bilecenoglu, M., Öztürk, B., & Can, A. (2006). New records of alien species on the Levantine coast of Turkey. Aquatic Invasions, 1(2), 84–90.

Côté, I. M., Green, S. J., & Hixon, M. A. (2013). Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. Biological Conservation, 164, 50–61. https://doi.org/10.1016/j.biocon.2013.04.014

Crall, A. W., Newman, G. J., Jarnevich, C. S., Stohlgren, T. J., Waller, D. M., & Graham, J. (2010). Improving and integrating data on invasive species collected by citizen scientists. Biological Invasions, 12, 3419–3428. https://doi.org/10.1007/s10530-010-9740-9

Dailianis, T., Akyol, O., Babali, N., Bariche, M., Crocetta, F., Gerovasileiou, V., Ghanem, R., Gokoglu, M., Hasiotis, T., Izquierdo-Munoz, A., Julian, D., Katsanevakis, S., Lipej, L., Mancini, E., Mytilineou, C., Ounifi Ben Amor, K., Ozgul, A., Ragkousis, M., Rubio-Portillo, E., … Trkov, D. (2016). New Mediterranean Biodiversity Records (July 2016). Mediterranean Marine Science, 17(2), 608–626.

Darling, E. S., Green, S. J., O'Leary, J. K., & Côté, I. M. (2011). Indo-Pacific lionfish are larger and more abundant on invaded reefs: A comparison of Kenyan and Bahamian lionfish populations. Biological Invasions, 13(9), 2045–2051. https://doi.org/10.1007/s10530-011-0020-0

de León, R., Vane, K., Bertuol, P., Chamberland, V., Simal, F., Imms, E., & Vermeij, M. (2013). Effectiveness of lionfish removal efforts in the southern Caribbean. Endangered Species Research, 22(2), 175–182. https://doi.org/10.3354/esr00542

Dimitriadis, C., Galanidi, M., Zenetos, A., Corsini-Foka, M., Giovos, I., Karachle, P. K., Fournari-Konstantinidoy, 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 the thermal boundaries and future range expansion. Mediterranean Marine Science, 21, 62–69.

Esri. (2020). ArcGIS Pro (2.5.1).

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, 107–123.

Galil, B., Marchini, A., Occhipinti-Ambrogi, A., & Ojaveer, H. (2017). The enlargement of the Suez Canal – Erythraean introductions and management challenges. Management of Biological Invasions, 8(2), 141–152. https://doi.org/10.3391/mbi.2017.8.2.02

Galil, B. S., Boero, F., Campbell, M. L., Carlton, J. T., Cook, E., Fraschetti, S., Gollasch, S., Hewitt, C. L., Jelmert, A., Macpherson, E., Marchini, A., McKenzie, C., Minchin, D., Occhipinti-Ambrogi, A., Ojaveer, H., Olenin, S., Piraino, S., & Ruiz, G. M. (2015). 'Double trouble': the expansion of the Suez Canal and marine bioinvasions in the Mediterranean Sea. Biological Invasions, 17, 973–976. https://doi.org/10.1007/s10530-014-0778-y

Galil, B. S., Marchini, A., Occhipinti-Ambrogi, A., Minchin, D., Narščius, A., Ojaveer, H., & Olenin, S. (2014). International arrivals: Widespread bioinvasions in

European Seas. Ethology Ecology and Evolution, 26(2–3), 152–171. https://doi.org/10.1080/03949370.2014.897651

Galloway, K. A., & Porter, M. E. (2019). Mechanical properties of the venomous spines of Pterois volitans and morphology among lionfish species. Journal of Experimental Biology, 222(6), jeb197905. https://doi.org/10.1242/jeb.197905

Gattuso, J. P., Magnan, A., Billé, R., Cheung, W. W. L., Howes, E. L., Joos, F., Allemand, D., Bopp, L., Cooley, S. R., Eakin, C. M., Hoegh-Guldberg, O., Kelly, R. P., Pörtner, H. O., Rogers, A. D., Baxter, J. M., Laffoley, D., Osborn, D., Rankovic, A., Rochette, J., … Turley, C. (2015). Contrasting futures for ocean and society from different anthropogenic CO2 emissions scenarios. Science, 349, aac4722. https://doi.org/10.1126/science.aac4722

Gewing, M.-T., Goldstein, E., Buba, Y., & Shenkar, N. (2019). Temperature resilience facilitates invasion success of the solitary ascidian Herdmania momus. Biological Invasions, 21, 339–351. https://doi.org/10.1007/s10530-018-1827-8

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/10.1017/CBO9781107415324.004

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). 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(Supplement B), 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. In Master's Thesis. Simon Fraser University.

Hermoso, M. I., Martin, V. Y., Gelcich, S., Stotz, W., & Thiel, M. (2021). Exploring diversity and engagement of divers in citizen science: Insights for marine management and conservation. Marine Policy, 124, 104316. https://doi.org/10.1016/j.marpol.2020.104316

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The Impact of Climate Change on the World's Marine Ecosystems. Science, 328, 1523–1528. https://doi.org/10.1016/s0262-4079(10)61509-6

Jimenez, C., Petrou, A., Andreou, V., Hadjioannou, L., Wolf, W., Koutsoloukas, N., & Alhaija, R. A. (2016). Veni, vidi, vici: the successful establishment of the lionfish pterois miles in Cyprus (Levantine Sea). Rapp. Comm. Int. Mer Medit., 41, 417. http://ciesm.org/online/archives/abstracts/pdf/41/CIESM_Congress_2016_Kiel_article_0417.pdf

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, 138–147. https://doi.org/10.1016/j.marpolbul.2014.09.013

Katsanevakis, S., Poursanidis, D., Hoffman, R., Rizgalla, J., Rothman, S. B., Levitt-Barmats, Y., Hadjioannou, L., Trkov, D., Garmendia, J. M., Rizzo, M., Bartolo, A. G., Bariche, M., Tomas, F., Kleitou, P., Schembri, P. J., Kletou, D., Tiralongo, F., Pergent, C., Pergent, G., … Zenetos, A. (2020). Unpublished Mediterranean records of marine alien and cryptogenic species. BioInvasions Records, 9(2), 165–182.

Kimball, M. E., Miller, J. M., Whitfield, P. E., & Hare, J. A. (2004). Thermal tolerance and potential distribution of invasive lionfish (Pterois volitans / miles complex) on the east coast of the United States. Marine Ecology Progress Series, 283, 269–278.

Kleitou, P., Hall-Spencer, J. M., Savva, I., Kletou, D., Hadjistylli, M., Azzurro, E., Katsanevakis, S., Antoniou, C., Hadjioannou, L., Chartosia, N., Christou, M., Christodoulides, Y., Giovos, I., Jimenez, C., Smeraldo, S., & Rees, S. E. (2021). The case of lionfish (Pterois miles) in the mediterranean sea demonstrates limitations in EU legislation to address marine biological invasions. Journal of Marine Science and Engineering, 9(325). https://doi.org/10.3390/jmse9030325

Kleitou, P., Savva, I., Kletou, D., Hall-Spencer, J. M., Antoniou, C., Christodoulides, Y., Chartosia, N., Hadjioannou, L., Dimitriou, A. C., Jimenez, C., Petrou, A., Sfenthourakis, S., & Rees, S. (2019). Invasive lionfish in the Mediterranean: Low public awareness yet high stakeholder concerns. Marine Policy, 104, 66–74. https://doi.org/10.1016/j.marpol.2019.02.052

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), 1–7. https://doi.org/10.1186/s41200-016-0065-y

Larson, E. R., Graham, B. M., Achury, R., Coon, J. J., Daniels, M. K., Gambrell, D. K., Jonasen, K. L., King, G. D., Laracuente, N., Perrin-stowe, T. I. N., Reed, E. M., Rice, C. J., Ruzi, S. A., Thairu, M. W., Wilson, J. C., & Suarez, A. V. (2020).

From eDNA to citizen science : emerging tools for the early detection of invasive species. Front Ecol Environ, 18(4), 194–202. https://doi.org/10.1002/fee.2162

Lesser, M. P., & Slattery, M. (2011). Phase shift to algal dominated communities at mesophotic depths associated with lionfish (Pterois volitans) invasion on a Bahamian coral reef. Biological Invasions, 13(8), 1855–1868. https://doi.org/10.1007/s10530-011-0005-z

Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M., & Bazzaz, F. A. (2000). Biotic Invasions: Causes, Epidemiology, Global Consequences, and Control. Ecological Applications, 10(3), 689–710. https://doi.org/10.1890/0012-9623(2008)89[341:iie]2.0.co;2

Magory, T., Kumar, R. S., Nair, M., Hauber, M. E., & Dor, R. (2020). Innovation and decreased neophobia drive invasion success in a widespread avian invader. Animal Behaviour, 163, 61–72. https://doi.org/10.1016/j.anbehav.2020.02.012

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(501). https://doi.org/10.1007/s00338-008-0372-9

Mavruk, S., & Avsar, D. (2008). Non-native fishes in the Mediterranean from the Red Sea, by way of the Suez Canal. Reviews in Fish Biology and Fisheries, 18(3), 251–262. https://doi.org/10.1007/s11160-007-9073-7

Mumby, P. J., Harborne, A. R., & Brumbaugh, D. R. (2011). Grouper as a natural biocontrol of invasive Lionfish. PLoS ONE, 6(6), 2–5. https://doi.org/10.1371/journal.pone.0021510

Myles-Gonzalez, E., Burness, G., Yavno, S., Rooke, A., & Fox, M. G. (2015). To boldly go where no goby has gone before: Boldness, dispersal tendency, and metabolism at the invasion front. Behavioral Ecology, 26(4), 1083–1090. https://doi.org/10.1093/beheco/arv050

Özbek, E. Ö., Mavruk, S., Saygu, İ., & Öztürk, B. (2017). Lionfish distribution in the eastern Mediterranean coast of Turkey. Journa of Black Sea/Mediterranean Environment, 23(1), 1–16.

Peake, J., Bogdanoff, A. K., Layman, C. A., Castillo, B., Jennifer, K. R., Kristen, C., William, D., Iii, F. P., Eddy, C., Ellis, R. D., Faletti, M., Higgs, N., Juan, S., Johnston, M. A., Mun, R. C., & Jr, J. A. M. (2018). Feeding ecology of invasive lionfish (Pterois volitans and Pterois miles) in the temperate and tropical western Atlantic. Bio Invasions, 20, 2567–2597. https://doi.org/10.1007/s10530-018-1720-5

Poursanidis, D. (2015). Ecological Niche Modeling of the invasive lionfish Pterois miles (Bennett, 1828) in the Mediterranean Sea. 11th Panhellenic Symposium on Oceanography and Fisheries, Mytilene, Levos Island, Greece, 621–624.

Poursanidis, D., Kalogirou, S., Azzurro, E., Parravicini, V., & Bariche, M. (2020). Habitat suitability , niche unfilling and the potential spread of Pterois miles in the Mediterranean Sea. Marine Pollution Bulletin, 154. https://doi.org/10.1016/j.marpolbul.2020.111054

Ramos-Ascherl, Z., Williams, E. H., Bunkley-Williams, L., Tuttle, L. J., Sikkel, P. C., & Hixon, M. A. (2015). Parasitism in Pterois volitans (Scorpaenidae) from coastal waters of Puerto Rico, the Cayman Islands, and the Bahamas. Journal of Parasitology, 101(1), 50–56. https://doi.org/10.1645/13-422.1

Riisgård, H. U., & Larsen, P. S. (2010). Particle capture mechanisms in suspension-feeding invertebrates. 418, 255–293. https://doi.org/10.3354/meps08755

Rius, M., & Shenkar, N. (2012). Ascidian introductions through the Suez Canal : The case study of an Indo-Pacific species. Marine Pollution Bulletin, 64(10), 2060–2068. https://doi.org/10.1016/j.marpolbul.2012.06.029

Rojas-Vélez, S., Tavera, J., & Acero, A. (2019). Unraveling lionfish invasion: Is Pterois volitans truly a morphologically novel predator in the Caribbean? Biological Invasions, 21(6), 1921–1931. https://doi.org/10.1007/s10530-019-01946-6

Sandahl, A., & Tøttrup, A. P. (2020). Marine Citizen Science: Recent Developments and Future Recommendations. Citizen Science: Theory and Practice, 5(1), 1–11.

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, April. https://doi.org/10.1111/jfb.14340

Sellers, A. J., Ruiz, G. M., Leung, B., & Torchin, M. E. (2015). Regional variation in parasite species richness and abundance in the introduced range of the invasive lionfish, Pterois volitans. PLoS ONE, 10(6), 1–15. https://doi.org/10.1371/journal.pone.0131075

Simberloff, D., Martin, J. L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., & Vilà, M. (2013). Impacts of biological invasions: What's what and the way forward. Trends in Ecology and Evolution, 28(1), 58–66. https://

doi.org/10.1016/j.tree.2012.07.013

Smith, N. S., & Côté, I. M. (2021). Biotic resistance on coral reefs? Direct and indirect effects of native predators and competitors on invasive lionfish. Coral Reefs. https://doi.org/10.1007/s00338-021-02117-7

Stern, N., Jimenez, C., Huseyinoglu, M. F., Andreou, V., Hadjioannou, L., Petrou, A., Öztürk, B., Golani, D., & Rothman, S. B. S. (2019). Constructing the genetic population demography of the invasive lionfish Pterois miles in the Levant Basin, Eastern Mediterranean. Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis, 30(2), 249–255. https://doi.org/10.1080/24701394.2018.1482284

Torchin, M. E., & Mitchell, C. E. (2004). Parasites, pathogens, and invasions by plants and animals. Frontiers in Ecology and the Environment, 2(4), 183–190. https://doi.org/10.1890/1540-9295(2004)002[0183:PPAIBP]2.0.CO;2

Turan, C. (2020). Species distribution modelling of invasive alien species; Pterois miles for current distribution and future suitable habitats. Global Journal of Environmental Science and Management, 6(4), 429–440. https://doi.org/10.22034/gjesm.2020.04.01

Ulman, A., Tunçer, S., Kizilkaya, I. T., Zilifli, A., Alford, P., & Giovos, I. (2020). The lionfish expansion in the Aegean Sea in Turkey: A looming potential ecological disaster. Regional Studies in Marine Science, 36, 101271. https://doi.org/10.1016/j.rsma.2020.101271

Weis, J. S., & Sol, D. (Eds.). (2016). Biological Invasions and Animal Behaviour. Cambridge University Press.

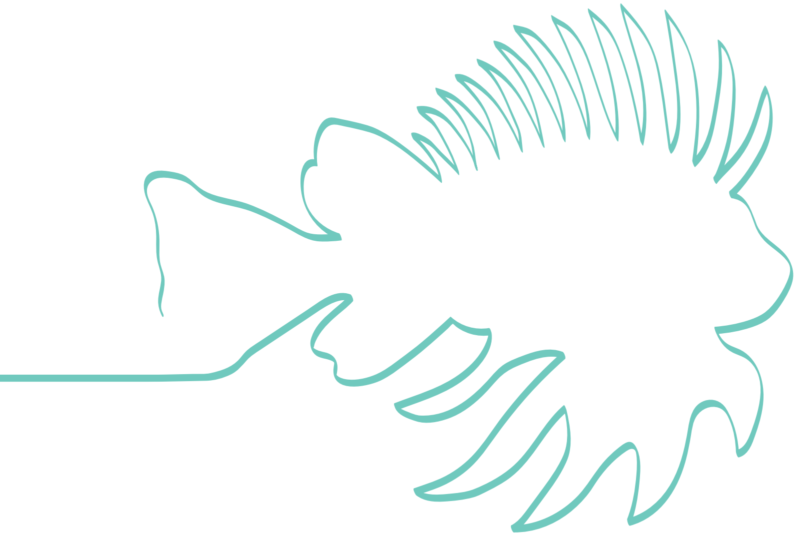
Whitfield, P. E., Muñoz, R. C., Buckel, C. A., Degan, B. P., Freshwater, D. W., & Hare, J. A. (2014). Native fish community structure and Indo-Pacific lionfish Pterois volitans densities along a depth-temperature gradient in Onslow Bay, North Carolina, USA. Marine Ecology Pr, 509, 241–254. https://doi.org/10.3354/meps10882

Chapter 3

Color Vision in Lionfish:

Gene Expression and Behavioural Evidence for Color Vision in a Crepuscular Aquatic Predator

Elizabeth W. Phillips, Miguel Augusto Vergara Ramirez, Hanna Douwes, Natasha Bloch, Alexander Kotrschal



Abstract

Color vision is widespread among animals. It is used for inter- and intra-species communication, foraging, hunting, and other situations in which animals depend on visual cues for input, creating a tight link between visual systems and the environment. Underwater, light attenuates with depth, causing vast differences in light environment that fishes need to adapt to. While diurnal coral reef fishes in shallow water typically possess tetrachromatic vision to utilize all available wavelengths, night-active or deeper habitat fish that preside in environments where light is drastically filtered by the water column are often sensitive to a more restrictive range of wavelengths. However, it remains enigmatic what the visual capabilities are of crepuscular species are. One Pacific coral reef fish, the red lionfish (Pterois volitans), is a highly invasive crepuscular predator in the Atlantic and Caribbean. While their invasive success has been attributed to traits such as their venomous spines, high reproductive output and its generalist predatory diet, we think their visual capabilities, specifically color vision, may be an additional factor that influences their invasive success. To investigate this, we used a combination of genomic and behavioral techniques to determine the color vision capabilities of lionfish. First, we sequenced the retinal transcriptome of lionfish and found evidence that lionfish express rhodopsins RH1-1 and RH1-2 and opsins RH2-1, RH2-2, RH2-3, RH2-4, SWS1, and SWS2A, indicating they indeed have color vision. Next, we therefore performed a two-choice associative learning task with colored disks to evaluate whether lionfish have color vision within the tested spectrum. We found that lionfish were able to learn to discriminate the two colors, even when luminance was varied to ensure chromaticity was the discriminating factor. Finally, we examine the evolutionary history of lionfish color vision in the context of other teleost fish using molecular evidence. All together, we show the first behavioral and molecular evidence that lionfish possess color vision in the tested range, a trait that could potentially increase their hunting efficiency in a range of different environments.

Introduction

The visual systems of animals are highly diverse. Visual abilities have often evolved as adaptations to different light environments, as vision is used by animals to better learn about and adapt to the challenges of their surroundings. For instance, ambient light levels, habitat coloration, and circadian rhythms can all influence the visual abilities of a given species (Bloch, 2015; Kelber et al., 2003; J. Marshall, 2010; Schmitz & Wainwright, 2011). In Old-World monkeys, for example, some species (including humans) possess trichromatic vision in response to their diurnal habits and diet, while most New World monkeys and other mammals only possess dichromatic vision (Jacobs, 1996; Thoreson & Dacey, 2019). Differences in visual capabilities are hypothesized to have evolved in primates due to differences in diet, as primates with trichromatic vision are often fructivorous, making it advantageous for these species to be able to distinguish brightly colored ripe fruit from other, less nutritious options like leaves (Dominy et al., 2001; Dominy & Lucas, 2001; Gilad et al., 2004). Outside of mammals, visual ability becomes even more complex and diverse, as many birds, reptiles, and fish are tetrachromatic, or possess four photoreceptors as opposed to mammals' two or three (Jacobs, 2018; Thoreson & Dacey, 2019). In extreme cases, animals can have even more types of photoreceptors, such as the mantis shrimp with 12 different photoreceptors (Thoen et al., 2014). Increases in number of photoreceptor types can improve the range of spectral sensitivity, as well as improve the discriminating ability of colors within the spectrum (Kelber et al., 2003; Thoen et al., 2014).

In contrast, color vision and the number of photoreceptors used to distinguish different wavelengths of light becomes less important when environmental light levels are low. Animals living in low-light conditions, such as nocturnal species, have adapted their visual abilities to reflect their low-light environments, causing variation in traits like eye morphology, pupil size, and rod to cone photoreceptor ration compared to diurnal species (Schmitz & Wainwright, 2011). Cones are the photoreceptors used by vertebrates to detect and respond to different wavelengths of light, thus providing color vision, while rod photoreceptors detect low amounts of light at any wavelength within a specie's visual spectrum, providing better low-light detection (Warrant, 2004). Therefore, both the conditions of the environment, as well as the way individuals interact with the environment can have profound impact of the evolution of visual abilities.

For fish, there is another important environmental factor for visual abilities a specie's evolves: light attenuation. As light travels through water, the amount of total light decreases resulting in bright shallow water and dark ocean depths. However, different wavelengths are also filtered at varying rates, which results in differences in color environment at different depths. Visual abilities in fish have adapted to these differences in light environments, and therefore can range from the limited color vision and dim-light adapted senses typically found in aquatic animals at lower depths, to the high spectral sensitivity found in coral reef fish (Cortesi et al., 2020). Indeed, most deep-water or nocturnal fish lack any color vision, while diurnal, shallow-water fish such as coral reef fish typically see the full spectrum of light available to them (J. Marshall, 2010). Similar to aquatic animals found at lower depths, nocturnal aquatic predators have been found to be less likely to have color vision than diurnally active fish, even at shallow depths, as the lack of visible colors in the low-light environment of the night makes possessing color vision less advantageous (Cortesi et al., 2020; Shand, 1997; Warrant, 2004).

However, the visual adaptations of crepuscular species, those that are active during the twilight hours, remains enigmatic. Crepuscular predators are faced with the unique challenge of having to begin hunting at relatively high light levels, but then quickly need to adjust as the light environment changes from light to dark. One terrestrial crepuscular species, the Red-eyed Treefrog (Agalychnis callidryas), possesses a mix of nocturnal and diurnal visual capabilities, with a high affinity for distinguishing brightness in dim light conditions, while also being able to distinguish chromaticity (Robertson et al., 2022). Therefore, it might be assumed that rather than specializing in one niche light environment like fully diurnal or nocturnal animals, crepuscular animals instead take a generalist approach and possess traits like general color vision with some dim-light visual capabilities.

Lionfish (Pterois spp.) are aquatic crepuscular hunters that are now invasive in the Caribbean and Mediterranean Seas (Bariche et al., 2013; Côté et al., 2013; Dimitriadis et al., 2020; E. W. Phillips & Kotrschal, 2021) They are considered to be one of the most invasive species, with its invasive success attributed to traits such as their venomous spines, high reproductive output, and its generalist behavior, including their highly varied diet and habitat usage (Côté et al., 2013). As lionfish's generalist behavior is considered to be one of the major factors impacting its invasive success, it could be predicted that lionfish may be generalist in other aspects of their behavior too, like vision. Having the color vision of a diurnal hunter combined with the dim-light visual acuity of a nocturnal predator would allow them to easily adapt to local environments and hunt when most beneficial. Indeed, we see that while lionfish in their native regions seem to hunt exclusively during the twilight periods,

lionfish in invasive regions in the Caribbean have been found to hunt during the day as well (Côté & Maljković, 2010; Morris Jr. & Akins, 2009). In addition, a recent study suggests that lionfish have the potential for color vision, as a study using corneal electroretinography has shown that lionfish corneas are sensitive to light in the spectrum 350-620nm with peak sensitivity at 510mn (Hasenei et al., 2020). While such corneal sensitivity is a good indicator for actual color vision properties it is indirect evidence. This therefore begs the question: What are the color vision capabilities of lionfish?

Here, we test the color vision capabilities of lionfish using transcriptomic and behavioral assays. First, we sequenced the retinal transcriptome of lionfish to investigate which opsin genes are expressed by this species. Opsins are proteins found in photoceptors that can be divided into various spectral classes that correlate highly with sensitivity to specific wavelengths of light. Therefore, determining how many and which spectral classes of opsins are expressed in the lionfish' retinas allows us to conclude whether they have the physiological basis for color vision. To then behaviorally test whether lionfish indeed have color vision, we performed an associative learning experiment. We presented lionfish with two color choices, green and blue, and trained them over a series of trials to associate a food reward with one of the two colors. The latter test is vital, as while physiological evidence can suggest the potential for certain visual capabilities in species, behavioral assays can determine if visual cues in the environment are not only detected in the eye, but processed further in the brain resulting in use of the information when interacting with one's environment (Kelber et al., 2003). We predict that lionfish can learn to distinguish the color cues from one another, which would demonstrate that lionfish are able to see a difference between the two colors and possess color vision in the evaluated wavelength range.

Finally, we carry-out a molecular evolution test, to investigate the evolutionary forces shaping the lionfish's visual system and understand their visual pigment molecular evolution in the context of other teleost fish. Testing the evolutionary history of lionfish visual capabilities in this way allows us to hypothesize on if lionfish developed their color vision abilities as a consequence of the invasive process, or if it is a trait previously held by lionfish that has become beneficial in the new invasive environment.

Methods

Retinal transcriptome sequencing and opsin identification

Sample acquisition and retinal RNA sequencing

Retinal samples were collected from Lionfish in Curaçao. We collected retina samples from three lionfish (standard lengths 7.5, 10.6, and 25.5cm) that were euthanized in an ice water bath, with retina dissections was carried out immediately after death. We obtained six retinas corresponding to the right and left eyes of the three lionfish we collected.

Retinal samples were preserved in RNAlater (Ambion, Austin, TX) and transported to Wageningen University & Research genome center. We extracted RNA for each retina following standard protocol and verifying RNA concentration and integrity. Out of the six retinas we collected, two had to be discarded as we obtained to little RNA for sequencing. For the four remaining samples, we prepared mRNAseq libraries for each retina following standard protocol for the illumina TruSeq Stranded mRNA kit. Samples were later sequenced using polyA mRNAseq on a Illumina platform obtaining reads between 35-151bp.

Data filtering and de novo assembly

We evaluated the quality of raw reads using FastQC v0.11.9 (Andrews, 2010) and trimmed reads with Trimmomatic v0.38 (Bolger et al., 2014), removing adapter sequences with the ILLUMINACLIP option and preloaded adapter sequences. We eliminated base pairs with low Phred scores using SLIDINGWINDOW 28 with a window size of 20 base pairs (Sewe et al., 2022). The resulting processed reads were later used to build a de novo assembly with Trinity v2.15.1 (Grabherr et al., 2011) using default settings. There is no reference genome for the lionfish or any closely related species, eliminating the possibility to perform a genome-guided assembly. Once we built a de novo assembly, we proceeded multiple steps of filtering and quality control to obtain a de novo reference transcriptome for lionfish retinas. Finally, we normalized transcript abundance using the TPM method

Table 3.1 *Metrics for the resulting lionfish de novo assembly.*

Illumina processed reads	
Number of reads	243,231,026
Lowest Phred score	36
Assembled contigs	
Number of transcripts	325,577
N50	2,297 bp
Ex90N50	2,501 bp
GC (%)	45.07
Length	447,302,069 bp
Largest contig	26,346 bp
Assembled contigs after depuration	
Number of transcripts	22,440
N50	1,671 bp
Ex90N50	1,812 bp
GC (%)	53.64
Length	31,563,378 bp
Largest contig	16,608 bp

Assembly completeness and transcript abundance estimation: We estimated transcript abundance and descriptive metrics for the assembly with Quast v5.2.0 (Gurevich et al., 2013)(Table 3.1). Additionally, we estimated Ex90N50 and transcript abundance using the Trinity toolkit v2.15.1 (Grabherr et al., 2011) and Kallisto v0.46. 2 (Bray et al., 2016). Ex90N50 is better than N50 to assess completeness in transcriptome assemblies as not necessarily the shortest contigs correspond to the most highly expressed transcripts. Ex90N50 is calculated exactly as the regular N50 metric but it considers only the transcripts that represent 90% of the total normalized expression level, avoiding an N50 statistic that is being dragged to smaller values by barely expressed genes (Hölzer & Marz, 2019). In order to improve the quality of our transcriptome assembly we estimated several measures of transcriptome completeness based on mapping of universal ortholog genes with BUSCO v5.4.6 (Simão et al., 2015). BUSCO uses the OrthoDB curated database providing a quantitative measure for completeness. We evaluated transcriptome completeness of the initial assembly and after following filtering procedures (Table 3.1).

Table 3.2 *Known opsin sequences used for assembly annotation.*

Opsin	Species	Accession
RH1-1	*D. rerio*	KT008392
RH1-2	*D. rerio*	KT008393
RH2-1	*P. reticulata*	LC127186
RH2-2	*D. rerio*	KT008395
RH2-3	*D. rerio*	KT008396
RH2-4	*D. rerio*	KT008397
SWS1	*P. reticulata*	LC127190
SWS2A	*P.caymanensis*	KX768635
SWS2B	*P. reticulata*	LC127189
LWS1	*P. reticulata*	KT905991
LWS2	*P. reticulata*	LC127183
LWS3	*P. reticulata*	LC127184

Non-coding RNA (ncRNA) filtering: We identified non-coding RNA (ncRNA) using known ncRNA sequences from the Channel bull blenny (Cottoperca gobio) and lumpfish (Cyclopterus lumpus, Perciformes) obtained from Ensembl v109.1 (Cunningham et al., 2022). Species were selected because of their closeness to our focal species and data availability. Our assembled transcriptome was compared to all ncRNA sequences for each reference species using NCBI BLAST (BLASTn v2.14.0) (P. J. A. Cock et al., 2015) and a custom python (v3.9.1) script.

Best isoform obtention and coding regions detection: It is important to keep only one isoform per gene, keeping those that are likely to be more relevant. Transcript abundance normalized value using the TPM method (Kallisto v0.46. 2,) (Bray et al., 2016), was used to determine and keep only the isoform with the highest average expression. In the case that two isoforms had the same level of expression, we kept the longest transcript. Finally, the assembly was analyzed with tansdecoder v5.5.0 (Haas et al., 2013) to maintain only those transcripts that correspond to an open reading frame or coding DNA.

Opsin annotation

To identify all transcripts that correspond to any opsin in our lionfish assembly, we used a representative sequence of each opsin type and subtype. We know fish have Rh1-1 and Rh1-2 and opsins Rh2-1, Rh2-2, Rh2-3, Rh2-4, Sws1, Sws2A, Sws2B, as well as Lws-1, Lws-2 and Lws-3 (Table 3.2). We thus used representatives of all known fish opsins to identify all opsin transcripts in our lionfish assembly. We performed a nucleotide blast (Blastn) against the lionfish assembly, keeping the best reciprocal hit for each opsin type among the assembly's transcripts. Then we confirmed our identification of each opsin by performing a Blast of each transcript against NCBI´s database and verify its best blast hits matched the expected opsin type and subtype.

Behavioral Analysis of Lionfish Color Vision

Animal Husbandry

The red lionfish used in this study (n=6) were supplied to us by the Coral and Fish Store (Breda, Netherlands). They originated from their native range in Indonesia and ranged from 7.9 - 10.4cm in length (average = 9.6cm). Fish were socially housed in 400 liter tanks in groups of three or four (some lionfish housed in tanks were not used in experiment), with the exception of one individual that was individually housed in a 60 liter tank (Simba). All tanks were connected by the same flow-through filtration system and kept at a temperature of ± 24°C, pH of ±8.0, and salinity of ±31ppt, with a 12:12 light/dark cycle. Each socially housed tank contained two PVC pipe structures for the fish to use as a shelter, while the tank housing the individual contained one PVC pipe segment. Fish were fed daily, Monday through Friday, with a varied diet of thawed pieces of shrimp, mackerel, and cod to meet the nutritional needs of lionfish. Fish were fed until their stomachs were bulging (sign of satiation in lionfish) to provide semi-regular food intake for learning experiments and to prevent overeating, a common problem in aquarium lionfish.

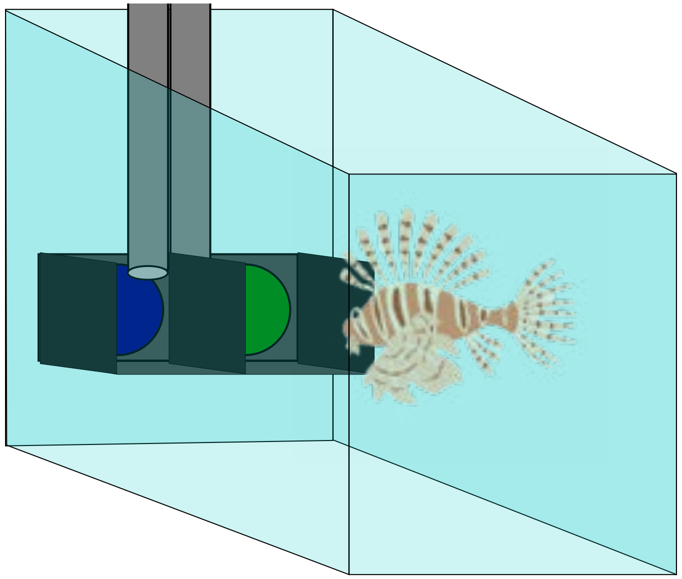


__Figure 3.1__ Diagram of the associative learning apparatus used to test for visual learning abilities in lionfish. View of the associative learning apparatus is from the fish's perspective. Black walls mark the walls of the apparatus, and the blue walls mark the walls of the tank. Food rewards were dropped through gray tubes and would land in compartments with corresponding color if the correct choice was made.

Experimental Apparatus

The learning apparatus used during the associative learning trials is shown in Figure 3.1. The body of the structure consisted of two square compartments, side-by-side, closed off by three walls and a bottom. The open side of each compartment pointed in the same direction so the content of both compartments could be seen at the same time by the approaching fish. Leaving the top of the compartment open allowed sufficient lighting for color-dependent tasks. At the top back of each compartment, a long tube was attached where food could be dropped by the experimenter as a reward. Both tubes were opaque except for the bottom 3cm so that fish associated seeing and receiving food with the inside of the compartments, rather than looking for food at the water line where food is initially dropped into the tube. Below each tube, a colored disk was hung on the back walls of the compartments during learning trials using metal hooks to be used as choice cues. The fish in this experiment had no previous trained association with the chosen colors, but were previously trained using the apparatus to differentiate black and white cues (de Groot, 2021). The learning trials in this study took place between December 2020 and January 2021.

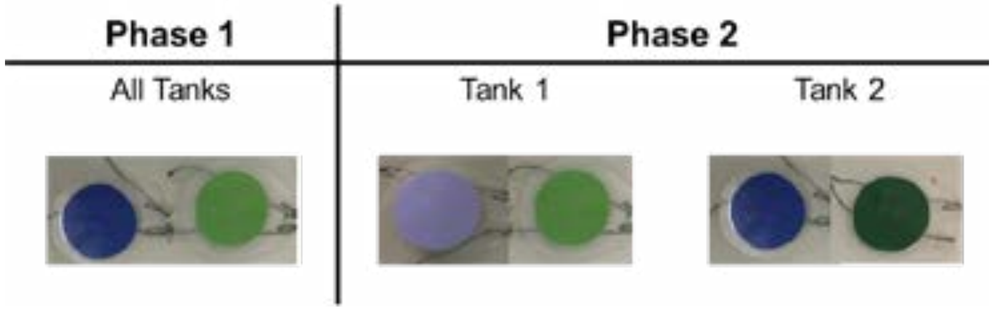


__Figure 3.2__ Color cues used during Phase 1 and Phase 2 of the experiment. The first image on the left shows the color cues used for all fish during the first nine days of learning trials (Phase 1). The other two images show the color cues used in Phase 2, with the center image of the color cues used in tank 1 (trained with green as the reward cue) and the right-most image of the color cues used in tank 2 (trained with blue as the reward cue).

Experimental Protocol

Fish were pre-trained to feed from the learning apparatus prior to the start of the experiment. During pre-training, fish were exposed to the apparatus within their housing tank and rewarded with food when they approached, regardless of the compartment they chose. Once all fish consistently approached the apparatus when food was offered, learning trials began. Trials were conducted daily, Monday through Friday, and individual fish participated in up to 19 trials per day (average = 4.9). The trials were carried out by one experimenter (H.D.), reducing observer bias. To start a trial, the apparatus was lowered into the tank with the open compartments aimed a target individual. Target individuals were chosen to ensure that all individuals were fed through learning trials. Two colors (blue and green, see Figure 3.2) were used as cues for associative learning and presented as colored disks, with one color randomly assigned to each compartment during a trial, with the limitation that colors were not placed on the same side for more than a maximum of three times in a row per fish. We chose these two colors as physiological research has shown that lionfish possess sensitivity peaks at these wavelengths (Hasenei et al., 2020).

For each fish one of the two colors was assigned to be the “rewarded color” and the other color assigned the “distractor”. We scored an approach as a ‘choice’ when a fish swam into a compartment of the apparatus and could no longer see the color cue in the other compartment. If the rewarded color was chosen, this was considered a correct choice, and a piece of food was dropped through the tube leading to the chosen compartment. If the distractor color was chosen, this was considered an incorrect choice; no food was given and the feeding apparatus was carefully lifted from the tank. Assigned rewarded and distractor colors for each fish were kept consistent throughout the learning trials, with two of the fish trained with blue as the rewarded color (Jaws and Luther), and the other four with green as the rewarded color (Nala, Sarabi, Scar, and Simba). We assigned the same reward color to all fish within one housing tank to minimize the chance of confusion due to social learning of other color associations from tankmates. After nine days of learning trials (Phase 1), luminance of the distractor color was altered to match the luminance of the rewarded color in order to test if fish used chromaticity alone to differentiate the color cues (Phase 2). This was done since luminance is an achromatic cue that lionfish could use to learn the association without actually possessing color vision (Kelber et al., 2003). Therefore, by removing the difference in luminance between the two color cues, continued success at the associative learning task would show that lionfish can use purely chromatic cues to distinguish the cues. Figure 3.2 shows the color cues used for each tank during the different phases.

Statistical Analysis

Data analysis was carried out in R Studio (R version 4.2.3). To test for learning in the lionfish, the likelihood of choosing the rewarded color was evaluated using a generalized linear mixed model (using R Studio package “lme4”) with trial number as a fixed factor, and Individual ID, reward color side, and phase as random factors. Individual lionfish were also tested for learning using a chi-squared test for goodness of fit that compared the proportion of correct and incorrect responses to chance.

Visual pigment molecular evolution

Opsin gene sequences and phylogeny

To carry out molecular evolution analyses, we built a phylogeny including our focal species, the lionfish. We initially constructed a teleost fish opsin sequence database with a total of 172 coding sequences for eight opsin gene types (Rh1-1, Rh1-2, Rh2-1, Rh2-2, Rh2-3, Rh2-4, Sws1 and Sws2A) based on available sequences (Sup. Table S3.1). Coding sequences were obtained from GenBank, previously published work and genomic analysis from Lin et al. (2017). We included the opsins sequences we identified for the lionfish in this database. Additionally, we used BLAST’s top 100 results using the lionfish coding sequence as a query to ensure we were not missing any species with available sequences. For every opsin, stop codons were eliminated and sequences were aligned using the MUSCLE algorithm, as implemented in MEGA 11 (Tamura et al., 2021).

Next, we built a phylogenetic tree that included all species in our database using The Fish Tree of Life, as implemented in R with packages fishtree and ape (Chang et al., 2019; Paradis et al., 2004)(Chang et al., 2019; Paradis et al., 2004). The spotted gar (Lepisosteus oculatus) was selected as an outgroup and is the only non-teleost fish included in the resulting phylogenetic tree. We used fossil calibration constraints for constructing the phylogeny as detailed in Rabosky et al. (2018).

Visual pigment molecular evolution

Selection tests were implemented in PAML (Yang, 2007) using the visual interface PAML-X (Xu & Yang, 2013) to investigate the patterns of molecular evolution is teleost fish opsins and the lionfish branch. We used random sites models and branch models, both implemented in PAML, running separate analyses were run for each opsin type.

We relied on codon-based random sites models to test for evidence of positive selection. The topology shown in Figure 3.3 was used as input in a framework consisting of alternative models differentially constraining ω and evaluating evidence for sites evolving under positive selection. The one-ratio model (M0), which assumes a constant ω for all sites in the alignment, was the simplest model included in our analysis (Yang & Nielsen, 1998). Four additional models were included to comparatively test for positive selection, particularly in neutral and selection pairs: M1a (neutral) vs. M2a (positive selection) (Wong et al., 2004; Yang et al., 2005) and M7 (beta) vs. M8 (beta + ω) (Yang, 2000). The five models estimated ω = dN /dS via a likelihood framework with multiple variables and we ran all of them with different starting ω values to reduce the chance of reaching local optima. To evaluate whether the more parameter-rich models (M2a and M8) fit the data better than their simpler counterparts (M1a and M7, respectively) we used likelihood ratio tests. Additionally, a Bayes Empirical Bayes (BEB) analysis allowed us to identify specific sites inferred to have evolved under positive selection (Zhang et al., 2005).

We also wanted to test for positive selection along the lionfish branch as evidence that this species visual system adapted to a variable light environment. For this purpose, we used branch models which allow us to evaluate whether there are positively selected sites along particular lineages (Yang & Nielsen, 1998). Again, likelihood ratio tests were used to evaluate if a parameter-rich model fit better than a null, simple model. Hence, the null model allowed all branches to share the same ω value while the alternative model assigned P. volitans as the foreground branch, allowing it to have a different ω value compared to the rest of the tree. By comparing these two models, we asked whether the lionfish lineage had a significantly higher ω than the background as well as checked if it was significantly higher than 1.0.

Results

Opsins expressed in the lionfish retina

After building a de novo assembly and performing all the appropriate filters, we obtained a de novo transcriptome of the lionfish retinas from three different individuals (Table 3.1). We used this transcriptome to identify all transcripts matching an opsin to identify which opsins are expressed in the retinas of lionfish. This was done using a reference opsin from other fish species as queries (Table 3.2). We looked for transcripts matching rhodopsins Rh1-1 and Rh1-2 and opsins Rh2-1, Rh2-2, Rh2-3, Rh2-4, Sws1, Sws2A, Sws2B, as well as Lws-1, Lws-2 and Lws-3 as detailed in Table 3.2. We identified multiple opsin types and subtypes in our final assembly (Table 3.3). It is worth noting that hits only reached identity values up to 81%, likely due to the phylogenetic distance between the lionfish and the species used as queries in our opsin blast. We found no trustable matches beyond weak hits for opsin Sws2-B or any of the Lws subtypes in our final assembly. To make sure we did not filter out reads matching these opsins during our assembly construction and quality processing, we performed a similar blast on the raw reads. Once more we did not find hits with a high percentage of identity for Sws2-B or any of the Lws subtypes. Our findings suggest these opsins are either not expressed or expressed at very low levels in the retinas of lionfish.

Table 3.3 *Opsin annotation using Blastn*

Opsin	Associated sensitivity	Transcript id	Percentage identity (%)	Hit length (bp)
RH1-1	Rhodopsin	DN2573_c0_g1_i5.p3	73.26	374
RH1-2	Rhodopsin	DN398_c0_g2_i1.p1	73.44	1073
SWS1	Short	DN32349_c0_g1_i2.p1	80.40	995
SWS2A	Short	DN3348_c0_g1_i6.p1	81.02	1080
SWS2B	Short	No relevant hits encountered		
RH2-1	Medium	DN26211_c0_g1_i2.p1	77.58	339
RH2-2	Medium	DN715_c0_g1_i2.p1	71.68	1045
RH2-3	Medium	DN10011_c0_g1_i1.p1	78.32	678
RH2-4	Medium	DN715_c0_g3_i5.p1	76.88	960
LWS-1	Long	No relevant hits encountered		
LWS-2	Long			
LWS-3	Long			

We estimated normalized expression levels for opsin transcripts that we identified in our lionfish assembly. We found rhodopsin RH1-2 has the highest expression levels (Figure 3.3), followed by cone opsin RH2-2 (Figure 3.3). This expression profile is consistent with what has been previously reported in nocturnal fish, in which rhodopsin expression is higher than cone opsins' (Luehrmann et al., 2018).

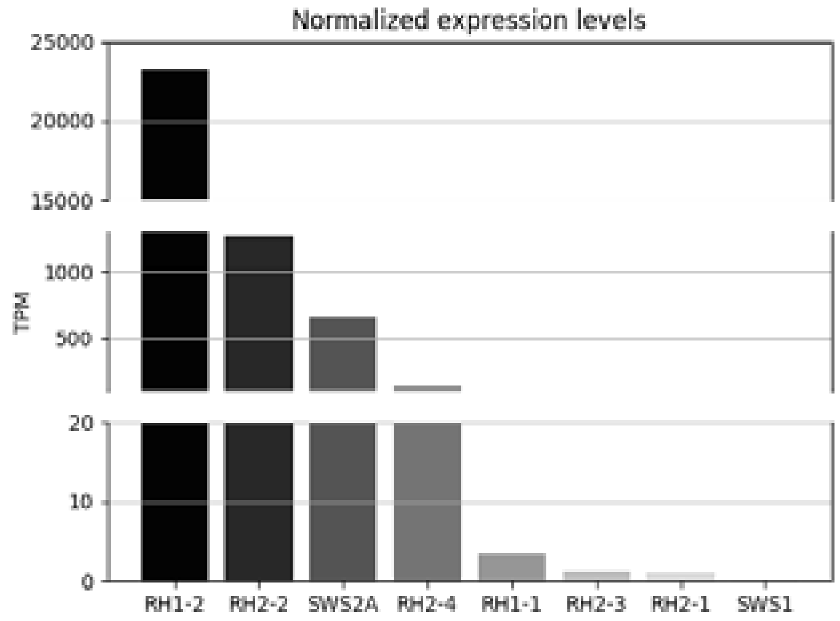


Figure 3.3 *Normalized expression levels for the Lionfish opsins identified in the assembly in transcripts per million (TPM).*

Behavioral Analysis of Lionfish Color Vision

We found that overall, lionfish showed evidence of learning, as success rate was significantly correlated with trial number (GzLMM; z = 2.78, p = 0.005; Figure 3.4). We found no significant effect of phase (df = 1, p = 1), on success rate, indicating that the change to controlled luminance did not impact lionfish's ability to choose the correct color. In contrast, the side at which we presented the reward significantly affected success rate (df = 1, p = 0.024), indicating a potential side preference. However, when we evaluated learning at the individual level using a chi-squared goodness of fit test, we found that five of the six fish showed learning as they chose the rewarded cue more often than would be expected by chance (Table 3.4). The one fish that did not learn (Scar) had an extreme side preference for the left and choose it almost every time, regardless of where the reward color was (x2 = 42.6, p < 0.001). We therefore re-ran the above model without including the outlier individual and found that while trial number was still significant (z = 2.56, p = 0.0105), side was no longer significant (df = 1, p = 1).

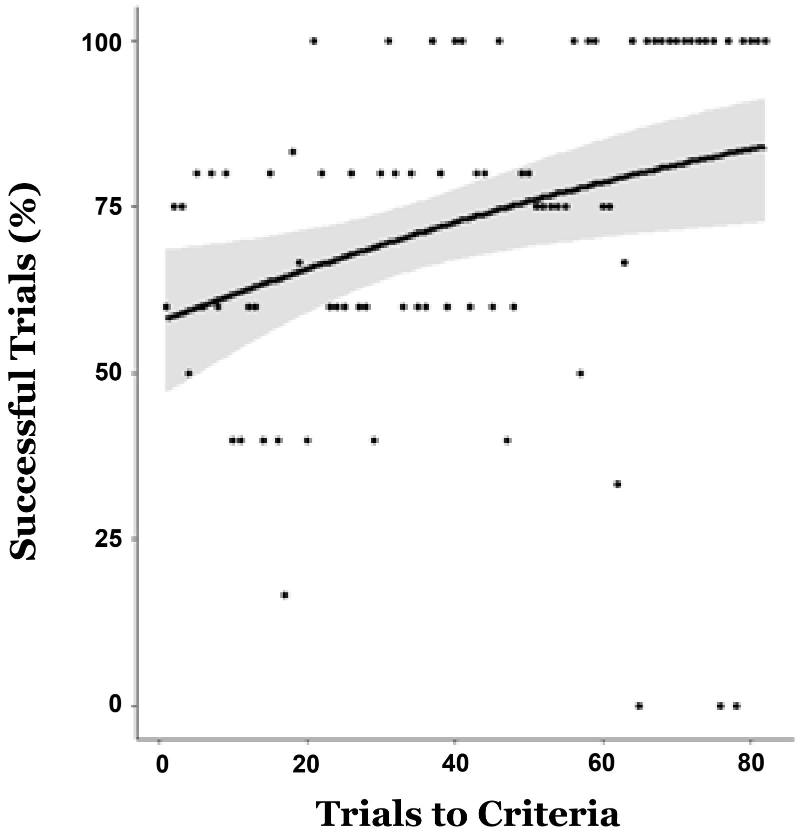


Figure 3.4 *Overall Learning Figure. The line indicates the success rate of each trial as determined by the generalized linear model. Each circle represents the mean success rate of all individual for each given trial.*

Table 3.4 *Individual Learning Table*

Individual	**Reward Color**	**Total Trials**	**Correct Choices**	**Incorrect Choices**	**x^2 statistic**	**p value**
Jaws	Blue	50	38	12	13.52	**0.00024**
Luther	Blue	82	54	28	8.24	**0.00409**
Nala	Green	64	45	19	10.56	**0.00115**
Sarabi	Green	63	44	19	9.92	**0.00163**
Scar	Green	71	37	34	0.13	0.72181
Simba	Green	61	43	18	10.25	**0.00137**

Visual pigment molecular evolution

Considering the species included in our database of teleost fish opsin sequences, we built a phylogeny that could be used in all our subsequent visual pigment molecular evolution analyses. The phylogeny we constructed is as shown in Figure 3.5, including 25 selected fish species with available coding sequences for all (or most) of the studied opsins. The resulting set of fishes represents a total of 15 families with available opsin coding sequences (Sup. Table S3.1).

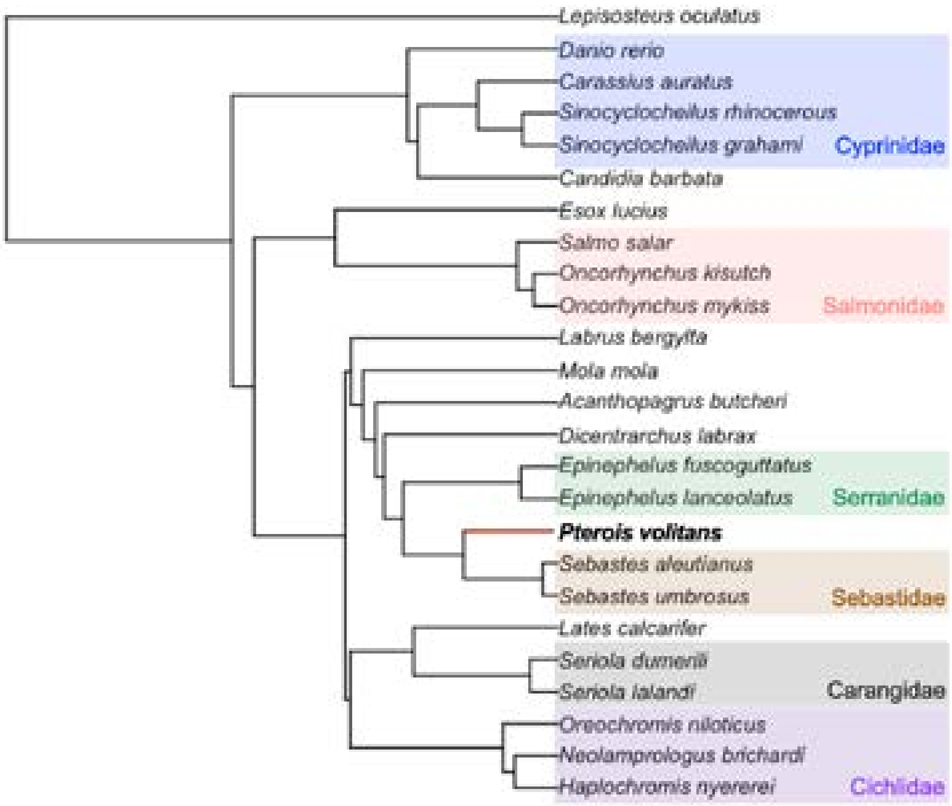


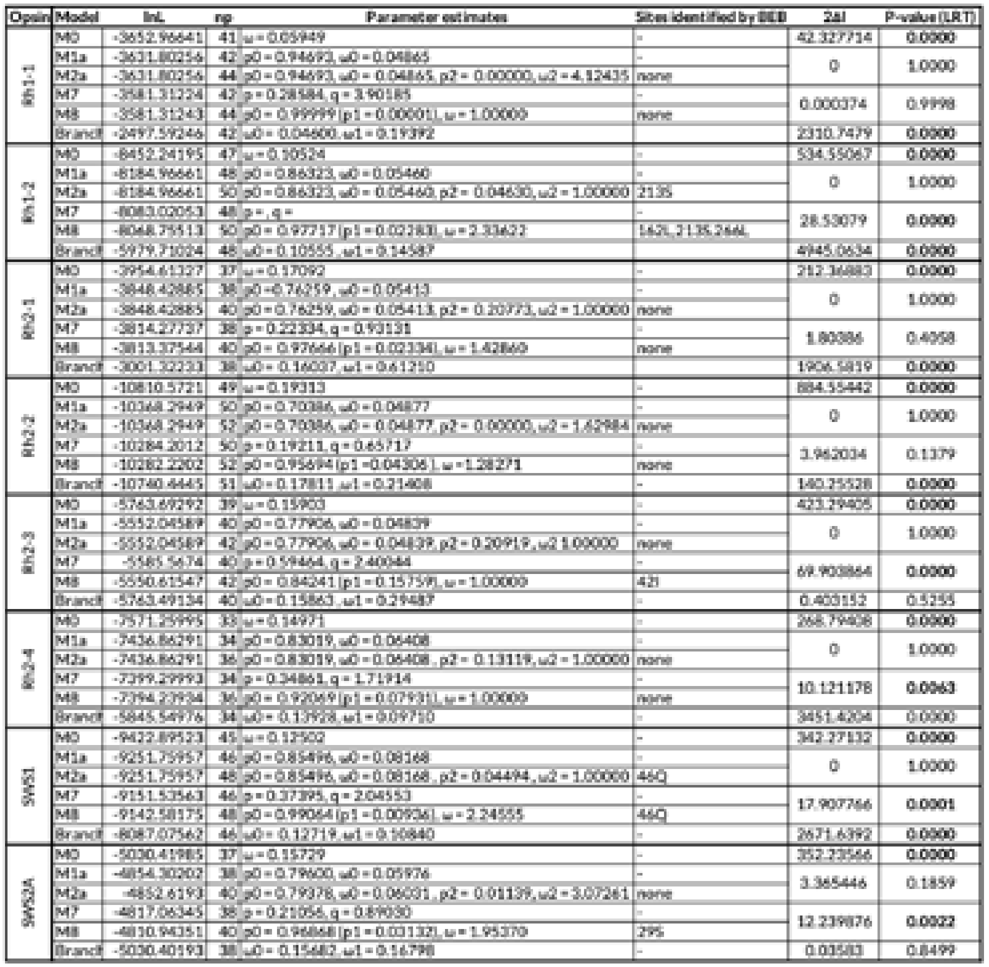
Figure 3.5 *Phylogeny including the 25 fish species used for visual pigment molecular evolution tests. The spotted gar (L. oculatus) was used as an outgroup. The lionfish (P. volitans) is highlighted in bold and was the branch set as foreground for branch models. All species not included in the Cyprinidae, Salmonidae, Serranidae, Sebastidae, Carangidae or Cichlidae families are the sole representatives of their respective family.*

Molecular evolution tests

We performed random sites models to test for evidence of different evolutionary forces across the tree for each opsin. We found the M1a models are a better fit the data that the M0 models, showing vast heterogeneity in the selective forces detected across sites (highly significant p-values <0.0001, Table 3.5). We carried out the corresponding likelihood-ratio tests (LRT) to evaluate whether the more parameter rich models were indeed a better fit to the data. However, we only found evidence that M8 is a better fit to the data when compared to its counterpart, M7. For the M1a-M2a comparisons the LRT were not significant in all cases, suggesting we cannot draw evidence of divergent selection across opsin genes from the comparison of M1a-M2a models (Table 3.5). We further performed a BEB procedure associated with M8 models identifying sites under selection for genes in Rh1-2, Rh2-3, SWS1 and SWS2A (Table 3.5).

We the carried-out branch models to detect selection along the lionfish lineage. In branch models ω is allowed to vary in the lineage selected as foreground, as opposed to the random sites models where all branches had a single ω value. Overall, these models showed significantly different ω along the lionfish lineage, however these values were not above 1 for any of the opsins. Values for the rate of nonsynonymous/synonymous mutations within the lionfish branch (ω1) were larger than in of the rest of the tree (ω0) for most opsins, but not above one. LRT tests were significant (p<0.01) for both Rh1 opsins, Rh2-1 and Rh2-2, and SWS1 (Table 3.5).

Table 3.5 *Results of PAML random sites and branch models for each opsin sequence. Significance based on Likelihood Ratio Test (LRT) which is twice the log-likelihood (lnL) difference between the two compared models. The associated p-values were calculated using a chi-square test, significant values (<0.01) are highlighted in bold. Tests were performed to compare M0-M1a, M1a-M2a and M7-M8. Positively selected sites by Bayes Empirical Bayes (BEB) identification are shown where applicable. All branch models selected the lionfish as foreground and results were compared to M0 for comparison.*



Opsin	Model	lnL	np	Parameter estimates	Sites identified by BEB	2Δl	P-value (LRT)
Rh1-1	M0	-3652.96641	41	ω = 0.05949	-	42.327714	**0.0000**
	M1a	-3631.80256	42	p0 = 0.94693, ω0 = 0.04865	-	0	1.0000
	M2a	-3631.80256	44	p0 = 0.94693, ω0 = 0.04865, p2 = 0.00000, ω2 = 4.12435	none		
	M7	-3581.31224	42	p = 0.28584, q = 3.90185	-	0.000374	0.9998
	M8	-3581.31243	44	p0 = 0.99999 (p1 = 0.00001), ω = 1.00000	none		
	Branch	-2497.59246	42	ω0 = 0.04600, ω1 = 0.19392		2310.7479	**0.0000**
Rh1-2	M0	-8452.24195	47	ω = 0.10524	-	534.55067	**0.0000**
	M1a	-8184.96661	48	p0 = 0.86323, ω0 = 0.05460	-	0	1.0000
	M2a	-8184.96661	50	p0 = 0.86323, ω0 = 0.05460, p2 = 0.04630, ω2 = 1.00000	213S		
	M7	-8083.02053	48	p = , q =	-	28.53079	**0.0000**
	M8	-8068.75513	50	p0 = 0.97717 (p1 = 0.02283), ω = 2.33622	162L, 213S, 266L,		
	Branch	-5979.71024	48	ω0 = 0.10555 , ω1 = 0.14587		4945.0634	**0.0000**
Rh2-1	M0	-3954.61327	37	ω = 0.17092	-	212.36883	**0.0000**
	M1a	-3848.42865	38	p0 = 0.76259, ω0 = 0.05413	-	0	1.0000
	M2a	-3848.42865	40	p0 = 0.76259, ω0 = 0.05413, p2 = 0.20773, ω2 = 1.00000	none		
	M7	-3814.27737	38	p = 0.22334, q = 0.93131	-	1.80386	0.4058
	M8	-3813.37544	40	p0 = 0.97666 (p1 = 0.02334), ω = 1.42860	none		
	Branch	-3001.32233	38	ω0 = 0.16037, ω1 = 0.61210		1906.5819	**0.0000**
Rh2-2	M0	-10810.5721	49	ω = 0.19313	-	884.55442	**0.0000**
	M1a	-10368.2949	50	p0 = 0.70386, ω0 = 0.04877	-	0	1.0000
	M2a	-10368.2949	52	p0 = 0.70386, ω0 = 0.04877, p2 = 0.00000, ω2 = 1.62984	none		
	M7	-10284.2012	50	p = 0.19211, q = 0.65717	-	3.962034	0.1379
	M8	-10282.2202	52	p0 = 0.95694 (p1 = 0.04306), ω = 1.28271	none		
	Branch	-10740.4445	51	ω0 = 0.17811, ω1 = 0.21408	-	140.25528	**0.0000**
Rh2-3	M0	-5763.69292	39	ω = 0.15903	-	423.29405	**0.0000**
	M1a	-5552.04589	40	p0 = 0.77906, ω0 = 0.04839	-	0	1.0000
	M2a	-5552.04589	42	p0 = 0.77906, ω0 = 0.04839, p2 = 0.20919 , ω2 1.00000	none		
	M7	-5585.5674	40	p = 0.59464, q = 2.40044	-	69.903864	**0.0000**
	M8	-5550.61547	42	p0 = 0.84241 (p1 = 0.15759), ω = 1.00000	42I		
	Branch	-5763.49134	40	ω0 = 0.15863 , ω1 = 0.29487	-	0.403152	0.5255
Rh2-4	M0	-7571.25995	33	ω = 0.14971	-	268.79408	**0.0000**
	M1a	-7436.86291	34	p0 = 0.83019, ω0 = 0.06408	-	0	1.0000
	M2a	-7436.86291	36	p0 = 0.83019, ω0 = 0.06408 , p2 = 0.13119, ω2 = 1.00000	none		
	M7	-7399.29993	34	p = 0.34861, q = 1.71914	-	10.121178	**0.0063**
	M8	-7394.23934	36	p0 = 0.92069 (p1 = 0.07931), ω = 1.00000	none		
	Branch	-5845.54976	34	ω0 = 0.13928, ω1 = 0.09710	-	3451.4204	0.0000
SWS1	M0	-9422.89523	45	ω = 0.12502	-	342.27132	**0.0000**
	M1a	-9251.75957	46	p0 = 0.85496, ω0 = 0.08168	-	0	1.0000
	M2a	-9251.75957	48	p0 = 0.85496, ω0 = 0.08168 , p2 = 0.04494 , ω2 = 1.00000	46Q		
	M7	-9151.53563	46	p = 0.37395, q = 2.04553	-	17.907766	**0.0001**
	M8	-9142.58175	48	p0 = 0.99064 (p1 = 0.00936), ω = 2.24555	46Q		
	Branch	-8087.07562	46	ω0 = 0.12719, ω1 = 0.10840	-	2671.6392	**0.0000**
SWS2A	M0	-5030.41985	37	ω = 0.15729	-	352.23566	**0.0000**
	M1a	-4854.30202	38	p0 = 0.79600, ω0 = 0.05976	-	3.365446	0.1859
	M2a	-4852.6193	40	p0 = 0.79378, ω0 = 0.06031 , p2 = 0.01139, ω2 = 3.07261	none		
	M7	-4817.06345	38	p = 0.21056, q = 0.89030	-	12.239876	**0.0022**
	M8	-4810.94351	40	p0 = 0.96868 (p1 = 0.03132), ω = 1.95370	29S		
	Branch	-5030.40193	38	ω0 = 0.15682, ω1 = 0.16798	-	0.03583	0.8499

Discussion

In our experiments, we provide some of the first evidence for color vision capabilities of lionfish through a mix of transcriptome and behavioral assays. First, by sequencing the retinal transcriptome of lionfish, we provide evidence that lionfish express two rhodopsins, Rh1-1 and Rh1-2, and at least six cone opsins (Sws1, Sws2A, Rh2-1, Rh2-2, Rh2-3, Rh2-4). In contrast, we did not find any evidence that they express any long-wavelength-sensitive opsin LWS. We identified transcripts corresponding to the two rhodopsin subtypes (Rh1-1 and Rh1-2), the latter one was also very highly expressed. Indeed, Rh1-2 was the most highly expressed opsin in the retinas we sequenced. Previous studies have identified similar patterns of expression in nocturnal teleost species, compared to diurnal species rhodopsin for which rhodopsin expression is generally lower (Luehrmann et al., 2018). We therefore find evidence that this crepuscular hunter species maintains visual system characteristics of nocturnal species that would allow them to hunt as light amount drastically changes at dawn and dusk. Interestingly, we see some convergence in what has been observed in terrestrial crepuscular hunters that have maintained their ability to see well in dim-light, scotopic environments (Robertson et al., 2022).

We also found that lionfish express multiple types of cone opsins. More specifically, four medium-wavelength sensitive opsins (Rh2-1, Rh2-2, Rh2-3, Rh2-4), one short-wavelength sensitive type 1 opsin (Sws1), and one short-wavelength sensitive type 2 opsin (Sws2-A). Of course, this set of opsins could change with age, and we are only able to ascertain adult lionfish like the ones we collected express the above-mention set of cone opsins. As one requirement for color vision is to possess more than two photoreceptor types and their corresponding visual pigments, our transcriptomic findings indicate that lionfish potentially have color vision as they have multiple visual pigments in their retinas.

On the other hand, we failed to find evidence that lionfish express Sws2-B, or any of the Lws subtypes. We know species tend to express either Sws1, Sws2-A or Sws2-B at high levels as the expression of any of these is upregulated the expression of the remaining two decreases (Luehrmann et al., 2018; O'Quin et al., 2010). Here, we see high levels of expression for Sws2-A, offering an explanation as to why we failed to see any expression of the Sws2-B opsin gene. We also failed to find transcripts matching any of the LWS opsin subtypes. These opsins could be expressed at very low levels or may be absent. However, this result is congruent with previous studies in other teleosts, in which Lws was found to be often absent or expressed at very low levels depending on the light spectrum that fish are being exposed to or their age (Flamarique, 2013; O'Quin et al., 2010; A. R. Smith et al., 2012).

Despite having molecular evidence that lionfish possess the cone opsins required for color vision, on its own these data are not enough to conclude that lionfish can see in color. Therefore, we performed a complimentary behavioral assay which revealed that lionfish can use color cues in a food learning trial. By showing the ability to associate color cues with a food reward through learning, we found substantial evidence that lionfish must be able to distinguish between the blue and green color stimuli used, and therefore demonstrate evidence of color vision capabilities in this part of the spectrum. Furthermore, this ability to distinguish and choose the rewarded stimuli remained even when luminance was switched between phase 1 and phase 2. These phases were added to control for luminance, as matching luminance between cues in phase 2 would ensure that lionfish were learning an association using chromaticity rather than other non-chromatic information, as only using chromatic cues would be indicative of color vision abilities (Kelber et al., 2003). While further methods have been developed to account differences in luminance sensitivity across species, this method has been previously used to explore the basic color vision and spectral sensitivities of many species (Kelber et al., 2003; Kelber & Pfaff, 1999). In this way, further work could build on our methodology to increase our current understanding of the chromatic and achromatic cues lionfish use during learning, as well as continue to investigate the spectral sensitivity of lionfish at different wavelengths.

Combined, our results provide strong evidence for color vision abilities in lionfish. Color vision could represent an important trait to lionfish, as it may be key in their ability to adapt to local environments and maximize their invasion success through hunting. Indeed, lionfish may be using visual capabilities to their advantage to efficiently hunt using chromatic cues during daylight hours in invasive ranges, such as the Caribbean (Côté & Maljković, 2010). For instance, lionfish typically hunt at close range to their prey, using a strategy where they slowly approach prey before lunging (Hixon et al., 2016). Previous research has proposed that animals may rely on more chromatic cues when hunting at close range versus at a distance (Potier et al., 2018), supporting our hypothesis that lionfish may benefit from color vision

while hunting. Additionally, one study found in a variety of environments within the Caribbean, fish were found in significantly lower densities during nocturnal periods in comparison to the daylight periods (Nagelkerken et al., 2000) While other studies have found that prey fish availability is even higher in crepuscular periods compared to daylight hours, this is also when most predatory fish are active, increasing potential competition between predatory fish for access to food (Green et al., 2011; Hobson, 1973). Therefore, lionfish might be able to take advantage of the decrease competition during diurnal periods due to their visual capabilities, leading to increased prey availability, and increased hunting and invasive success for this species. However, further testing is needed to determine if lionfish's visual capabilities have any influence on their hunting efficiency, or more generally their invasive success.

Next, as an insight into how lionfish vision may have evolved, we traced sequence evolution of visual pigments across 25 species of teleost fish, including the lionfish, and found strong conservatism for all opsins. We considered the possibility that rates of evolution were faster in the lionfish clade, as a signature of adaption as it invaded diverse environments. We did not however find evidence of positive selection in any of the opsins. Values were slightly more elevated for Rh2-1 ($\omega 1 = 0.61$ compared to $\omega 0 = 0.16$), but remains below 1. This is consisted with a hypothesis where these generalists have a visual system that allows them to invade multiple environments without evolving special adaptations for them. It is important to keep in mind that our sequences come from a small sample of lionfish, all originating from the same population. It is possible a larger sample could reveal other mutations in populations that have invaded other habitats.

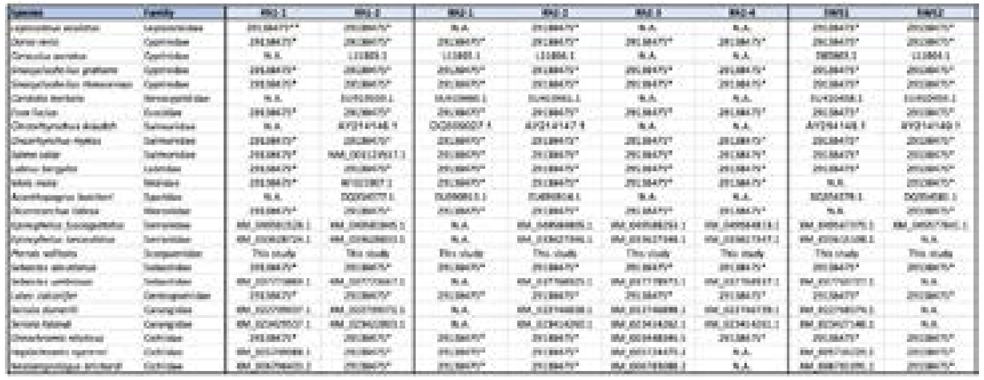
Opsin evolution is constrained by multiple selective pressures imposed by the environment and an organism's ecology, in addition to the function of the molecule. Constraints that arise from the function of visual pigments and complexity of the visual system are likely to result in strong purifying selection on opsins (Bloch, 2015). This expected conservatism is exactly what we see across the teleost fish we examined. We did find, however, evidence for some positively selected sites for RH1-2, RH2-3, SWS1 and SWS2-A as shown in Table 3.5. One site in particular, amino acid site 46, which was found to be positively selected for SWS1, is a site known to shift the spectral sensitivity of this opsin in mammals and of particular evolutionary importance (Hagen et al., 2023; Mitchell et al., 2021; Yokoyama et al., 2007)Sws1 tuning is known to have a string link to nocturnality across vertebrates (Hagen et al., 2023), and it is thus of great interest to find it varying and under positive selection across this dataset. Sites 162 and 213, under positive selection in RH1-2, were also identified to be under positive selection across anurans (Wan et al., 2023), and could very well be sites with a role in spectral tuning or visual pigment function that have yet to be studied in depth.

Based on these results, we can conclude that the color vision capabilities of lionfish in their invasive range are not a result of evolutionary processes during invasion, but rather that this is a conserved trait when compared to other teleost fish. This is interesting to note, as this further supports our hypothesis that lionfish may possess generalist visual capabilities that allow them to behaviorally adapt to local environments, rather than having evolved to specialize in a specific environment. This may be key to their invasive success, as behavioral flexibility of this kind has been predicted to be a strong factor impacting invasive success (Wright et al., 2010). Future studies can further investigate the implications of color vision in lionfish invasive success.

Overall, our findings support our hypothesis that crepuscular species, such as the lionfish, may show traits of both diurnal and nocturnal species rather than specialize in one of the other. This lack of specialization could potentially allow for flexibility in visual capabilities and may therefore lead crepuscular species to have a more generalist behaviors, such as in their hunting style. However, this may not always be the case, as other crepuscular hunters, such as the honey possum (Tarsipes rostratus) show specialized visual capabilities for the light levels at twilight, suggesting not all crepuscular species have generalist visual capabilities (Arrese et al., 2002). Indeed, we find that even some exclusively nocturnal species, such as the nocturnal helmet gecko (Tarentola chazaliae), possess some level of color vision capabilities, indicating some species might be more specialized and other more generalist, regardless of their diurnal cycle (Kelber & Roth, 2006; Roth & Kelber, 2004). For lionfish in particular though, we predict that this species has more generalist visual capabilities, complementing its generalist hunting style, and predict this may be beneficial to lionfish as an invasive species, since they can easily adapt to local light environments in invasive regions and have the opportunity to exploit unique hunting niches that other predatory species might not be able to. However, our research represents just the beginning of potential research questions to be answered about lionfish vision, and future research can investigate how the visual capabilities of lionfish impact their hunting ability and invasive success, as well as more generally about crepuscular predator vision.

Supplementary Table

Table S3.1 *Species with available opsin sequences used in PAML evolutionary analyses. All references are NCBI Accession numbers except those marked with an asterisk (*), which represent PubMed Unique identifiers (PMIDs) for their corresponding publications.*



References

Andrews, S. (2010). FastQC A Quality Control tool for High Throughput Sequence Data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Arrese, C., Archer, M., & Beazley, L. D. (2002). Visual capabilities in a crepuscular marsupial, the honey possum (Tarsipes rostratus): A visual approach to ecology. Journal of Zoology, 256(2), 151–158. https://doi.org/10.1017/s0952836902000183

Bariche, M., Torres, M., & Azzurro, E. (2013). The presence of the invasive Lionfish Pterois miles in the Mediterranean Sea. Mediterranean Marine Science, 14(2), 292–294.

Bloch, N. I. (2015). Evolution of opsin expression in birds driven by sexual selection and habitat. Proceedings of the Royal Society B: Biological Sciences, 282, 20142321. https://doi.org/10.1098/rspb.2014.2321

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics, 30(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bray, N. L., Pimentel, H., Melsted, P., & Pachter, L. (2016). Near-optimal probabilistic RNA-seq quantification. Nature Biotechnology, 34(5), 525–527. https://doi.org/10.1038/nbt.3519

Chang, J., Rabosky, D. L., Smith, S. A., & Alfaro, M. E. (2019). An r package and online resource for macroevolutionary studies using the ray-finned fish tree of life. Methods in Ecology and Evolution, 10(7), 1118–1124. https://doi.org/10.1111/2041-210X.13182

Cock, P. J. A., Chilton, J. M., Grüning, B., Johnson, J. E., & Soranzo, N. (2015). NCBI BLAST+ integrated into Galaxy. GigaScience, 4(1). https://doi.org/10.1186/s13742-015-0080-7

Cortesi, F., Mitchell, L. J., Tettamanti, V., Fogg, L. G., Busserolles, F. De, Cheney, K. L., & Marshall, N. J. (2020). Visual system diversity in coral reef fishes. Seminars in Cell and Developmental Biology, 106, 31–42. https://doi.org/10.1016/j.semcdb.2020.06.007

Côté, I. M., Green, S. J., & Hixon, M. A. (2013). Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. Biological Conservation, 164, 50–61. https://doi.org/10.1016/j.biocon.2013.04.014

Côté, I. M., & Maljković, 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

Cunningham, F., Allen, J. E., Allen, J., Alvarez-Jarreta, J., Amode, M. R., Armean, I. M., Austine-Orimoloye, O., Azov, A. G., Barnes, I., Bennett, R., Berry, A., Bhai, J., Bignell, A., Billis, K., Boddu, S., Brooks, L., Charkhchi, M., Cummins, C., Da Rin Fioretto, L., … Flicek, P. (2022). Ensembl 2022. Nucleic Acids Research, 50(Database issue), D988–D995. https://doi.org/10.1093/nar/gkab1049

de Groot, C. (2021). Exploring the cognitive landscape of an invasive species: reversal learning in the red lionfish (Pterois volitans). Wageningen University.

Dimitriadis, C., Galanidi, M., Zenetos, A., Corsini-Foka, M., Giovos, I., Karachle, P. K., Fournari-Konstantinidoy, 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 the thermal boundaries and future range expansion. Mediterranean Marine Science, 21, 62–69.

Dominy, N. J., & Lucas, P. W. (2001). Ecological importance of trichromatic vision to primates. Letters to Nature, 410, 363–366.

Dominy, N. J., Lucas, P. W., Osorio, D., & Yamashita, N. (2001). The Sensory Ecology of Primate Food Perception. Evolutionary Anthropology, 10, 171–186.

Flamarique, I. N. (2013). Opsin switch reveals function of the ultraviolet cone in fish foraging. Proceedings of the Royal Society B, 280, 20122490.

Gilad, Y., Wiebe, V., Przeworski, M., Lancet, D., & Pääbo, S. (2004). Loss of olfactory receptor genes coincides with the acquisition of full trichromatic vision in primates. PLoS Biology, 2(1), 0120–0125. https://doi.org/10.1371/journal.pbio.0020005

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., Di Palma, F., Birren, B. W., Nusbaum, C., Lindblad-Toh, K., … Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnology, 29(7), 644–652. https://doi.org/10.1038/nbt.1883

Green, S. J., Akins, J. L., & CôtÉ, I. M. (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

Gurevich, A., Saveliev, V., Vyahhi, N., & Tesler, G. (2013). QUAST: Quality assessment tool for genome assemblies. Bioinformatics, 29(8), 1072–1075. https://doi.org/10.1093/bioinformatics/btt086

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., Couger, M. B., Eccles, D., Li, B., Lieber, M., Macmanes, M. D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C. N., … Regev, A. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nature Protocols, 8(8), 1494–1512. https://doi.org/10.1038/nprot.2013.084

Hagen, J. F. D., Roberts, N. S., & Johnston, R. J. (2023). The evolutionary history and spectral tuning of vertebrate visual opsins. Developmental Biology, 493, 40–66. https://doi.org/10.1016/j.ydbio.2022.10.014

Hasenei, A., Kerstetter, D. W., Horodysky, A. Z., & Brill, R. W. (2020). Physiological limits to inshore invasion of Indo-Pacific lionfish (Pterois spp.): insights from the functional characteristics of their visual system and hypoxia tolerance. Biological Invasions, 22, 2079–2097. https://doi.org/10.1007/s10530-020-02241-5

Hixon, M. A., Green, S. J., Albins, M. A., Akins, J. L., & Morris, J. A. (2016). Lionfish: A major marine invasion. Marine Ecology Progress Series, 558, 161–165. https://doi.org/10.3354/meps11909

Hobson, E. S. (1973). Diel feeding migrations in tropical reef fishes. Helgolander Wiss. Meeresunters, 24, 361–370.

Hölzer, M., & Marz, M. (2019). De novo transcriptome assembly: A comprehensive cross-species comparison of short-read RNA-Seq assemblers. GigaScience, 8, 1–16. https://doi.org/10.1093/gigascience/giz039

Jacobs, G. H. (1996). Primate photopigments and primate color vision. Proceedings of the National Academy of Sciences of the United States of America, 93, 577–581.

Jacobs, G. H. (2018). Photopigments and the dimensionality of animal color vision. Neuroscience and Biobehavioral Reviews, 86, 108–130. https://doi.org/10.1016/j.neubiorev.2017.12.006

Kelber, A., & Pfaff, M. (1999). True Colour Vision in the Orchard Butterfly, Papilio aegeus. In Naturwissenschaften (Vol. 86). Springer-Verlag.

Kelber, A., & Roth, L. S. V. (2006). Nocturnal colour vision – not as rare as we might think. The Journal of Experimental Biology, 209, 781–788. https://doi.org/10.1242/jeb.02060

Kelber, A., Vorobyev, M., & Osorio, D. (2003). Animal colour vision - Behavioural tests and physiological concepts. Biological Reviews of the Cambridge Philosophical Society, 78(1), 81–118. https://doi.org/10.1017/S1464793102005985

Lin, J. J., Wang, F. Y., Li, W. H., & Wang, T. Y. (2017). The rises and falls of opsin genes in 59 ray-finned fish genomes and their implications for environmental adaptation. Scientific Reports, 7, 15568. https://doi.org/10.1038/s41598-017-15868-7

Luehrmann, M., Stieb, S. M., Carleton, K. L., Pietzker, A., Cheney, K. L., & Marshall, N. J. (2018). Short-term colour vision plasticity on the reef: Changes in opsin expression under varying light conditions differ between ecologically distinct fish species. Journal of Experimental Biology, 221, jeb175281. https://doi.org/10.1242/jeb.175281

Marshall, J. (2010). Why Are Animals Colourful? Sex and Violence, Seeing and Signals. Colour: Design & Creativity, 5(8), 1–8.

Mitchell, L. J., Cheney, K. L., Lührmann, M., Marshall, J., Michie, K., & Cortesi, F. (2021). Molecular Evolution of Ultraviolet Visual Opsins and Spectral Tuning of Photoreceptors in Anemonefishes (Amphiprioninae). Genome Biology and Evolution, 13(10). https://doi.org/10.1093/gbe/evab184

Morris Jr., J. A., & Akins, J. L. (2009). Feeding ecology of invasive lionfish (Pterois volitans) in the Bahamian archipelago. Environmental Biology of Fishes, 86, 389–398. https://doi.org/10.1007/s10641-009-9538-8

Nagelkerken, I., Dorenbosch, M., Verberk, W., Cocheret de la Moriniere, E., & van der Velde, G. (2000). Day-night shifts of fishes between shallow-water biotopes of a Caribbean bay, with emphasis on the nocturnal feeding of Haemulidae and Lutjanidae. Marine Ecology Progress Series, 194, 55–64.

O'Quin, K. E., Hofmann, C. M., Hofmann, H. A., & Carleton, K. L. (2010). Parallel Evolution of opsin gene expression in African cichlid fishes. Molecular Biology and Evolution, 27(12), 2839–2854. https://doi.org/10.1093/molbev/msq171

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. Bioinformatics, 20(2), 289–290. https://doi.org/10.1093/bioinformatics/btg412

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

Potier, S., Mitkus, M., & Kelber, A. (2018). High resolution of colour vision, but low contrast sensitivity in a diurnal raptor. Proceedings of the Royal Society B: Biological Sciences, 285, 20181036. https://doi.org/10.1098/rspb.2018.1036

Rabosky, D. L., Chang, J., Title, P. O., Cowman, P. F., Sallan, L., Friedman, M., Kaschner, K., Garilao, C., Near, T. J., Coll, M., & Alfaro, M. E. (2018). An inverse latitudinal gradient in speciation rate for marine fishes. Nature, 559(7714), 392–395. https://doi.org/10.1038/s41586-018-0273-1

Robertson, J. M., Bell, R. C., & Loew, E. R. (2022). Vision in dim light and the evolution of color pattern in a crepuscular/nocturnal frog. Evolutionary Ecology, 36(3), 355–371. https://doi.org/10.1007/s10682-022-10173-w

Roth, L. S. V., & Kelber, A. (2004). Nocturnal colour vision in geckos. Proceedings of the Royal Society B: Biological Sciences, 271(Suppl.), S485–S487. https://doi.org/10.1098/rsbl.2004.0227

Schmitz, L., & Wainwright, P. C. (2011). Nocturnality constrains morphological and functional diversity in the eyes of reef fishes. BMC Evolutionary Biology, 11, 338. https://bmcecolevol.biomedcentral.com/articles/10.1186/1471-2148-11-338#additional-information

Sewe, S. O., Silva, G., Sicat, P., Seal, S. E., & Visendi, P. (2022). Trimming and Validation of Illumina Short Reads Using Trimmomatic, Trinity Assembly, and Assessment of RNA-Seq Data. In Plant Bioinformatics (pp. 211–232). http://www.springer.com/series/7651

Shand, J. (1997). Ontogenetic changes in retinal structure and visual acuity: a comparative study of coral-reef teleosts with differing post-settlement lifestyles. Z Environmental Biology of Fishes, 49, 307–322.

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics, 31(19), 3210–3212. https://doi.org/10.1093/bioinformatics/btv351

Smith, A. R., Ma, K., Soares, D., & Carleton, K. L. (2012). Relative LWS cone opsin expression determines optomotor thresholds in Malawi cichlid fish. Genes, Brain and Behavior, 11(2), 185–192. https://doi.org/10.1111/j.1601-183X.2011.00739.x

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution, 38(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Thoen, H. H., How, M. J., Chiou, T. H., & Marshall, J. (2014). A different form of color vision in mantis shrimp. Science, 343(6169), 411–413. https://doi.org/10.1126/science.1245824

Thoreson, W. B., & Dacey, D. M. (2019). Diverse Cell Types, Circuits, and Mechanisms for Color Vision in the Vertebrate Retina. Physiological Reviews, 99(3), 1527–1573. https://doi.org/10.1152/physrev.00027.2018

Wan, Y. C., Méndez, M. J. N., O'Connell, L. A., Uricchio, L. H., Roland, A. B., Maan, M. E., Ron, S. R., Betancourth-Cundar, M., Pie, M. R., Howell, K. A., Richards-Zawacki, C. L., Cummings, M. E., Cannatella, D. C., Santos, J. C., & Tarvin, R. D. (2023). Selection on Visual Opsin Genes in Diurnal Neotropical Frogs and Loss of the SWS2 Opsin in Poison Frogs. Molecular Biology and Evolution, 40(10), msad206. https://doi.org/10.1093/molbev/msad206

Warrant, E. (2004). Vision in the dimmest habitats on Earth. J Comp Physiol A, 190, 765–789. https://doi.org/10.1007/s00359-004-0546-z

Wong, W. S. W., Yang, Z., Goldman, N., & Nielsen, R. (2004). Accuracy and power of statistical methods for detecting adaptive evolution in protein coding sequences and for identifying positively selected sites. Genetics, 168(2), 1041–1051. https://doi.org/10.1534/genetics.104.031153

Wright, T. F., Eberhard, J. R., Hobson, E. A., Avery, M. L., & Russello, M. A. (2010). Behavioral flexibility and species invasions: the adaptive flexibility hypothesis: Ethology Ecology and Evolution, 22(4), 393–404. https://doi.org/10.1080/03949370.2010.505580

Xu, B., & Yang, Z. (2013). PamlX: A graphical user interface for PAML. Molecular Biology and Evolution, 30(12), 2723–2724. https://doi.org/10.1093/molbev/mst179

Yang, Z. (2000). Complexity of the simplest phylogenetic estimation problem. https://royalsocietypublishing.org/

Yang, Z. (2007). PAML 4: Phylogenetic analysis by maximum likelihood. Molecular Biology and Evolution, 24(8), 1586–1591. https://doi.org/10.1093/molbev/msm088

Yang, Z., & Nielsen, R. (1998). Synonymous and Nonsynonymous Rate Variation in Nuclear Genes of Mammals. J Mol Evol, 46, 409–418.

Yang, Z., Wong, W. S. W., & Nielsen, R. (2005). Bayes empirical Bayes inference of amino acid sites under positive selection. Molecular Biology and Evolution, 22(4), 1107–1118. https://doi.org/10.1093/molbev/msi097

Yokoyama, S., Takenaka, N., & Blow, N. (2007). A novel spectral tuning in the short wavelength-sensitive (SWS1 and SWS2) pigments of bluefin killifish (Lucania goodei). Gene, 396(1), 196–202. https://doi.org/10.1016/j.gene.2007.03.019

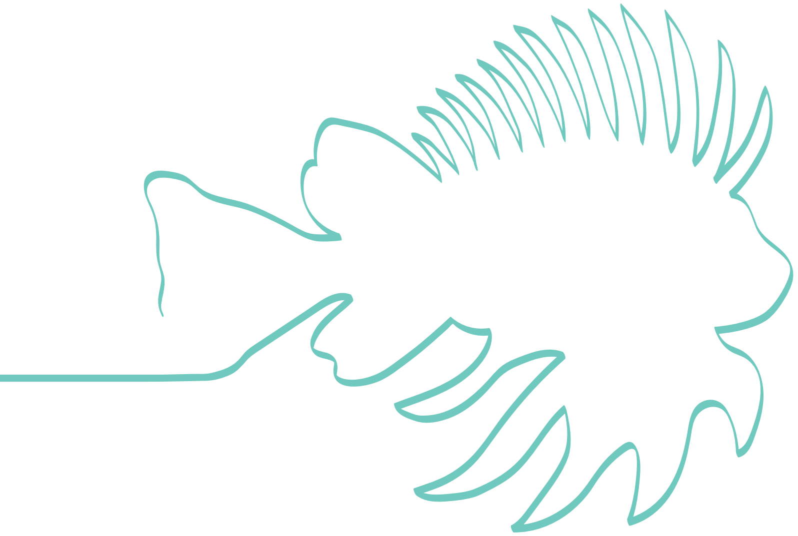
Zhang, J., Nielsen, R., & Yang, Z. (2005). Evaluation of an improved branch-site likelihood method for detecting positive selection at the molecular level. Molecular Biology and Evolution, 22(12), 2472–2479. https://doi.org/10.1093/molbev/msi237

Chapter 4

Lionfish can detect UV light:

UV Vision in an Invasive Aquatic Predator

Elizabeth W. Phillips, Karen E. de Wit, Alexander Kotrschal



Abstract

UV vision is widespread across animals. Many coral reef fish species use the reflection of UV light to communicate with conspecifics, as most aquatic predators are UV-blind. This creates a "secret" communication system for prey fish to signal to others while minimizing their risk of predation. Exploitation of this system by a predator that can detect UV light would likely help facilitate prey detection and increase predator efficiency. Recently, indirect evidence has emerged that the Indo-Pacific lionfish (Pterois volitans), a highly invasive predator in the Caribbean, might be able to see into the UV spectrum. We propose that UV vision might be an important, and so far overlooked, reason for lionfish's predatory success. Using an associative learning assay, we investigated lionfish's ability to discriminate UV from non-UV light and found the first behavioral evidence that lionfish can indeed see UV light. To further investigate the potentials of UV vision in lionfish ecology, we also measured lionfish's UV body reflectance and found that lionfish reflect UV light. Further studies should continue to investigate the function of UV vision in lionfish, as well as investigate if the UV vision is more widespread in marine predators than originally thought.

Introduction

Animals can see light in a wide range of spectra. Humans see light in the spectrum of 400-700nm (called the visual spectrum). The ultra-violet (UV) spectrum (100-400nm) constitutes a range that humans and most other mammals are blind to, but a wide range of insects, crustaceans, birds, and fishes can detect (Cortesi et al., 2020; Ulrike E. Siebeck & Marshall, 2001; Tovée, 1995). The function of UV vision in these species has been extensively studied and, in general, falls into five broad categories: navigation, foraging, communication, mate attraction, and circadian rhythms regulation (Tovée, 1995). Of these functional uses, fishes have been shown to use UV vision mainly during mate attraction, while foraging to better detect plankton, and for short range communication between conspecifics (Flamarique, 2013; G. S. Losey et al., 1999; Rick & Bakker, 2008; Ulrike E. Siebeck, Parker, Sprenger, Mäthger, & Wallis, 2010; Ulrike E Siebeck, 2004). However, in marine systems, many predatory marine fish show characteristics in their eye morphology that would prevent the absorbance and detection of UV light (Cummings, Rosenthal, & Ryan, 2003; G S Losey et al., 2003; Lythgoe, Muntz, Partridge, Shand, & Williams, 1994; U. E. Siebeck & Marshall, 2000). When investigating the UV transmittance of ocular media in coral reef fish, it was found that around half the 130 predators surveyed showed complete blockage of light below 405nm (G S Losey et al., 2003; U. E. Siebeck & Marshall, 2000). Of the predatory species whose ocular media did transmit UV light, most limited UV light transmittance to wavelengths above 360nm (the average peak absorbance of short-wavelength receptors found in fish with UV vision), indicating UV sensitivity, but not necessarily specialized UV vision (Douglas & Mcguigan, 1989; G S Losey et al., 2003; U. E. Siebeck & Marshall, 2000). Blockage of UV light at the eye can increase predators' prey detection at long distances, but it also prevents long-term damage to the eye caused by UV light (Carleton, Escobar-Camacho, Stieb, Cortesi, & Marshall, 2020; Ivanov, Mappes, Schaupp, Lappe, & Wahl, 2018; Ulrike E Siebeck, 2004; Zigman, 1993). Prey species can benefit from predators' lack of UV vision, as it allows for more elaborate coloration in the UV spectrum and therefore increased opportunity for mate attraction and communication with conspecifics, without risk of increased predation, a trade-off well-documented in the visual spectrum (Burk, 1982; Endler, 1987; Magnhagen, 1991). Even though most predatory fish seem UV insensitive, 24 species of predatory fish were identified with a high likelihood for UV vision based on UV transmittance of ocular media (transmitted UV light under 355nm). Most species were from the families Apogonidae (Cardinalfish) and Holocentridae (Squirrelfish and Soldierfish); small nocturnal fish that feed mainly on crustaceans and invertebrates. Most likely, these species never evolved to block UV light due to the lack of UV light during the night, unlike diurnal predators that can be harmed by the high levels of UV light during the day (G S Losey et al., 2003). However, two families of predators, Synodontidae (Lizardfish) and Scorpaenidae (Scorpionfish), that show a high likelihood for UV vision, eat mainly small fish, and hunt nocturnally and in daylight (Cure et al., 2012; Esposito et al., 2009; Morris Jr. et al., 2008; Morris Jr. & Akins, 2009; Soares et al., 2002). These predators also largely attack prey at close range, making them less likely to be impacted by the decreased visual clarity at long distances caused by UV vision, while also potentially benefitting from the ability to tap into the "secret" UV communication channel used by prey fish. Indeed, a freshwater predator with UV ability, the brown trout (Salmo trutta), is more likely to attack three-spined sticklebacks (Gasterosteus aculeatus) that reflect UV light than members of the same species that don't (Modarressie et al., 2013).

Lionfish (Pterois volitans) are scorpionfish present in coral reefs and are infamous for their incredible invasive success in the Atlantic and Caribbean. Recently, physiological evidence has emerged revealing the potential for UV vision in lionfish. Corneal electroretinography showed that lionfish corneas are sensitive to light in the spectrum 350-620nm, and that lionfish also possess UV-transmissible lenses with 50% transmittance down to 310nm (Hasenei et al., 2020). We predict that UV vision may play a role in their invasive ability. The lionfish's invasion began in 2000 with sightings first recorded in Florida, USA. They rapidly spread up the east coast of the United States and south through the Caribbean towards South and Central America over the next decade (Côté et al., 2013). Another systematically close, ecologically and behaviorally similar lionfish species (Pterois miles) has also recently begun invading the Mediterranean after introduction from the Red Sea through the Suez Canal (Bariche et al., 2013; Dimitriadis et al., 2020; E. W. Phillips & Kotrschal, 2021). A thorough understanding of what underlies lionfish's high invasive success is paramount for management efforts, as lionfish invasions have caused dramatic ecological and economic damage to local areas (Côté et al., 2013; Lesser & Slattery, 2011). Their ability to so successfully invade and expand into new habitats is often attributed to their high reproductive ability, lack of natural predators due to venomous dorsal spines, and their generalist diet (Côté et al., 2013; Galloway & Porter, 2019; Hixon et al., 2016). We suggest that their hunting technique may also crucially contribute to their invasive success. Lionfish use a variety of techniques when hunting, all of them requiring close proximity of lionfish to their prey (Hixon et al., 2016). This proximity opens up the potential for lionfish to take advantage of the normally secret short distance UV signaling used by prey fish and may hence improve lionfish hunting ability through better prey detection.

While physiological evidence of UV transmittance in ocular media and the presence of UV wavelength absorption in visual receptors suggests lionfish possess UV vision, it is currently unknown whether UV light is further processed in the brain and can be functionally used by this species. Behavioral experiments are one way to test for the use of certain visual spectrums in a species (Kelber et al., 2003). For example, in a color association learning task, individuals are presented with two or more colors and taught to associate one of those colors with a food reward. If the animals can learn to discriminate between those colors, then they must be able to perceive them as different. Therefore, such an assay can be used to test if animals of one species can distinguish between the cues within the light spectrum of interest. Lionfish possess learning ability similar to other fish species, including in an associative learning assay using colors in the visual spectrum (de Groot, 2021; Deroy et al., 2020). Here, we use an associative learning assay to determine if lionfish can differentiate between light containing UV and light without UV as a measure of lionfish's ability to see into the UV spectrum.

We also measured the UV reflectance of lionfish using UV photography. Previous research has shown UV body reflectance in fish that use UV vision as a means of species recognition and communication (Cummings et al., 2003; George S. Losey, 2003; Ulrike E. Siebeck et al., 2010; Ulrike E Siebeck, 2004; Smith et al., 2002), so we predict if lionfish have UV vision, they may also possess UV reflectance. In this way, our study aims to be a first step in exploring the potential of UV vision in hunting and communication among predatory fish.

Methods

Animal Husbandry

The lionfish used in this study were supplied by the Coral and Fish Store (Breda, Netherlands), originally caught within their native range in Indonesia. The fish ranged in size from 3–12cm SL at the time of the experiments and were individually housed in 100-liter tanks equipped with a PVC pipe shelter and an air stone. All tanks were connected by a flow-through filtration system and kept at a temperature of 24°C, pH of 8.0, and salinity of 31ppt. The room housing the lionfish was equipped with overhead fluorescent lights (400-700nm), as well as LED lights above the bottom row of tanks (380-740nm), all set to the same 12:12 light/dark cycle. To minimize the impact of daytime lighting conditions on experimental results, the experiments were conducted only during the dark cycle when no overhead lights were on. Fish were fed once a day, five days a week during the first experiment, and twice a week during the second experiment. They were fed with a varied diet of shrimp, mackerel, and cod, and were fed each day until their stomachs were bulging (sign of satiation in lionfish) or until they stopped showing interest in food when presented. The associative learning experiment took place between August 2021 and October 2021 and data collection of UV body reflectance took place in February 2022.

Experiment 1: Associative Learning with UV and Non-UV Light as Cues

Experimental Apparatus

The learning apparatuses used were introduced to housing tanks prior to the start of the experiment and left in the tank for the entire duration. The body of the structure consisted of two rectangular compartments, side-by-side, closed off by three walls and a bottom (Figure 4.1). The side walls were made from black opaque PVC, while the back wall was made from transparent PVC plate. This side was positioned against the tank wall to allow the observer to score associative learning trials in real-time. The front side of the compartment was left open, allowing fish to enter a compartment and make a choice during learning trials, but was closed off with two doors (one opaque and one transparent) outside of trials.

During learning trials, light was reflected off of paper and into each compartment to create light cues, as shining light directly into the water disperses it, making spectral differences more difficult to see. Light cues were created using two flashlights (PNQ UV zaklampen) that emit light between 360nm and 395nm. One was covered by a lens that blocks only UV light below 390nm and allows all other wavelength to pass with >95% intensity (Edmund Optics), so is referred to as the "non-UV light", while the other one was left uncovered and is referred to as the "UV light". Because we used a filter to eliminate UV light from one of the light sources, which leads to a slightly less bright light field, we cannot exclude that instead of light spectrum, lionfish may pick up on minute brightness differences when learning. However, being able to discriminate such small brightness differences is likely beyond the resolution of the visual system of most fishes (Blaxter, 1980; Guthrie, 1986).

Pre-training Phase

Prior to the start of experimental trials, lionfish (n=19) were pre-trained to feed from the learning apparatus. During pre-training trials, the doors were lifted one at a time (first the opaque door, then the transparent door five seconds later) and the fish were allowed to inspect the apparatus. If the fish entered either of the two compartments in the apparatus, they were rewarded with food inside the compartment. A fish was considered to have entered a compartment if, when viewing from above, their head up until the base of the pectoral fins was within the walls of the compartment. If after two minutes the fish did not enter the apparatus, a food reward was presented in one of two compartments, determined randomly with the limitation that one side could not be used more than two times in a row. Once food was presented, the trial ended, and the doors were closed. A break of about two minutes was given between subsequent trials. Pre-training continued until the individual swam into either compartment, unprompted, within two minutes for three trials in a row. The eight individuals that did not complete pre-training were not used in the subsequent phase of the experiment. Trials were conducted daily, Monday through Friday. Individuals participated in zero to six trials per day, depending on their hunger level, as trials were stopped each day when the focal fish showed signs of satiation.

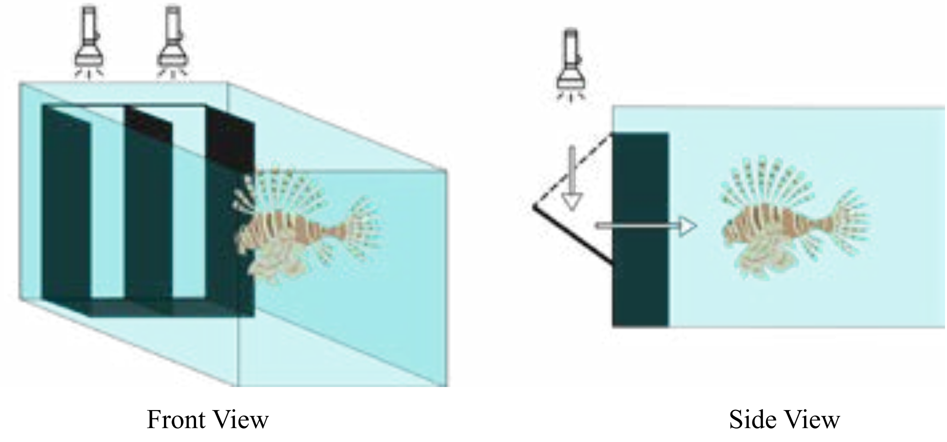


Figure 4.1 *Experimental Apparatus. View of the associative learning apparatus from the fish's view (left) and the side (right). Black walls mark the opaque walls of the apparatus, and the blue walls mark the walls of the tank. Flashlights indicate light source locations from each view, and white arrows indicate the path the light takes when shown to the fish.*

Associative Learning Phase

Once an individual completed pre-training, the next day marked the start of the associative learning phase. Like during pre-training, a trial started when the opaque door of the apparatus was lifted, followed by the transparent door five seconds later.

During these trials, UV and non-UV lights were presented as cues for associative learning. Half of the individuals (n=6) were assigned UV light as the rewarded cue, and the other half (n=5) were assigned non-UV light. Each light was randomly assigned to a compartment during each a trial, with the limitation that a cue could not be placed on the same side more than three times in a row for an individual. Individuals were given two minutes to make a choice between the two compartments. If the compartment with the rewarded cue was chosen, this was considered a "correct" choice and food was presented in the compartment as a reward. If the other compartment was chosen, this was considered an "incorrect" choice and individuals were given the chance to correct themselves within the two minutes of the trial. In this case, either food was presented when they entered the rewarded compartment, or at the end of the two minute trial in the rewarded compartment. If no choice was made within those two minutes, food was presented in the compartment with the rewarded cue and the trial outcome marked as "no choice". Once the food was presented, the trial ended, and the doors were closed. Associative learning trials continued until either the individual reached the learning criteria (seven correct choices in a row), or until the experimental time limit was reached (three months to complete both the pre-training and associative learning phases). The 'seven in a row' learning criteria is a common learning criterion in associative learning tasks (Vila Pouca et al., 2021) as it is significant according to binomial probability.

Statistical Analysis

To test for learning in the lionfish, we used a one-tailed one-sample Wilcox rank test to determine if the mean success rate of the group was different than what would be expected by chance. Individual lionfish were also tested for learning using a chi-squared test for goodness of fit, comparing the proportion of correct and incorrect responses to chance. Additionally, we also used chi-square tests of independence to determine if lionfish prefer UV or non-UV light by comparing the likelihood of making a correct or incorrect choice in the first trial, first five trials, first ten trials, or overall depending on the light assigned as the rewarded cue. Statistical analyses were conducted in R Studio Version 1.1.463.

Experiment 2: UV Body Reflectance

To determine the UV reflectance of lionfish, photos were taken of free-swimming lionfish (n=15) in their tanks in both the visual and UV spectrum. Similar to Kodric-Brown & Johnson (Kodric-Brown & Johnson, 2002), we took photos using filters that either block or absorb light in the UV spectrum to create a colored visual spectrum photo and a black & white UV spectrum photo of each lionfish. For all photos, we used a Nikon D40 camera body with Nikon AF 50mm f/1.8D lens. Visual spectrum photos were taken with light shown directly onto the focal fish from above in an otherwise dark room, and with a UV/IR Cut Filter (Kolari Vision) used on the lens to limit the light captured by the camera to 400-700nm. For the UV spectrum photo, light was provided by two UV flashlights (PNQ UV zaklamp) that emitted light between 360nm and 395nm, and an Ultraviolet Bandpass Transmission Filter (Kolari Vision) was used to limit light captured by the camera to 340-380nm. These UV-spectrum photos were then imported into Adobe Photoshop and converted to black & white images to reveal areas of UV reflectance (white) and areas of no UV reflectance (black). Due to limited lighting during shooting, the brightness of some images was adjusted to better visualize the differences between UV and non-UV reflecting areas.

Results

Experiment 1: Associative Learning with UV and non-UV Light as Cues

We found that as a group, lionfish chose the correct cue more often than chance (V = 55, p = 0.003), indicating a learned preference for the rewarded cue. However, only three of the eleven individuals tested reached learning criteria (seven correct choices in a row with no incorrect choices) by the end of the experiment (Figure 4.2). Of these three, two showed learning at an individual level (Fish 2: x2 = 4.5, df = 1, p = 0.034; Fish 4: x2 = 7, df = 1, p = 0.008; Table 4.1).

Additionally, we evaluated the effect of reward cue (UV vs. non-UV light) on the likelihood of a fish to make a correct choice during the first trial, first five trials, first ten trials, and overall. We found that in all cases, the assigned reward cue had no effect on the likelihood of fish making the correct choice (first trial: x2 = 0, df = 1, p =

1; first five trials: x2 = 0, df = 1, p = 0.989; first ten trials: x2 = 0.802, df = 1, p = 0.371; overall: x2 = 0, df = 1, p = 1).

Table 4.1 *Individual Learning Data. Chi-squared tests for goodness of fit were used to determine individual learning for each fish that participated in the associative learning phase (n = 11). Calculated values for each individual are listed. P-values in bold with an asterisk are significant (p ≤ 0.05).*

Fish ID	Reward Cue	Total Trials	Correct Choices	Incorrect Choices	No Choice	x^2 statistic	p value
2	UV	20	7	1	12	4.500	**0.034***
3	UV	44	4	3	37	0.143	0.705
4	UV	10	7	0	3	7.000	**0.008***
5	Non-UV	45	2	2	41	0.000	1.000
7	Non-UV	53	8	5	40	0.692	0.405
10	UV	54	18	12	24	1.200	0.273
11	Non-UV	27	9	3	15	3.000	0.083
12	Non-UV	32	11	5	16	2.250	0.134
17	UV	55	12	11	32	0.043	0.835
18	UV	61	11	9	41	0.200	0.655
20	Non-UV	43	9	8	26	0.059	0.808

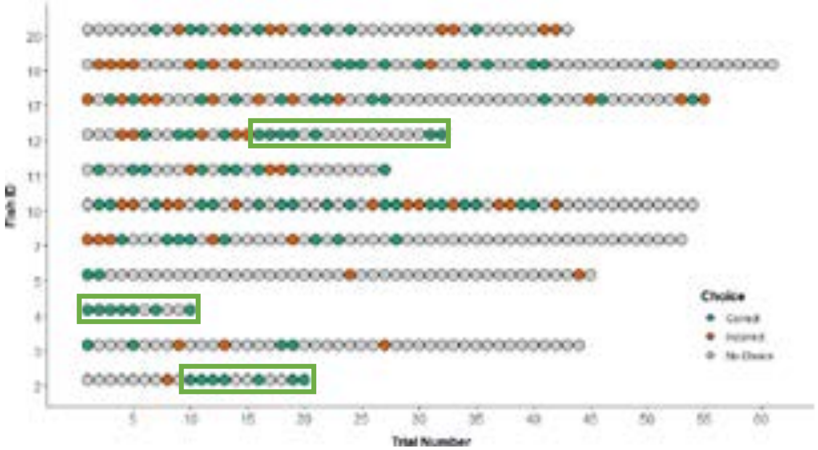


Figure 4.2 *Associative Learning Data. Each circle represents one trial. The color of the circle indicates the choice made by the individual during that trial: green indicates a correct choice, red indicates an incorrect choice, and grey represents when no choice was made. Green boxes are added for individuals that reached learning criteria and encircle the seven correct trials in a row made by the individual.*

Experiment 2: UV Body Reflectance

We found that when photographed in the UV spectrum, all lionfish were visible in photos, indicating they reflect at least some UV light (Figure 4.3). When comparing UV and visible spectrum photos, we found that the UV-reflective portions of the body match with the white portions of the body in the visible spectrum, while the darker parts do not seem to reflect UV within the spectrum 365-380nm.

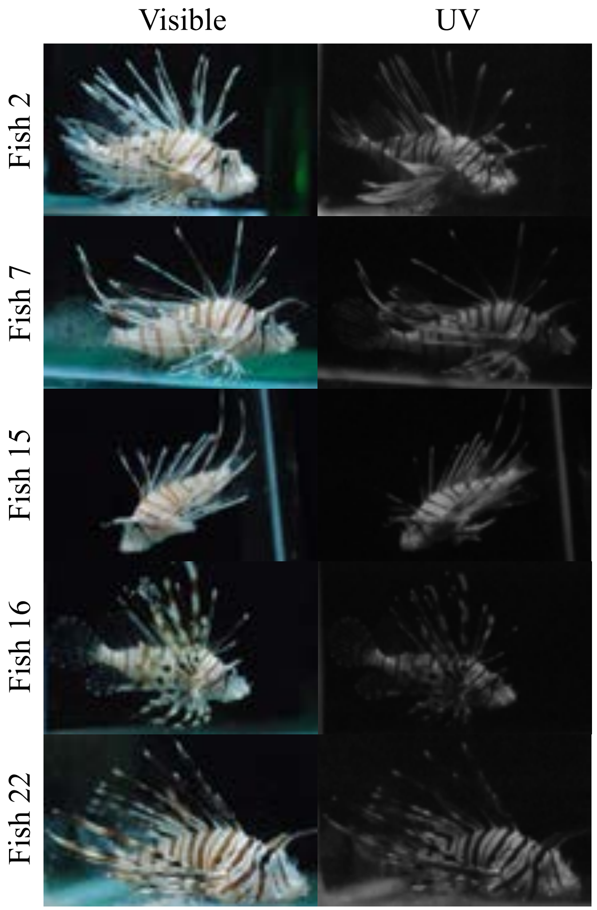


Figure 4.3 *UV Body Reflectance. A subset of lionfish photographed with visual spectrum and UV spectrum photography. Pictures on the left are of lionfish in visual spectrum, and pictures on the right are from the UV spectrum. UV spectrum photos have been converted to black and white for clarity: white area indicate UV reflectance, while black areas indicate no reflectance. Note that the silicone of the fish tank reflects UV light.*

Discussion

Here, we show that lionfish are likely able to distinguish between UV and non-UV light using an associative learning task. Overall, lionfish chose the correct cue more often than what would be expected by chance, indicating an ability to distinguish differences between the cues. This is the first behavioral evidence of UV light detection in lionfish, and corroborates previous physiological evidence that lionfish have the potential to see UV light (Hasenei et al., 2020; Ulrike E. Siebeck & Marshall, 2001).

Though our data shows a clear trend of learning at the group level when UV and non-UV cues are used, only two of the eleven individuals tested showed learning at an individual level (Table 4.1). These low learning rates were most likely caused by the high number of no choices per individual, creating a low number of total trials where choices were made. One reason for the low number of choices could be that, as this is one of the first attempts to investigate associative learning in lionfish, the learning apparatus may not have been completely optimized for lionfish's learning ability and feeding ecology, leading to reduced success by individual fish. Indeed, only eleven of the nineteen lionfish that started the experiment made it to the associative learning phase and eight where unable to successfully complete the pre-training. This may indicate that learning to partially enter one of the two compartments in the apparatus to earn a food reward was too difficult for the fish. Alternatively, lionfish might not have been fully motivated to participate in daily trials due to satiation effects, increasing the number of "no choices" in our data set. We suggest future research investigate the optimal learning apparatus for lionfish that provides higher success rates in trial participation. However, our findings should be general for lionfish despite the relatively low number of individuals showing clear learning as traits such as visual capabilities typically vary little within a species. Therefore, it can be reasonably assumed that if some individuals show the ability to see UV light, most others will as well.

We propose that a function of UV vision in lionfish may be to increase hunting efficiency, as many prey fish reflect UV light in coral reefs, a natural habitat of lionfish (G. S. Losey et al., 1999; Ulrike E. Siebeck & Marshall, 2001). One of the ways in which planktivorous prey species use UV vision is for foraging of plankton due to the contrast plankton have with their background in the UV spectrum (Flamarique, 2013; G. S. Losey et al., 1999). UV light attenuates rapidly in the water column, creating a bright UV-illuminated background against which non-UV reflective plankton are easy to spot. A similar, but opposite effect could explain a potential function of UV vision in lionfish. In contrast to the water column, corals often absorb UV light as a way to mitigate the harmful impacts of UV radiation (Reef et al., 2009). This would provide a darker background on which UV-reflective prey would shine more brightly at close distances. Therefore, lionfish that possess UV vision may have increased hunting success with UV-reflecting prey. Furthermore, prey species in invasive regions that rely heavily on camouflage as their main predatory defense may not be well-adapted to evade a predator that can see into the UV-spectrum, like the lionfish (Sih et al., 2010). This effect would further increase lionfish hunting ability in invasive regions and could be a potential cause of the extreme impact lionfish have had on native ecosystems (Cure et al., 2012; D'Agostino et al., 2020; Rojas-Vélez et al., 2019). However, as our study only tests the potential of UV vision and not its function for lionfish, additional testing is needed.

One caveat to our hypothesis may be that lionfish typically hunt during the crepuscular period rather than during the day in their native ranges, potentially reducing the effectiveness of UV vision during hunting. However, we argue this does not take away from the potential benefits of UV vision in lionfish for several reasons. In the Caribbean, lionfish have been reported to hunt both diurnally and during twilight hours when UV light is more abundant (Cure et al., 2012; Morris Jr. & Akins, 2009). Even during twilight periods, lionfish might still use UV vision to hunt as even though there is less UV light than during the day, there is still some UV light that is reflected by the moon (Robinson et al., 2011). It is unclear how much nocturnal animals use UV light to their advantage, but it remains possible that lionfish utilize UV light during twilight when there is still some amount of light coming directly from the sun, and some reflected off the moon.

In our study, we used relatively small, potentially juvenile lionfish of around 8 cm. This may be important for our findings as in many piscivorous fish species, such as salmon or rainbow trout, juveniles exhibit UV vision during early life stages, but lose the ability to detect UV light in adulthood (Bowmaker & Kunz, 1987; Flamarique, 2013; Losey et al., 1999). This is theorized to be due to a shift from a largely planktivorous diet, where UV vision can aid in detection of plankton, to a piscivorous diet in adulthood. However, in lionfish larvae tend to settle around 26 days when they are about 1-2 cm in length and from settlement onwards seem to display similar behaviors throughout their lifetime in regards to hunting, rather than have a distinct juvenile and adulthood phases (Ahrenholz & Morris, 2010; Côté et al., 2013; Cure et al., 2012; del Río et al., 2022). While lionfish are a gape-limited predators and show a change preference for fish over other prey such as crustaceans and mollusks at larger sizes due to reduced restriction of fish prey due to gape size, lionfish as small as 5 cm in length were shown to have diets similar to those of larger lionfish (Côté et al., 2013; del Río et al., 2022). Therefore, we argue the results of our study are representative of lionfish adult visual abilities in general.

Furthermore, to test for the potential of communication with conspecifics via UV vision and reflectance, we used UV photography to take photos of lionfish in the UV spectrum and evaluated their UV reflectance. We found that all the lionfish photographed reflect in the UV spectrum (Figure 4.3). Notably, all UV-reflecting areas of the lionfish coincided with areas that are white in the visual spectrum, while all UV-absorbing areas were contained within the darker colored regions. This is typical of many UV-reflecting species, as unique patterns that are only visible in the UV spectrum are rare (G. S. Losey et al., 1999; Ulrike E Siebeck, 2004). Instead, UV-reflectance often enhances the contrast between different areas also visible in the visual spectrum, improving communication through signaling. For lionfish, having this UV reflectance to enhance the already stark contrast in stripes seen in their body coloration within the visual spectrum may increase visibility of their coloration when signaling to conspecifics or during threat displays. It may also increase their crypsis through alternating patterns of UV and non-UV stripes, as is hypothesized to be the purpose of their stripes in the visual spectrum. Alternatively, lionfish UV reflectance may not represent a functional use of their UV vision, but rather be the byproduct of their evolved coloration in the visual spectrum. For example, in one study over half of the investigated species that very likely do not possess UV vision (as light transmittance in their eyes is above 411nm) show reflectance within the UV spectrum (N. Marshall et al., 2003). Hence, it is clear that additional research is needed to test the functional significance of UV vision, including if lionfish communicate using UV vision and reflectance.

We also noted that the two individuals that showed learning at an individual level were both trained with UV as the rewarded cue. We would expect that if UV light had a functional significance while hunting, lionfish might be more attracted to UV light than non-UV light initially - before an association with a light cue is learned. With our data we could test for such a general preference in the first trial, first five trials, first ten trials, or overall during the associative learning experiment. In all cases, we did not find a significant choice for one over the other, indicating no inherent preference for UV light.

Overall, we show the first behavioral evidence that is consistent with UV light detection in lionfish. This is unique, as most marine predatory fish do not possess the capacity for UV vision and instead rely on long-distance detection of prey in the visual spectrum. Lionfish, however, capture prey at short-ranges and therefore have the potential to utilize UV vision to better increase their hunting ability. More research is needed, however, to determine whether lionfish fully possess UV vision and what the function of their UV vision is.

References

Ahrenholz, D. W., & Morris, J. A. (2010). Larval duration of the lionfish, Pterois volitans along the Bahamian Archipelago. Environmental Biology of Fishes, 88(4), 305–309. https://doi.org/10.1007/s10641-010-9647-4

Bariche, M., Torres, M., & Azzurro, E. (2013). The presence of the invasive Lionfish Pterois miles in the Mediterranean Sea. Mediterranean Marine Science, 14(2), 292–294.

Blaxter, J. (1980). Vision and the Feeding of Fishes. In Fish Behavior and its Use in the Capture and Culture of Fishes (pp. 32–56).

Bowmaker, J., & Kunz, Y. (1987). Ultraviolet receptors, tetrachromatic colour vision and retinal mosaics in the brown trout (Salmo trutta): Age-dependent changes. Vision Research, 27(12), 2101–2108.

Burk, T. (1982). Evolutionary Significance of Predation on Sexually Signalling Males. The Florida Entomologist, 65(1), 90–104.

Carleton, K. L., Escobar-Camacho, D., Stieb, S. M., Cortesi, F., & Marshall, N. J. (2020). Seeing the rainbow: Mechanisms underlying spectral sensitivity in teleost fishes. Journal of Experimental Biology, 223, jeb193334. https://doi.org/10.1242/jeb.193334

Cortesi, F., Mitchell, L. J., Tettamanti, V., Fogg, L. G., Busserolles, F. De, Cheney, K. L., & Marshall, N. J. (2020). Visual system diversity in coral reef fishes. Seminars in Cell and Developmental Biology, 106, 31–42. https://doi.org/10.1016/j.semcdb.2020.06.007

Côté, I. M., Green, S. J., & Hixon, M. A. (2013). Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. Biological Conservation, 164, 50–61. https://doi.org/10.1016/j.biocon.2013.04.014

Cummings, M. E., Rosenthal, G. G., & Ryan, M. J. (2003). A private ultraviolet channel in visual communication. Proc. R. Soc. Lond. B, 270, 897–904. https://doi.org/10.1098/rspb.2003.2334

Cure, K., Benkwitt, C. E., Kindinger, T. L., Pickering, E. A., Pusack, T. J., McIlwain, J. L., & Hixon, M. A. (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. A. (2020). Behavioural traits and feeding ecology Mediterranean lionfish and naiveté native species lionfish predation. Marine Ecology Progress Series, 638, 123–135.

de Groot, C. (2021). Exploring the cognitive landscape of an invasive species: reversal learning in the red lionfish (Pterois volitans). Wageningen University.

del Río, L., Navarro-Martínez, Z. M., Ruiz-Abierno, A., Chevalier-Monteagudo, P. P., Angulo-Valdes, J. A., & Rodriguez-Viera, L. (2022). Feeding ecology of invasive lionfish in the Punta Frances MPA, Cuba: insight into morphological features, diet and management. PeerJ, 10, e14250. https://doi.org/10.7717/peerj.14250

Deroy, E. M., Hussey, N. E., & Macisaac, H. J. (2020). Behaviourally-mediated learning ability in an invasive marine fish. Biological Invasions, 0123456789. https://doi.org/10.1007/s10530-020-02329-y

Dimitriadis, C., Galanidi, M., Zenetos, A., Corsini-Foka, M., Giovos, I., Karachle, P. K., Fournari-Konstantinidoy, 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 the thermal boundaries and future range expansion. Mediterranean Marine Science, 21, 62–69.

Douglas, R. H., & Mcguigan, C. M. (1989). The spectral transmission of freshwater teleost ocular media - an interspecific comparison and a guide to potential ultraviolet sensitivity. Vision Research, 29(7), 871–879.

Endler, J. A. (1987). Predation , light intensity and courtship behaviour in Poecilia reticulata (Pisces : Poeciliidae). Animal Behaviour, 35, 1376–1385.

Esposito, V., Battaglia, P., Castriota, L., Finoia, M. G., Scotti, G., & Andaloro, F. (2009). Diet of Atlantic lizardfish, Synodus saurus (Linnaeus, 1758) (Pisces: Synodontidae) in the central Mediterranean Sea. Scientia Marina, 73(2), 369–376. https://doi.org/10.3989/scimar.2009.73n2369

Flamarique, I. N. (2013). Opsin switch reveals function of the ultraviolet cone in fish foraging. Proceedings of the Royal Society B, 280, 20122490.

Galloway, K. A., & Porter, M. E. (2019). Mechanical properties of the venomous spines of Pterois volitans and morphology among lionfish species. Journal of Experimental Biology, 222(6), jeb197905. https://doi.org/10.1242/jeb.197905

Guthrie, D. M. (1986). Role of Vision in Fish Behaviour. In T. J. Pitcher (Ed.), The Behaviour of Teleost Fishes (1st ed., pp. 75–113). Springer New York, NY.

Hasenei, A., Kerstetter, D. W., Horodysky, A. Z., & Brill, R. W. (2020). Physiological limits to inshore invasion of Indo-Pacific lionfish (Pterois spp.): insights from the functional characteristics of their visual system and hypoxia tolerance.

Biological Invasions, 22, 2079–2097. https://doi.org/10.1007/s10530-020-02241-5

Hixon, M. A., Green, S. J., Albins, M. A., Akins, J. L., & Morris, J. A. (2016). Lionfish: A major marine invasion. Marine Ecology Progress Series, 558, 161–165. https://doi.org/10.3354/meps11909

Ivanov, I. V, Mappes, T., Schaupp, P., Lappe, C., & Wahl, S. (2018). Ultraviolet radiation oxidative stress affects eye health. Journal of BioPhotonics, 11, e201700377. https://doi.org/10.1002/jbio.201700377

Kelber, A., Vorobyev, M., & Osorio, D. (2003). Animal colour vision - Behavioural tests and physiological concepts. Biological Reviews of the Cambridge Philosophical Society, 78(1), 81–118. https://doi.org/10.1017/S1464793102005985

Kodric-Brown, A., & Johnson, S. C. (2002). Ultraviolet reflectance patterns of male guppies enhance their attractiveness to females. Animal Behaviour, 63, 391–396. https://doi.org/10.1006/anbe.2001.1917

Lesser, M. P., & Slattery, M. (2011). Phase shift to algal dominated communities at mesophotic depths associated with lionfish (Pterois volitans) invasion on a Bahamian coral reef. Biological Invasions, 13(8), 1855–1868. https://doi.org/10.1007/s10530-011-0005-z

Losey, G. S. (2003). Crypsis and communication functions of UV-visible coloration in two coral reef damselfish, Dascyllus aruanus and D. reticulatus. Animal Behaviour, 66(2), 299–307. https://doi.org/10.1006/anbe.2003.2214

Losey, G. S., Cronin, T. W., Goldsmith, T. H., Hyde, D., Marshall, N. J., & McFarland, W. N. (1999). The UV visual world of fishes: A review. Journal of Fish Biology, 54(5), 921–943. https://doi.org/10.1006/jfbi.1998.0919

Losey, G. S., McFarland, W. N., Loew, E. R., Zamzow, J. P., Nelson, P. A., & Marshall, N. J. (2003). Visual Biology of Hawaiian Coral Reef Fishes I. Ocular Transmission and Visual Pigments. Copeia, 3, 433–454.

Lythgoe, J. N., Muntz, W. R. A., Partridge, J. C., Shand, J., & Williams, D. M. B. (1994). The ecology of the visual pigments of snappers (Lutjanidae) on the Great Barrier Reef. Journal of Comparative Physiology A, 174(4), 461–467. https://doi.org/10.1007/BF00191712

Magnhagen, C. (1991). Predation Risk as a Cost of Reproduction. Trends in Ecology & Evolution, 6(6), 183–186.

Marshall, N., Jennings, K., McFarland, W., Loew, E., & Losey, G. (2003). Visual Biology of Hawaiian Coral Reef Fishes. II. Colors of Hawaiian Coral Reef Fish. Copeia, 2003(3), 455–466. https://doi.org/https://doi.org/10.1643/01-055

Modarressie, R., Rick, I. P., & Bakker, T. C. M. (2013). Ultraviolet reflection enhances the risk of predation in a vertebrate. 59(2), 151–159.

Morris Jr., J. A., & Akins, J. L. (2009). Feeding ecology of invasive lionfish (Pterois volitans) in the Bahamian archipelago. Environmental Biology of Fishes, 86, 389–398. https://doi.org/10.1007/s10641-009-9538-8

Morris Jr., J. A., Akins, J. L., Barse, A., Cerino, D., & Freshwater, D. W. (2008). Biology and Ecology of the Invasive Lionfishes, Pterois miles and Pterois volitans. Proceedings of the 61st Gulf and Caribbean Fisheries Institute.

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

Reef, R., Kaniewska, P., & Hoegh-Guldberg, O. (2009). Coral Skeletons Defend against Ultraviolet Radiation. PLoS ONE, 4(11), e7995. https://doi.org/10.1371/journal.pone.0007995

Rick, I. P., & Bakker, T. C. M. (2008). UV wavelengths make female three-spined sticklebacks (Gasterosteus aculeatus) more attractive for males. Behavioral Ecology and Sociobiology, 62, 439–445. https://doi.org/10.1007/s00265-007-0471-6

Robinson, M. S., Denevi, B. W., Sato, H., Hapke, B. W., & Hawke, B. R. (2011). LROC WAC Ultraviolet Reflectance of the Moon. Lunar and Planetary Science Conference, 6, 15–16.

Rojas-Vélez, S., Tavera, J., & Acero, A. (2019). Unraveling lionfish invasion: Is Pterois volitans truly a morphologically novel predator in the Caribbean? Biological Invasions, 21(6), 1921–1931. https://doi.org/10.1007/s10530-019-01946-6

Siebeck, U. E. (2004). Communication in coral reef fish: the role of ultraviolet colour patterns in damselfish territorial behaviour. Animal Behaviour, 68, 273–282. https://doi.org/10.1016/j.anbehav.2003.11.010

Siebeck, U. E., & Marshall, N. J. (2000). Transmission of ocular media in labrid fishes. Philosophical Transactions of the Royal Society B: Biological Sciences, 355(1401), 1257–1261. https://doi.org/10.1098/rstb.2000.0679

Siebeck, U. E., & Marshall, N. J. (2001). Ocular media transmission of coral reef fish - Can coral reef fish see ultraviolet light? Vision Research, 41(2), 133–149. https://doi.org/10.1016/S0042-6989(00)00240-6

Siebeck, U. E., Parker, A. N., Sprenger, D., Mäthger, L. M., & Wallis, G. (2010). A Species of Reef Fish that Uses Ultraviolet Patterns for Covert Face

Recognition. Current Biology, 20(5), 407–410. https://doi.org/10.1016/j.cub.2009.12.047

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

Smith, E. J., Partridge, J. C., Parsons, K. N., White, E. M., Cuthill, I. C., Bennett, A. T. D., & Church, S. C. (2002). Ultraviolet vision and mate choice in the guppy (Poecilia reticulata). 13(1), 11–19.

Soares, M. S. C., Barreiros, J. P., Sousa, L., & Santos, R. S. (2002). Agonistic and predatory behaviour of the lizardfish Synodus saurus (Linnaeus, 1758) (Actinopterygii : Synodontidae) from the Azores. Journal of Ichthyology and Aquatic Biology, 6(2), 53–60.

Tovée, M. J. (1995). Ultra-violet photoreceptors in the animal kingdom: their distribution and function. Trends in Ecology & Evolution, 10(11), 455–460. https://doi.org/10.1016/S0169-5347(00)89179-X

Vila Pouca, C., Mitchell, D. J., Lefèvre, J., Vega-Trejo, R., & Kotrschal, A. (2021). Early predation risk shapes adult learning and cognitive flexibility. Oikos, 130(9), 1477–1486. https://doi.org/10.1111/oik.08481

Zigman, S. (1993). Ocular Light Damage. Photochemistry and Photobiology, 57(6), 1060–1068.

Chapter 5

Limited effects of culling on the behavior of invasive lionfish (Pterois miles) in the Mediterranean

Elizabeth W. Phillips, Davide Bottacini, Anna N. M. Schoonhoven,
Ynze J. J. Kamstra, Hannah De Waele, Carlos Jimenez, Louis
Hadjioannou, Alexander Kotrschal

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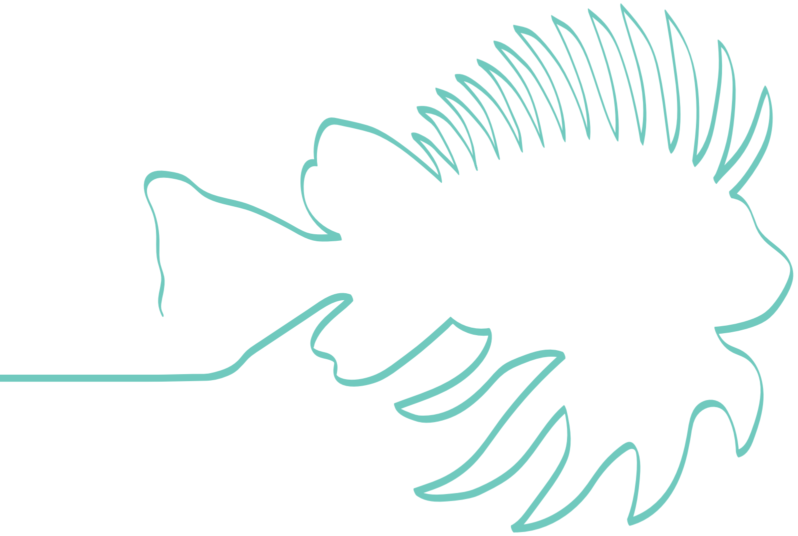


Abstract

Invasive species pose serious threats to ecosystems. To reduce ecological and economic consequences of invasions, efforts are made to control invaders and evaluating the effects of such efforts is paramount. Lionfishes (Pterois volitans and Pterois miles) are native to the Indo-Pacific Ocean and pose a major threat to local ecosystems in the invaded Atlantic and Mediterranean. Culling via spearfishing is a widespread measure to limit lionfish population size in invaded ranges. However, like most hunted fishes, lionfish alter their behavior after repeated culling, potentially decreasing the effectiveness of future culls. Previous studies on lionfish in the Caribbean have shown that lionfish are less bold after repeated culling. However, the impact of culling on lionfish in their newest invasive range, the Mediterranean, remains enigmatic. To determine the behavioral changes in response to culling in this second area of invasion, we tested for effects of culling on the behavior of lionfish in Cyprus, a region heavily impacted by the lionfish invasion in the Mediterranean. We compared the response of lionfish to an approaching free diver holding a metal pole (imitating a spear fisher) between protected areas where spearfishing is restricted and areas where culls are frequently conducted. We also assessed whether activity, hiding pattern, and site fidelity differed between these culled and unculled sites. Overall, we found limited effects of culling on the traits measured, indicating surprising resistance to culling-induced behavioral changes in Mediterranean lionfish. Future studies should monitor invasive lionfish population densities and the effects of culling in more detail to tailor management plans and reduce the negative effects of these fish in specific invaded ranges.

Introduction

Species that are found outside of their native range are known as exotic, alien or allochthonous (J. H. Brown & Sax, 2004), and are termed invasive when they establish and cause ecological or economic damage to the new local area (Carey et al., 2012). Due to potential catastrophic effects of invasive species on local ecosystems, steps to prevent invasions or limit their spread are often taken (M. J. W. Cock et al., 2010; Hewitt & Campbell, 2007). Although complete eradication may be achieved in areas where invasive expansion is limited or during the early stages of the invasion, controlling the population size of an invader is often seen as a more realistic goal (Rita et al., 2022). For example, the release of natural enemies is often used against invasive arthropod pests in agriculture and has been successful in controlling major invasions (M. J. W. Cock et al., 2010).

Lionfishes (Pterois spp.) are a prominent example of a highly invasive species. Since the start of their invasion in the 1990s, the Indo-Pacific red lionfish (Pterois volitans) have been found in invaded ranges spanning from the northeast United States to northern Brazilian coast (Côté et al., 2013; Ferreira et al., 2015).More recently, another species of lionfish (Pterois miles) have started to invade the Eastern Mediterranean after introduction via the Suez Canal in 2012 (Bariche et al., 2013; Dimitriadis et al., 2020; Jimenez et al., 2016; E. W. Phillips & Kotrschal, 2021). Within both of these invaded regions, lionfish have caused great disturbances to the social-economic landscape due to their overconsumption of commercially important prey fish crucial to the local fishing industry, as well as to the ecosystem itself through biodiversity loss and reef deterioration (Côté et al., 2013; Galanidi et al., 2018; Lesser & Slattery, 2011). As such, control measures have been devised to limit their spread in the Atlantic and Eastern Mediterranean Sea (Forrester et al., 2021; Kleitou, Rees, et al., 2021; Kletou et al., 2016; Morris et al., 2011). Culling via spearfishing is considered the best practice to control invasive lionfish populations and is commonly promoted through lionfish culling tournaments that raise awareness and encourage the public to participate in large-scale spearfishing events to reduce lionfish numbers in focal areas (Barbour et al., 2011; Jiménez et al., 2018; Kleitou et al., 2019; Malpica-Cruz et al., 2021). However, as is the case with most hunting activities, spearfishing alters the behavior of fishes when conducted regularly (Casas et al., 2009; Sbragaglia et al., 2018). For example, fishes living in spearfished areas show a longer flight initiation distance to approaching divers and more effective escape responses compared to conspecifics living in unfished waters (Guidetti et al., 2008; Sbragaglia et al., 2018). Lionfish in culled reefs in the Caribbean are more wary towards approaching divers than in reefs where culling does not occur (Côté et al., 2014). Regular culling also resulted in originally crepuscular lionfish becoming more nocturnal and hiding in deeper spots, making encounters with spear fishers less likely (Côté et al., 2014). These behavioral changes can have negative impacts on control strategies that rely on spearfishing by reducing their overall effectiveness if changes persist long-term.

However, as previous studies have only investigated culling-induced behavioral changes in Caribbean lionfish, the presence of such behavioral changes in Mediterranean lionfish remains enigmatic. While both the Caribbean and the Mediterranean possess ecological traits that allow for the spread and establishment of lionfish in their waters, these seas differ greatly in their ecology. For example, in the Caribbean, lionfish are found in a wide range of habitats, from coral reefs to seagrass beds, and even mangroves and estuarine rivers, while in the Mediterranean, lionfish are often found in rocky reefs and underwater caves, and more rarely in sandy bottom areas, seaweed beds, or algal patches (Côté et al., 2013; Dimitriadis et al., 2020). Similarly, while P. miles and P. volitans are nearly identical in their morphology and ecology, they are two separate species and may react differently to stressor such as culling (Schultz, 1986). Little information is known about the general behavior and reactivity of lionfish, so it remains to be explored how these species may differ from one another.

We predict that, despite these differences, behavioral changes due to culling will be present in Mediterranean lionfish. Fishes in general have been shown to retain learned behaviors for extended periods of time (Brown, 2015). For example, cleaning wrasses (Labroides dimidiatus) maintained fear towards divers carrying nets for almost a year after being caught and released (Triki & Bshary, 2020), while hook shyness in carp (Cyprinus carpio) previously caught by fisherman has been shown to last for at least a year (Beukema, 1970). Moreover, for lionfish learning and behaviorally adjusting to local dangers such as spearfishing may be crucial for survival as lionfish often have very small home ranges (typically less than 50 meters in diameter) and can reach up to 30 years of age (Bos et al., 2018; Jud & Layman, 2012; Potts et al., 2010). This means that lionfish will remain in the same local environment long-term, so can benefit from investing into attaining knowledge of their local environment to better survive and avoid dangers. Therefore, we predict that culled populations of lionfish will retain any culling-induced behavioral changes, regardless of the invasive range.

Here, we investigate the presence of culling-induced behavioral changes in lionfish in Cyprus. Cypriot waters are among the most heavily invaded by lionfish in the Mediterranean, as they are close to the Suez Canal, the route of entry for lionfish into the Mediterranean, and offer similar environmental conditions to the Red Sea (Jimenez et al., 2016; Kletou et al., 2016). Lionfish populations have been well established in Cyprus since 2015, particularly in the Southern and Eastern parts of the island (Jimenez et al., 2016). With numbers predicted to increase to the high densities currently observed in the Caribbean, culling tournaments are regularly organized within the framework of the Relionmed-LIFE project within these parts of Cyprus to promote invasive management efforts (D'Agostino et al., 2020; Jiménez et al., 2018; Kleitou, Rees, et al., 2021; Savva et al., 2020).

To investigate the effects of culling on behavior, we tested whether lionfish activity, hiding pattern, and reaction to an approaching diver differed between sites that are regularly culled, and sites where no organized culling of lionfish has occurred. If culling-induced behavioral changes are present in Mediterranean lionfish populations, we predict that lionfish in previously culled areas will hide deeper in hiding spots, be less active, and be more wary towards approaching free-divers than lionfish in areas without a history of culling.

Materials and Methods

Study sites

We conducted observations in Protaras on the eastern coast of Cyprus in September and October 2021. Two of the sites were located in areas where major culling events in the form of lionfish tournaments are commonly held (culled sites) and the other two sites (unculled sites) are part of marine protected areas where recreational fishing is forbidden and major culling events have never occurred (Figure 5.1 & S5.1). Figure 5.2 shows a timeline of the lionfish hunting tournaments that have occurred in the areas, marking the large-scale removal events of lionfish that have taken place here. Given the typically small home ranges of lionfish and the long distance between our study sites (Akins et al., 2014; Jud & Layman, 2012), it is unlikely that lionfish moved among sites during our study.

At each site, we created an underwater grid using labelled white pebbles to delimit quadrants of five meters by five meters to allow for precise location of individual lionfish. We chose the location of the grids based on surveys across a larger area to ensure that at least five lionfish could be found within the area of the grids. The area of the grids ranged between 1350 and 1575 m2 between 0 and 10 meters depth.

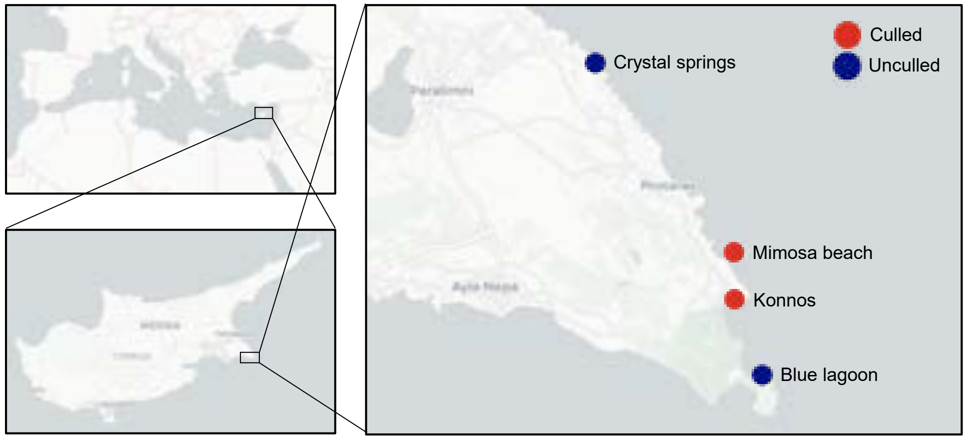


Figure 5.1 *Study sites in South-eastern Cyprus.*

Behavioral assays

We surveyed each site over a period of four weeks while snorkeling above the grid with a torch. Surveys started at 18:30 and ended at 20:30, with a 30-minute break in between. We chose the survey times to maximize the number of lionfish visible from the surface, as most lionfish are crepuscular (D'Agostino et al., 2020). Over the course of our study, we surveyed each grid between five and six times, and during each survey the grid was checked four times. During surveys, two free divers snorkeled above adjacent rows of quadrants. The divers looked in all directions while swimming along a row of quadrants, which allowed them to check for lionfish under crevices not visible from directly above, often in adjacent quadrants. When a lionfish was found, one diver approached it with a torch and camera and filmed both sides of the fish (when possible) for later identification. Meanwhile, the other diver scored the size class, activity, location of the lionfish, and hiding score from 0 to 4 (see Table .51 for descriptions). The same diver then approached the lionfish with a metal pole, simulating a spear fisher in action and the estimated flight initiation distance and burst distance. The behavioral parameters and hiding score were selected and determined after preliminary observations of lionfish along the coast and at different sites.

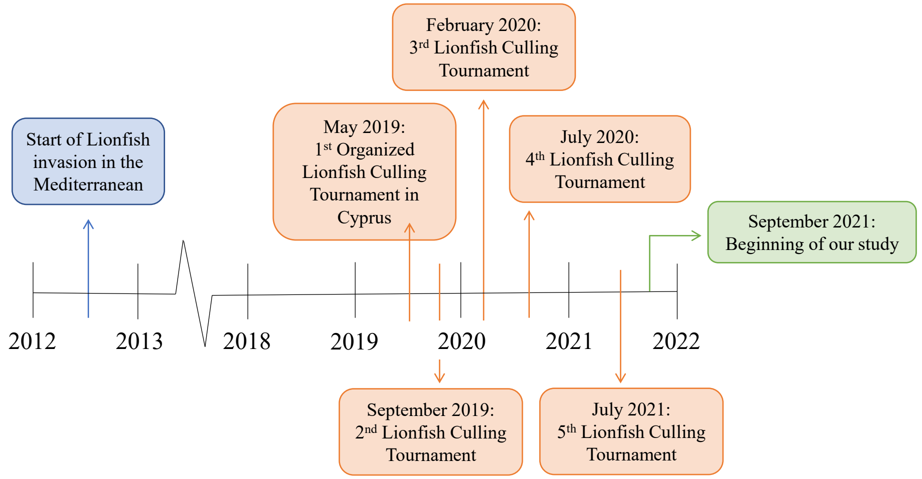


Figure 5.2 *Timeline of lionfish invasion and start of culling events in Cyprus.*

Table 5.1 *Description of behavioral measures.*

Measurement	**Description**
Size Class	Estimation of standard length to the nearest 5 cm
Activity	Movement of lionfish when first spotted; either "swimming" if actively moving, or "resting" if they remained in one place (includes either resting on an object or hovering in one place)
Location	Cartesian coordinates as determined by grid
Hiding Score	Scale of 0-4: 0 = Further than 1 m from any hiding spot (i.e., any spot that could provide cover, including crevices, ledges, spaces between/below boulders, between/behind sponges, etc.) 1 = < 1 m from a hiding spot 2 = Partly hidden when viewed from above 3 = Completely hidden when viewed from above; located in covered hiding spot, but within 50 cm from entrance when viewed from side 4 = Completely hidden when viewed from above; >50cm from entrance of hiding spot when viewed from side
Flight Initiation Distance	Distance between the tip of the pole and the lionfish when an escape burst was triggered (estimated to the nearest 10 cm)
Burst Distance	Length of the escape burst (estimated to the nearest 10 cm)

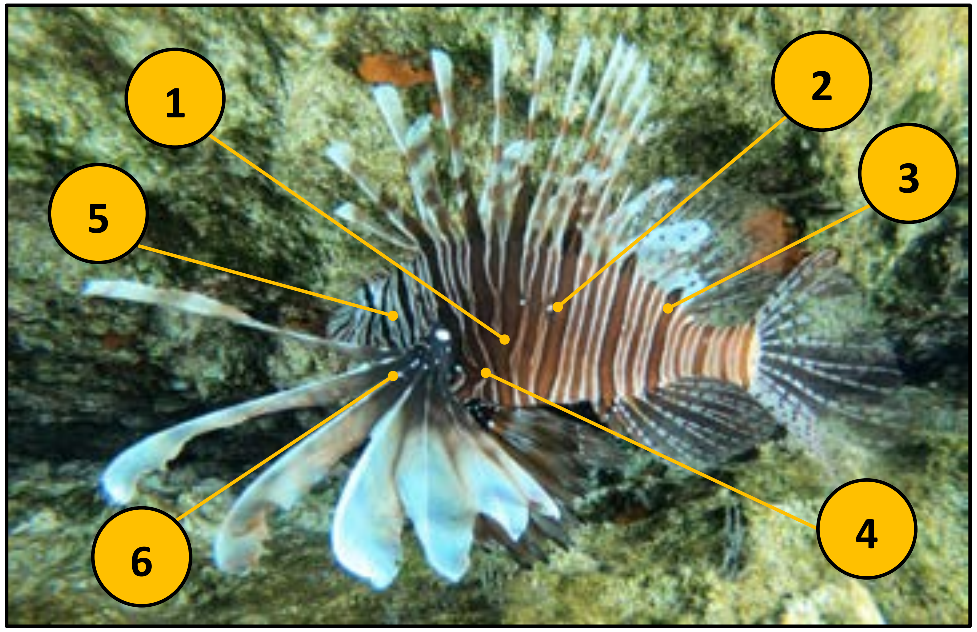


***Figure 5.3** Salient features of lateral pattern used for lionfish identification. Width and length of the Y-shaped bar (present on virtually every lionfish) (**1**), shape and position of white dots on the body (**2**), width and shape of bars on the tail region (**3**), peculiar features of the pattern such as merging bars (**4**), width and shape of bars on the head region (**5**) shape and position of white dots on the fins (**6**).*

Lionfish identification and population density

Each lionfish was assigned an identity based on visual image analysis. Lionfish possess a unique lateral color pattern, consisting mainly of brown-red bars on a pale background (Schultz, 1986). The shape, width and sequence of these bars, together with other salient features of the pattern, allow for precise identification of lionfish individuals through comparisons of lateral images (see Figure 5.3 & S5.2) (Chaves et al., 2016). When the quality of the images and videos did not allow for complete exclusion of matching identities, the fish was classified as unidentified (7.4% of filmed individuals). The Schnabel method was then used to estimate population densities, similar to that of capture-recapture studies (Carvalho et al., 2013; Lukey et al., 2006).

Statistical analyses

In total, we recorded 277 lionfish sightings and assigned identities to 257 of them, as some lionfish could not be identified due to low video quality or lionfish fleeing mid-trial. Through comparison of individual identities, we found 61 unique identities. Of these, 42 individuals (23 from culled and 19 from unculled sites) were encountered more than once. On average, lionfish individuals were encountered 4.51 times during the one month of observations.

Behavioral responses (activity, hiding score, flight initiation distance, and burst distance) were evaluated with generalized linear mixed models in RStudio (R version 4.2.3) with the package “lme4” (Bates et al., 2015). Activity and hiding score were recorded for all lionfish sightings with identified lionfish, while flight initiation distance was only recorded for 162 trials and burst distance for 128 trials. In addition, during measures of flight initiation distance, most fish reacted only when touched by the metal pole (46%) or when their distance from the tip was between 1 and 10 cm (48%). Therefore, we decided to calculate flight initiation distance as a binary variable with lionfish either “touched” or “untouched” by the metal pole when approached. Models for all behavioral responses were evaluated using culling and trial number per individual as fixed factors, and lionfish ID nested in site and size as random factors. Random effects were evaluated for significance using the ANOVA function from the “car” package (Fox & Weisberg, 2019)and subsequently removed if not significant. In all cases, both random factors were non-significant, resulting in activity, hiding score, and flight initiation distance being modelled as a binary variable in a generalized linear model with culling and trial number as fixed factors, and burst distance modelled as a continuous variable in a linear model with culling and trial number as fixed factors.

Cartesian coordinates were used to calculate the distance between consecutive sightings of the same individual and were used as a measure of site fidelity. Only individuals that were identified multiple times were used in this analysis, as a minimum of two sightings is necessary to determine distance between sightings. Multiple site fidelity calculations were also taken for an individual if encountered three or more times, with distance between sightings calculated for each consecutive sighting. We modelled the distance between sightings (site fidelity) in a LMM where Culling was used as a fixed factor and Lionfish ID, nested in Site, as a random factor.

There was no correlation between distance and time between sightings (Figure S5.3) and therefore no reason to account for time between sightings.

We made all graphs and maps and performed statistical analyses in RStudio. We used the packages 'lme4' and 'car' to fit and test the models, 'leaflet' (Cheng et al., 2001) for the maps and 'tidyverse' (Wickham et al., 2019) for summary statistics, data wrangling and graphs. We used the package 'fishmethods' (Nelson, 2022) to estimate lionfish population densities with the Schnabel method.

Results

Lionfish in culled and unculled sites showed no difference in hiding score (GLM: p = 0.648, Figure 5.4a) or flight initiation distance to an approaching free diver (GLM: p = 0.848, Figure 5.4b). However, burst distance showed signs of habituation as it decreased over time (LM: p = 0.01, Figure 5.4d), though no overall difference between culled and unculled sites (GLM: p = 0.900). Activity level on the other hand was decreased in culled populations (GLM: p = 0.004, Figure 5.4c) and decreased over trials (GLM: p = 0.031).

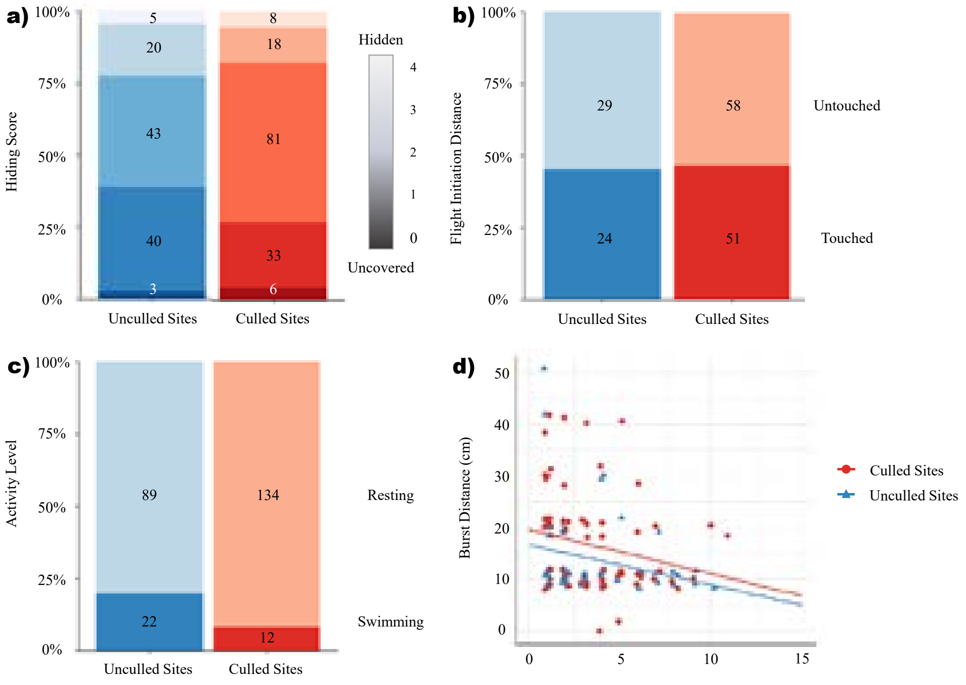


Figure 5.4 *Behaviour of lionfish in culled and unculled sites. Shown are hiding score (**a**), flight initiation distance (touched vs untouched) (**b**), activity level (swimming vs resting) (**c**), and burst distance (**d**).*

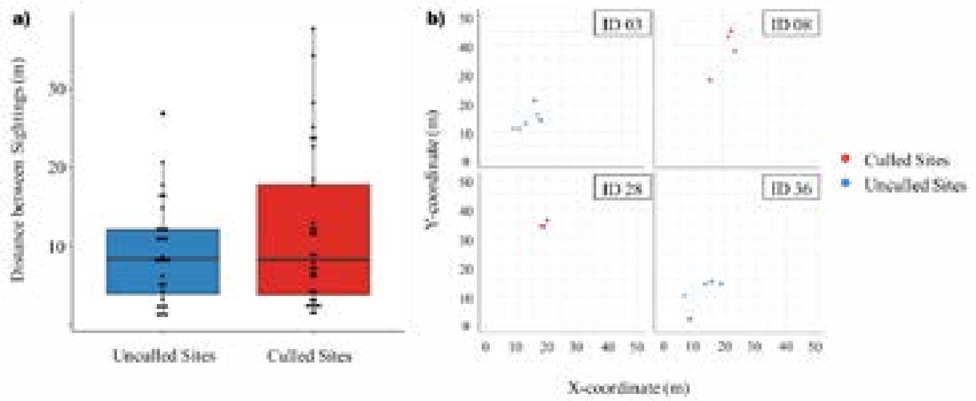


Figure 5.5 *Site fidelity of lionfish. Shown are the distances between consecutive sightings of lionfish in culled and unculled areas (**a**) and the sighting locations of four representative individuals in culled and unculled sites with each dot marking a different sighting within the individual grid (**b**).*

Table 5.2 *Estimates of lionfish population size and population density at our four sites in Cyprus. Population size shows the estimated number of lionfish on a grid calculated with the Schnabel method and it is reported with a confidence interval (CI). Area indicates the surface of a grid and Population density is the ratio between Population size and Area.*

See Figure 5.1 for location of sites.

Site	**Population size**	**CI**	**Area** (m^2)	**Population density** (fish/ha)
Unculled Sites				
Blue lagoon	20.25	10-81	1575	129
Crystal springs	11.46	8-18	1400	82
Culled Sites				
Konnos	25.62	17-41	1575	163
Mimosa beach	17.73	11-39	1350	131

The mean distance between consecutive sightings for all fish was 10.5 m (sd = 8.6 m, n = 60) and was not affected by culling (LMM: p = 0.39, Figure 5.5). Estimated population densities ranged between 82 and 163 lionfish/ha (Table 5.2).

Discussion

We investigated the effects of culling on the behavior of invasive lionfish in the Mediterranean and found that in this population, repeated culling resulted in subtle behavioral differences when compared to lionfish that had not experienced intensive culling events. This is remarkable as strong behavioral changes caused by spearfishing are well-established in fishes in general and for lionfish, and are often retained for years (Côté et al., 2014; Januchowski-hartley et al., 2011; Satterfield & Johnson, 2020; Sbragaglia et al., 2018). In our study, activity level was the only measure significantly affected by culling, while other measures such as flight initiation distance, burst distance, and hiding score showed no significant difference between culled and unculled sites. Flight initiation distance might have been unaffected by culling since lionfish are well-protected by their venomous spines and typically stand their ground against potential predators rather than fleeing (Côté et al., 2014). However, hiding score, burst distance, and site fidelity, are all predicted to be indicators of wariness in lionfish, so their lack of difference supports the hypothesis that lionfish in the Mediterranean either do not experience or do not retain pronounced culling-induced behavioral changes. In the following, we discuss several reasons why lionfish in the Mediterranean show remarkably little long-term behavioral response to culling and why this seems to differ from Caribbean findings.

Our study was not set up for a direct comparison between Caribbean and Mediterranean lionfish' response to culling pressure. For such a comparison the methods would have needed to be closer matched. For instance, we surveyed in late afternoon and early night using snorkeling techniques, while Coté et al. (2014) used diurnal SCUBA diving censuses. Our aim was rather to determine long term behavioral consequences of hunting pressure on lionfish, for which we compared culled and unculled sites. Nevertheless, we here use the Caribbean situation as point of reference for our own results. For instance, although previous studies have found that invasive lionfish show similar behavioral and ecological traits across ranges (Cure et al., 2012; D'Agostino et al., 2020; McTee & Grubich, 2014), the lionfish populations of Cyprus and the Caribbean are of two closely related, but separate species. In the Caribbean, most invasive lionfish are of the species P. volitans, while in the Mediterranean, the invading lionfish are P. miles (Bariche et al., 2017; Hamner et al., 2007). Based on their similar morphology and ecology, these species were assumed to be the same until the 1980s (Schultz, 1986). Only more recently more attention has been paid to how these species differ, resulting in debate on how closely related they are, and if P. volitans and P. miles hybridize in invasive ranges (Hamner et al., 2007; Wilcox et al., 2018). In other taxa, however, it has been shown that even closely related species can differ in their behavior. For example, in Drosophila, two closely related species (D. subquinaria and D. recens) were found to have differences mating signals and preferences, even though the species have overlapping geographical ranges and are almost indistinguishable in their morphology (Giglio & Dyer, 2013). Also, in parasitic wasps, memory after experience with olfactory cues differed between the closely related species Cotesia glomerata and Cotesia rubecula, with C. glomerata retaining the olfactory memory trace for 5 days after a single associative learning experience, while C. rubecula's memory began to fade after the first day (Bleeker et al., 2006). Differences in learning ability between P. miles and P. volitans might play a role in the differences in behavior observed after culling as we predict that learning may be the mechanism through which lionfish obtain these culling-induced behavioral changes. In our study, we show that multiple contacts with the same diver resulted in lionfish showing shorter burst distances through habituation learning (Figure 5.4). Learning is a widespread phenomenon among fishes (Brown, 2003), and lionfish in particular have been shown to possess the capacity for associative learning (de Groot, 2021; E. W. Phillips et al., 2022). Moreover, in another study, short-term effects of culling were evident when several sites of major removal activities were revisited after 2-3 days, with lionfish behaving more wary when approached by divers and hiding surprisingly fast even at a distance of 5 m from divers (Jimenez in prep.). This may mean that just like the parasitic wasps discussed above, P miles and P. volitans, may differ in their retention of these learned behaviors, rather than in their acquisition.

Culling intensity is known to determine the strength of behavioral changes in fishes targeted by spear fishers, and if culling is done at a low enough intensity, it can prevent enough pressure from being exerted to change behavior (Januchowski-hartley et al., 2011; Sbragaglia et al., 2018). In our sites, the frequency of culling events was reduced in 2020 and 2019 due to the covid-19 pandemic, which may have relaxed culling pressure, and ultimately led to the lack of behavioral differences seen in Mediterranean lionfish from culled and unculled areas. We think that this is unlikely, however, as lionfish have been previously reported to show no behavioral differences between reefs where culling occurred every three versus every six months (Côté et al., 2014), indicating that lionfish may be less susceptible to differences in culling intensities compared to other fish species. Similarly, studies on lionfish culling-induced behavioral changes in the Caribbean took place around three weeks after the last major culling event, while in our study in the Mediterranean, the last culling event was two months prior to the start of our behavioral studies, implying that lionfish may only retain behavioral changes for a short period of time. We consider this explanation unlikely, though, as fishes have been shown to have robust memories that persist over several months or years, especially for signals that indicate danger such as the presence of hunters (Beukema, 1970; Brown, 2015; Triki & Bshary, 2020). Future studies investigating the effects of culling on the same population at different time scales (i.e., both long- and short-term) can elucidate how long the effects of regular culling persist in lionfish. Our study took place during the evening in low-light conditions, while culling tournaments are organized during the day (Kleitou et al., 2021). Potentially, lionfish at culled sites may have learnt to avoid divers only during day time. While we think this is an unlikely explanation for our results, additional trials including day and night sampling will be necessary to determine whether lionfish indeed do not generalize predation threat across day time.

Alternatively, the Mediterranean and Caribbean offer very different environments that could influence the number and accessibility of suitable hiding places for lionfish when they perceive a threat. For example, lionfish are typically found in different types of habitats between the two invasive ranges: in the Caribbean lionfish are typically found in a wide range of habitats, including coral reefs, seagrass meadows, and even mangroves, while the Mediterranean typically inhabit rocky structures, such as rocky reefs and crevices, or underwater caves (Côté et al., 2013; Dimitriadis et al., 2020). This could account for the differences in behavior seen between Caribbean and Mediterranean lionfish after culling, as Mediterranean lionfish may be more hidden from divers naturally, resulting in less selection for learned avoidance behavior as they are less likely to be found by hunters in the first place. Meanwhile, in the Caribbean lionfish may be more exposed and therefore rely on the behavioral adaptations to survive when faced with threats, like spear fishers. Future studies on how habitat structure affects lionfish behavior are needed to explore this idea further.

Additionally, in our study we show it is possible to identify lionfish at the individual level in the wild without physical tags or other markers. Image-based identification offers several advantages compared to tagging methods used in other studies as fish are not handled during identification, resulting in decreased safety risk to operators, limited stress to the fish and no risk of inducing barotrauma (Akins et al., 2014; Chaves et al., 2016). This identification method could be used in future experiments for repeated observations accounting for identity that could provide information on whether changes in behavior happen at the individual level, indicating a role of learning in culling-induced behavioral changes.

With this methodology of individual photoidentification, we found that lionfish densities in our study sites ranged from 82 to 163 lionfish/ha. Previous studies in the Mediterranean have found that lionfish population densities near our study sites range from 25 to 100 lionfish/ha prior to major removal events, similar to our estimates (Kleitou, Rees, et al., 2021). Meanwhile in the Western Atlantic, higher densities are reported (max. 400 lionfish/ha), while in the native range, densities tend to be lower than in either invasive range (max. 26 lionfish/ha) (Kulbicki et al., 2012). Therefore, our results agree with previous reports in the Mediterranean relative to other lionfish ranges showing that population numbers within the Mediterranean invasive range fall in the middle of those in the Caribbean and native ranges. Considering that the Mediterranean has been invaded more recently than the Atlantic, it could be predicted that the Mediterranean population density will grow in the coming years and reach similar levels to those in the Atlantic. However, given the different habitat that the Mediterranean offers compared to the coral reefs of the Caribbean and the Red Sea, it is also possible that the population density in Cyprus is already stable. Future studies on lionfish population dynamics in the Mediterranean are necessary.

In conclusion, we showed that Mediterranean lionfish differ from their Caribbean counterparts in their resilience behavior in reaction to regular culling. We suggest these differences may be due to the fact that different species are invading the Caribbean and Mediterranean (P. volitans and P. miles, respectively), due to differences in habitat composition of the two invasive areas, or due to differences in methodologies. We successfully used individual photoidentification of lionfish in the wild to estimate the lionfish density at our study sites and our density estimates are in agreement with previous reports from this region. This methodology therefore can be applied in future studies to answer a wide range of research questions about this species.

Supplementary Material

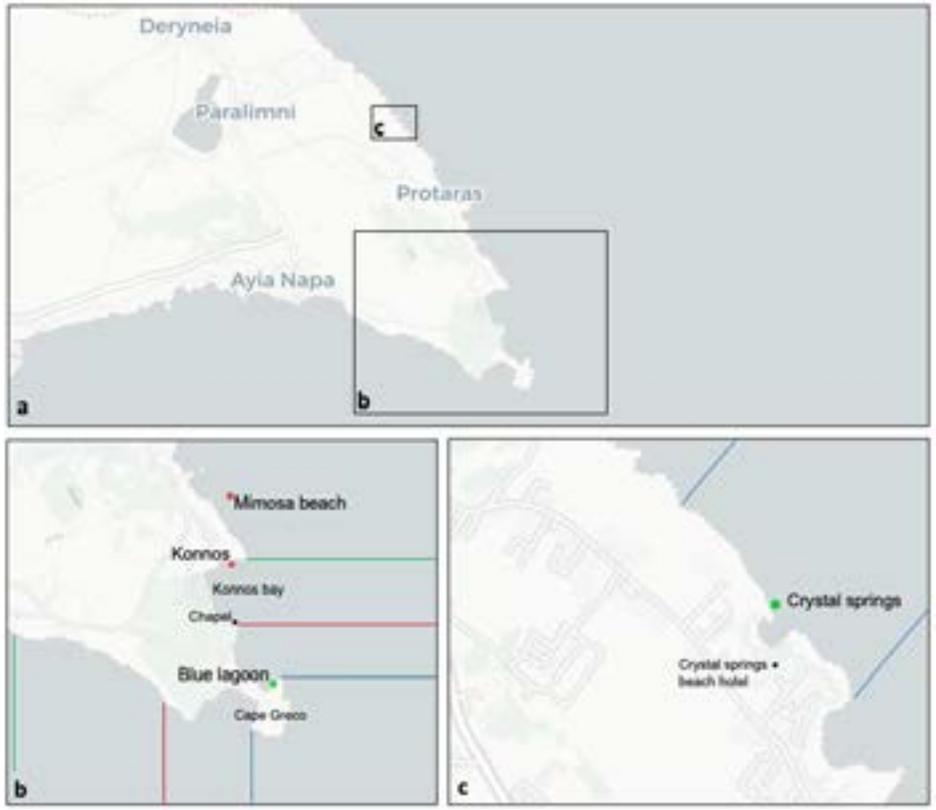


Figure S5.1 *Location of the study sites (***a***) and their position relative to two marine protected areas: 'Cavo Greco marine protected area' (***b***) and 'Marine protected area with artificial reefs in Paralimni' (***c***). Blue lines delimit areas where fishing is forbidden; red lines delimit areas where recreational fishing is forbidden; green lines delimit the 'peripheral zone' of the Cavo Greco marine protected area, where no fishing restrictions are enforced. Recreational fishing includes both angling and spearfishing.*

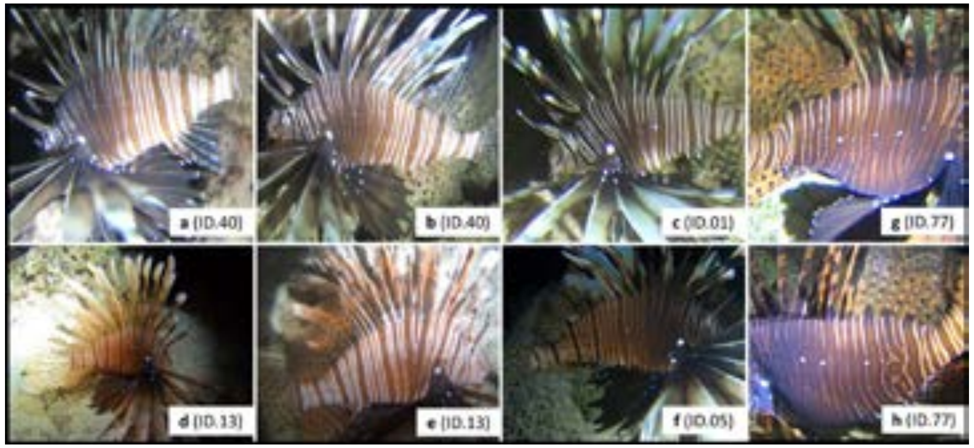


Figure S5.2** Examples of image-based lionfish identification. The Y-shaped bar and the position of white dots indicate that picture* **a** *and* **b** *portray the same individual (ID.40). The Y-shaped bar is variable in shape and width, as illustrated by the comparison between ID.40 (a** *and* **b***) and ID.01 (***c***). ID.13 (***d** *and* **e***) shows a peculiar bending on one of the bars towards the tail. The complex pattern with bars in the tail region merging at several points in ID.05 (***f***) makes it clearly different from ID.13. Importantly, the pattern on the two sides of the same individual can be markedly different, as illustrated by* **g** *and* **h***, both showing ID.77.*

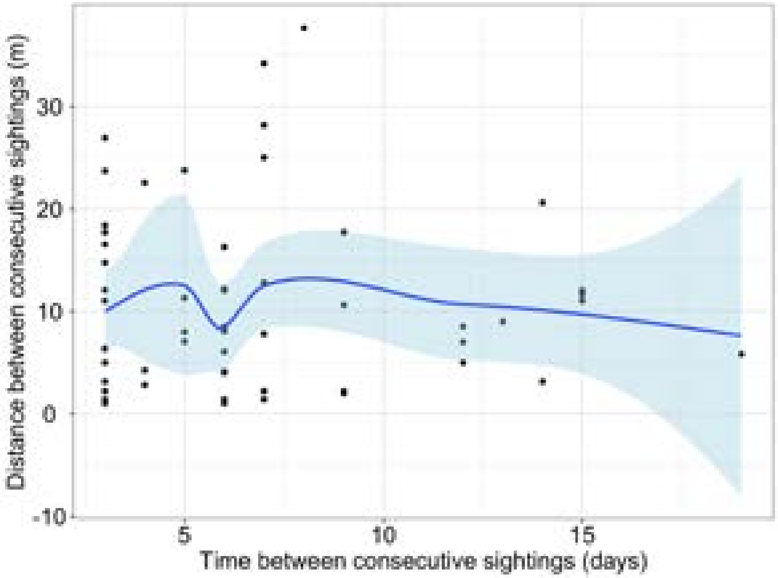


***Figure S5.3** Relationship between time and distance between consecutive sightings (p = 0.96, R2 < 0.001).*

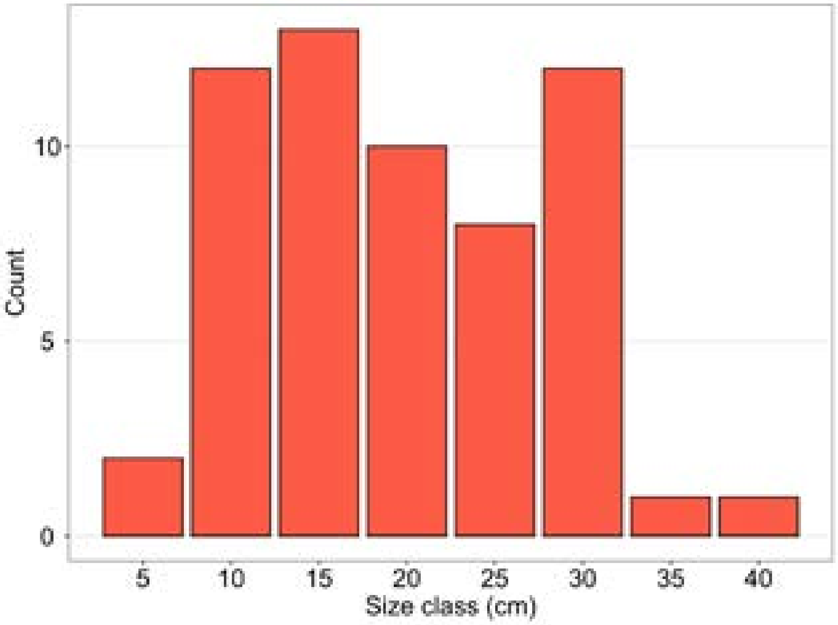


***Figure S5.4** Number of identified lionfish in each size class. The x axis shows the upper limit of each size class (e.g., class 25 = fish between 20 and 25 cm of standard length).*

References

Akins, J. L., Morris Jr, J. A., & Green, S. J. (2014). In situ tagging technique for fishes provides insight into growth and movement of invasive lionfish. Ecology and Evolution, 4(19), 3768–3777. https://doi.org/10.1002/ece3.1171

Barbour, A. B., Allen, M. S., Frazer, T. K., & Sherman, K. D. (2011). Evaluating the potential efficacy of invasive lionfish (Pterois volitans) removals. PLoS ONE, 6(5), e19666. https://doi.org/10.1371/journal.pone.0019666

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(6782), 1–6. 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), 292–294.

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Beukema, J. J. (1970). Angling experiments with carp (Cyprinus carpio L). Decreasing catchability through one-trial learning. Netherlands Journal of Zoology, 20(1), 81–92. https://doi.org/doi.org/10.1163/002829670X00088

Bleeker, M. A. K., Smid, H. M., Steidle, J. L. M., Kruidof, H. M., van Loon, J. J. A., & Vet, L. E. M. (2006). Differences in memory dynamics between two closely related parasitoid wasp species. Animal Behaviour, 71, 1343–1350. https://doi.org/10.1016/j.anbehav.2005.09.016

Bos, A. R., Grubich, J. R., & Sanad, A. M. (2018). Growth, site fidelity, and grouper interactions of the Red Sea lionfish Pterois miles (Scorpaenidae) in its native habitat. Marine Biology, 165(10), 1–10. https://doi.org/10.1007/s00227-018-3436-6

Brown, C. (2015). Fish intelligence, sentience and ethics. Animal Cognition, 18(1), 1–17. https://doi.org/10.1007/s10071-014-0761-0

Brown, G. E. (2003). Learning about danger : chemical alarm cues and local risk assessment in prey fishes. Fish and Fisheries, 4, 227–234.

Brown, J. H., & Sax, D. F. (2004). An Essay on Some Topics Concerning Invasive Species. Austral Ecology, 29, 530–536.

Carey, M. P., Sanderson, B. L., Barnas, K. A., & Olden, J. D. (2012). Native invaders – challenges for science, management, policy, and society. Frontiers in Ecology and the Environment, 10(7), 373–381. https://doi.org/10.1890/110060

Carvalho, D. de A., Collins, P. A., & de Bonis, C. J. (2013). The mark-recapture method applied to population estimates of a freshwater crab on an alluvial plain. Marine and Freshwater Research, 64, 317–323.

Casas, F., Mougeot, F., Viñuela, J., & Bretagnolle, V. (2009). Effects of hunting on the behaviour and spatial distribution of farmland birds: importance of hunting-free refuges in agricultural areas. Animal Conservation, 12, 346–354. https://doi.org/10.1111/j.1469-1795.2009.00259.x

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, 800–804. https://doi.org/10.1111/jfb.12857

Cheng, J., Karambelkar, B., & Xie, Y. (2001). Leaflet: create interactive web maps with the JavaScript “Leaflet” library. R package version 2.0.4.1.

Cock, M. J. W., van Lenteren, J. C., Brodeur, J., Barratt, B. I. P., Bigler, F., Bolckmans, K., Cônsoli, F. L., Haas, F., Mason, P. G., & Parra, J. R. P. (2010). Do new Access and Benefit Sharing procedures under the Convention on Biological Diversity threaten the future of biological control? BioControl, 55, 199–218. https://doi.org/10.1007/s10526-009-9234-9

Côté, I. M., Darling, E. S., Malpica-Cruz, L., Smith, N. S., Green, S. J., Curtis-Quick, J., & Layman, C. (2014). What Doesn’t Kill You Makes You Wary? Effect of Repeated Culling on the Behaviour of an Invasive Predator. PLoS ONE, 9(4), e94248. https://doi.org/10.1371/journal.pone.0094248

Côté, I. M., Green, S. J., & Hixon, M. A. (2013). Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. Biological Conservation, 164, 50–61. https://doi.org/10.1016/j.biocon.2013.04.014

Cure, K., Benkwitt, C. E., Kindinger, T. L., Pickering, E. A., Pusack, T. J., McIlwain, J. L., & Hixon, M. A. (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. A. (2020). Behavioural traits and feeding ecology Mediterranean lionfish and naiveté native species lionfish predation. Marine Ecology Progress Series, 638, 123–135.

de Groot, C. (2021). Exploring the cognitive landscape of an invasive species: reversal learning in the red lionfish (Pterois volitans). Wageningen University.

Dimitriadis, C., Galanidi, M., Zenetos, A., Corsini-Foka, M., Giovos, I., Karachle, P.

K., Fournari-Konstantinidoy, 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 the thermal boundaries and future range expansion. Mediterranean Marine Science, 21, 62–69.

Ferreira, C. E. L., Luiz, O. J., Floeter, S. R., & Lucena, M. B. (2015). First Record of Invasive Lionfish (Pterois volitans) for the Brazilian Coast. PLoS ONE, 10(4), e0123002. https://doi.org/10.1371/journal.pone.0123002

Forrester, G. E., Dzilenski, K. B., & Gleeson, D. W. (2021). Use of social-media networking to facilitate a grass-roots lionfish removal program in the British Virgin Islands. Management of Biological Invasions, 12.

Fox, J., & Weisberg, S. (2019). An {R} companion to applied regression, third edition. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

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, 107–123.

Giglio, E. M., & Dyer, K. A. (2013). Divergence of premating behaviors in the closely related species Drosophila subquinaria and D. recens. Ecology and Evolution, 3(2), 365–374. https://doi.org/10.1002/ece3.477

Guidetti, P., Vierucci, E., & Bussotti, S. (2008). Differences in escape response of fish in protected and fished Mediterranean rocky reefs. Journal of the Marine Biological Association of the United Kingdom, 88(3), 625–627. https://doi.org/10.1017/S0025315408000933

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(Supplement B), 214–222. https://doi.org/10.1111/j.1095-8649.2007.01575.x

Hewitt, C. L., & Campbell, M. L. (2007). Mechanisms for the prevention of marine bioinvasions for better biosecurity. Marine Pollution Bulletin, 55, 395–401. https://doi.org/10.1016/j.marpolbul.2007.01.005

Januchowski-hartley, F. A., Graham, N. A. J., Feary, D. A., Morove, T., & Cinner, J. E. (2011). Fear of Fishers: Human Predation Explains Behavioral Changes in Coral Reef Fishes. PLoS ONE, 6(8). https://doi.org/10.1371/journal.pone.0022761

Jiménez, C., Çiçek, B. ali, Jiménez, J. U., Hadjioannou, L., & Huseyinoglu, M. F. (2018). What is the roar about? Lionfish targeted removals in Costa Rica (Central America) and Cyprus (Mediterranean Sea). In M. F. Hüseyinoglu & B. Öztürk (Eds.), Lionfish Invasion and Its Management in the Mediterranean Sea (Issue 49, pp. 34–45).

Jimenez, C., Petrou, A., Andreou, V., Hadjioannou, L., Wolf, W., Koutsoloukas, N., & Alhaija, R. A. (2016). Veni, vidi, vici: the successful establishment of the lionfish pterois miles in Cyprus (Levantine Sea). Rapp. Comm. Int. Mer Medit., 41, 417. http://ciesm.org/online/archives/abstracts/pdf/41/CIESM_Congress_2016_Kiel_article_0417.pdf

Jud, Z. R., & Layman, C. A. (2012). Site fidelity and movement patterns of invasive lionfish, Pterois spp., in a Florida estuary. Journal of Experimental Marine Biology and Ecology, 414–415, 69–74. https://doi.org/10.1016/j.jembe.2012.01.015

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, 1–13. https://doi.org/10.1002/aqc.3669

Kleitou, P., Savva, I., Kletou, D., Hall-Spencer, J. M., Antoniou, C., Christodoulides, Y., Chartosia, N., Hadjioannou, L., Dimitriou, A. C., Jimenez, C., Petrou, A., Sfenthourakis, S., & Rees, S. (2019). Invasive lionfish in the Mediterranean: Low public awareness yet high stakeholder concerns. Marine Policy, 104, 66–74. https://doi.org/10.1016/j.marpol.2019.02.052

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), 1–7. https://doi.org/10.1186/s41200-016-0065-y

Kulbicki, M., Beets, J., Chabanet, P., Cure, K., Darling, E., Floeter, S. R., Galzin, R., Green, A., Harmelin-Vivien, M., Hixon, M., Letourneur, Y., De Loma, T. L., McClanahan, T., McIlwain, J., MouTham, G., Myers, R., O'Leary, J. K., 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

Lesser, M. P., & Slattery, M. (2011). Phase shift to algal dominated communities at mesophotic depths associated with lionfish (Pterois volitans) invasion on a Bahamian coral reef. Biological Invasions, 13(8), 1855–1868. https://doi.org/10.1007/s10530-011-0005-z

Lukey, J. R., Booth, A. J., & Froneman, P. W. (2006). Fish population size and movement patterns in a small intermittently open South African estuary.

Estuarine Coastal and Shelf Science, 67, 10–20. https://doi.org/10.1016/j.ecss.2005.10.021

Malpica-Cruz, L., Fulton, S., Quintana, A., Zepeda-Domínguez, J. A., Quiroga-García, B., Tamayo, L., Canto Noh, J. Á., & Côté, I. M. (2021). Trying to collapse a population for conservation: commercial trade of a marine invasive species by artisanal fishers. Reviews in Fish Biology and Fisheries, 31(May), 667–683. https://doi.org/10.1007/s11160-021-09660-0

McTee, S. A., & Grubich, J. R. (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., Shertzer, K. W., & Rice, J. A. (2011). A stage-based matrix population model of invasive lionfish with implications for control. Biological Invasions, 13(1), 7–12. https://doi.org/10.1007/s10530-010-9786-8

Nelson, G. A. (2022). Fishmethods: fishery science methods and models. R package version 1.11.3.

Phillips, E. W., de Wit, K. E., & Kotrschal, A. (2022). Lionfish (Pterois volitans) can see UV light: UV Vision in an Invasive Aquatic Predator. Preprint. https://www.biorxiv.org/content/10.1101/2022.09.09.507242v1

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

Potts, J. C., Berrane, D., & Morris, Jr., J. A. (2010). Age and Growth of Lionfish from the Western North Atlantic. Proceedings of the 63rd Gulf and Caribbean Fisheries Institute.

Rita, J., Capó, M., & Cursach, J. (2022). Eradication of rabbits from islets is essential for conservation of microinsular vegetation and narrow endangered flora: the case of Medicago citrina (Fabaceae) in s'Espartar islet (Balearic Islands, Western Mediterranean Basin). Biodiversity and Conservation, 31, 779–796. https://doi.org/10.1007/s10531-022-02362-4

Satterfield, D., & Johnson, D. W. (2020). Local adaptation of antipredator behaviors in populations of a temperate reef fish. Oecologia, 194, 571–584. https://doi.org/10.1007/s00442-020-04757-y

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, April. https://doi.org/10.1111/jfb.14340

Sbragaglia, V., Morroni, L., Bramanti, L., Weitzmann, B., Arlinghaus, R., & Azzurro, E. (2018). Spearfishing modulates flight initiation distance of fishes: the effects of protection, individual size, and bearing a speargun. ICES Journal of Marine Science, 75(5), 1779–1789. https://doi.org/10.1093/icesjms/fsy059

Schultz, E. T. (1986). Pterois volitans and Pterois miles : Two Valid Species. Copeia1, 3, 686–690.

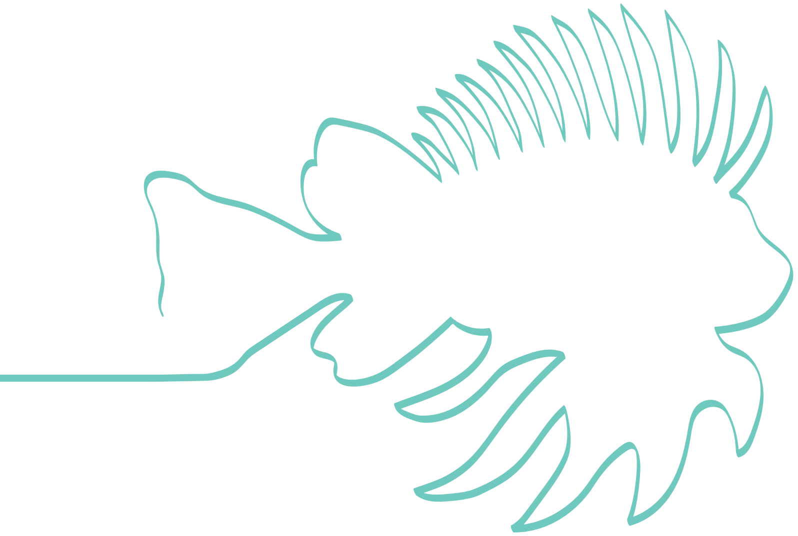
Triki, Z., & Bshary, R. (2020). Long□term memory retention in a wild fish species Labroides dimidiatus eleven months after an aversive event. Ethology, 126, 372–376. https://doi.org/10.1111/eth.12978

Wickham, H., Averick, M., Bryan, J., Chang, W., Mcgowan, L. D. A., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Lin, T., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., … Woo, K. (2019). Welcome to the Tidyverse Tidyverse package. 4, 1–6. https://doi.org/10.21105/joss.01686

Wilcox, C. L., Motomura, H., Matsunuma, M., & Bowen, B. W. (2018). Phylogeography of Lionfishes (Pterois) Indicate Taxonomic Over Splitting and Hybrid Origin of the Invasive Pterois volitans. Journal of Heredity, 162–175. https://doi.org/10.1093/jhered/esx056

Chapter 6

General Discussion



Lionfish are considered to be one of the most successful marine invaders in the world, having covered an impressive invasive range in the Caribbean and Atlantic Ocean, while also continuing to expand into the Mediterranean (Côté et al., 2013; Schofield, 2010). While several factors that are predicted to impact lionfish's invasive success are well studied, such as their unique morphology, high fecundity, and overall generalist behavior, many traits of lionfish, such their behavior and cognitive abilities, remain enigmatic. In this thesis, I begin to explore different facets of lionfish behavior and cognition to investigate why lionfish are such successful invaders.

What factors have led to lionfish's invasive success and continued range expansion?

First, to provide an up-to-date record on the boundaries of lionfish's expansion into their newest invasive range, I conducted a citizen science survey of dive centers in the Mediterranean asking respondents to report if they have seen lionfish in their local area, and if so, when (**Chapter 2**). Over 200 dive centers across the Mediterranean responded to my survey, revealing that lionfish at the invasion fronts are continuing to expand north into the Aegean and Ionian Seas and west through the Mediterranean Sea, even into regions previously deemed unsuitable for lionfish. Lionfish were predicted to have limited settlement in these areas due to multiple ecological factors, including average temperature, habitat suitability, and connectivity between these areas (Johnston & Purkis, 2014; Poursanidis, 2015; Poursanidis et al., 2020). In other studies, lionfish have also shown significant range expansion in the Caribbean, again passing over predicted barriers to expansion such as the Amazon-Orinoco Plume – a biogeographically division between the Caribbean and Brazil that is often considered an obstacle to many marine species' expansions (Ferreira et al., 2015; Luiz et al., 2021; Rocha, 2003). While climate change is predicted to change some of the factors, such as average temperature, in ways that would benefit lionfish expansion into these areas, it does not seem to explain the whole story. I propose that several factors could contribute to lionfish's surprising ability to expand into new areas, including their behavior and cognitive abilities. Below, I discuss a few of these potential factors that I explored in my thesis, as well as provide future directions research could take to continue investigating what makes lionfish so invasive.

Sensory Capabilities

Starting first with species-level adaptions that can make lionfish more success invaders, I explore the visual capabilities of lionfish, as I predict increased spectral sensitivities through color vision and UV detection could be contributor towards lionfish's hunting success in invasive regions, which in turn increases their invasive success. To investigate this, I first conduct an RNA analysis of opsin gene expression in the retina of lionfish (**Chapter 3**). In this analysis, I found evidence for expression of short-wavelength-sensitive opsins SWS1 ("ultraviolet") and SWS2 ("violet-blue"), middle-wavelength-sensitive rhodopsin-like RH2 ("blue-green"), and rhodopsin/rod opsin RH1 (dim-light vision), supporting my hypothesis that lionfish possess color vision, and potentially UV vision as well. However, while physiological evidence can suggest at the potential for certain visual capabilities in species, and further testing with behavioral assays is needed to determine if visual cues in the environment are not only detected in the eye, but processed further in the brain resulting in use of the information when interacting with one's environment (Kelber et al., 2003). Therefore, I used an associative learning assay to test for:

1) the ability to distinguish the colors blue and green from one another (**Chapter 3**), and

2) the ability to detect and discriminate UV versus non-UV light (**Chapter 4)**.

Since the ability to distinguish the two stimuli in each experiment would be required to learn which of the stimuli produces a reward when chosen, evidence of learning in these experiments would also be evidence that lionfish are able to detect differences in the stimuli and therefore have the potential for either color vision or UV vision, respectively. In the first experiment (**Chapter 3**), I found that overall, lionfish were able to learn the associative learning task, even when luminance was controlled for, supporting my hypothesis that lionfish can differentiate colors in the visual spectrum and therefore possess color vision. In the second experiment (**Chapter 4**), while evidence should be interpreted cautiously, I conclude that some individuals were able to successfully learn to associate either a UV or non-UV light with a food reward, supporting the hypothesis that lionfish possess at least some capacity for UV detection.

How do these visual capabilities impact the invasive success of lionfish? Most predatory fish have a more limited spectral sensitivity when compared to prey fish, due in part to most predators hunting during crepuscular or nocturnal hours when this increased spectral sensitivity is less useful (Lythgoe et al., 1994; Mccomb et al., 2010; Siebeck & Marshall, 2000). While there are many advantages to having reduced spectral sensitivity for predators that hunt nocturnally, such as increased prey detection at long distances or reduced risk of damage from long-term UV exposure in the eye, lionfish instead may benefit from having more “generalist” visual abilities that allow them to more readily adapt to different hunting niches. Indeed, lionfish have been shown to hunt diurnally in invasive ranges, though it is commonly assumed that they are crepuscular or nocturnal hunters in native ranges (Côté & Maljković, 2010; Morris Jr. & Akins, 2009), supporting my hypothesis that lionfish may have more flexibility in their hunting strategies than is common in other fish predators. Similarly, chromatic visual signals like color have been proposed to be used more when detecting large or close-range objects(Potier et al., 2018), so improvements to this type of vision could be predicted to enhance lionfish’s close-range hunting.

The benefits lionfish may gain by maximizing hunting success through vision might be even further enhanced by the effects of prey naiveté in invasive fronts. Previous studies have shown that in invasive ranges, prey fish often do not recognize lionfish as a predator due to their unfamiliarity with lionfish’s unique morphology, and this naiveté is predicted to be one of the main reasons for lionfish’s large impact of native ecosystems via predation (Anton et al., 2016; Benkwitt, 2017; D’Agostino et al., 2020). Combined with the hunting advantages lionfish may have over other native predators through improved spectral sensitivity, this could lead to increased invasive success for lionfish. However, further research should investigate the direct relationship between visual capabilities in lionfish, their hunting ability, and their invasive success.

Behavioral Flexibility

Next, I investigate how the individual behavior of lionfish may impact their invasive success, specifically their behavioral flexibility. One of the most common methods for controlling lionfish populations in invasive areas is culling via spearfishing (Barbour et al., 2011; Jiménez et al., 2018). However, one of the potential downsides of systematic culling is that the target species may change their behavior after subsequent cullings in ways that result in less successful removals over time (Casas et al., 2009; Guidetti et al., 2008; Sbragaglia et al., 2018). Indeed, in a study on lionfish in the Caribbean, researchers found that lionfish in areas regularly culled showed more wary behavior than areas that had no or limited history of culling (Côté et al., 2014). However, no studies have investigated this effect on lionfish in the Mediterranean, despite its importance in the effectiveness of management strategies. Therefore, in **Chapter 5** I conduct a behavioral experiment investigating the response of lionfish to an approaching free diver in both previously culled and unculled areas. I found that despite my predictions, lionfish in the Mediterranean show little differences in activity, hiding behavior, or flight response between culled and unculled areas.

While at first glance, this may seem to reveal a lack of behavioral flexibility in Mediterranean lionfish, I argue it may actually show lionfish’s ability to independently respond to each new invasive environment and behave adaptively in that specific environment. When comparing my results to those found in the Caribbean study on lionfish wariness behavior after culling (Côté et al., 2014), it is clear that lionfish in the twos areas show different responses to threats by humans. This may have occurred for a variety of reasons, some of which may be due to environmental differences that lionfish are responding to. For example, the Mediterranean Sea environment is much rockier, with more readily-available nooks and crevices for lionfish to hide in compared to the Caribbean environment that has a larger range of environments that provide various degrees of the cover (Côté et al., 2013; Dimitriadis et al., 2020). Therefore, lionfish in the Mediterranean may already be able to avoid major negative effects from culling without having to increase their hiding ability, while Caribbean lionfish may have to adapt to their local environment and hide more when threatened to escape culling. Thus, this may show increased behavioral flexibility in the species, as lionfish populations are able to tailor their behavior to the environment and the threats they experience in each location.

However, there is a caveat to this conclusion, as there are other potential reasons for differences in my study and the one conducted in the Caribbean. For one, while closely related, the lionfish invading the Mediterranean and Caribbean are two different species, Pterois miles and Pterois volitans, respectively (Bariche et al., 2017; Hamner et al., 2007). Therefore, differences in study results could represent a difference in behavioral flexibility between two closely related species of lionfish, rather than differences between two populations of invasive lionfish that are otherwise similar. In addition, the areas of each study were managed by different organizations, leading to differences in culling strategies, such as frequency of culls. However, it's unlikely this was the cause of differences in the studies, as lionfish in the Caribbean were shown to have similar responses to culling, regardless of intensity (Côté et al., 2014), so I predict the same would be true of Mediterranean lionfish. Future studies on lionfish management strategies in the Mediterranean can further investigate these differences in Caribbean and Mediterranean lionfish and how that might impact culling strategies.

Cognition

An often overlooked topic in invasive ecology is how cognition can impact invasion success in different species (reviewed in Szabo et al., 2020). As such, only one study to date has investigated the link between individual behavioral traits and learning ability in lionfish (Deroy et al., 2020). In my thesis, I begin to explore some of the basic cognitive abilities of lionfish, namely their associative learning ability in **Chapters 3 and 4**. In these experiments, I repeatedly presented the lionfish with two stimuli (color cues in **Chapter 3** and UV and non-UV lights in **Chapter 4**) and rewarded individuals when they would approach the correct stimulus, training them to associate one stimulus with a food reward. Lionfish were successful in learning the association during the first experiment, as all but one individual chose the correct stimuli significantly more often than what would be predicted by chance (**Chapter 3**). In this way, I show that lionfish are capable of associative learning in apparatuses similar to other fish species, such as guppies (Poecilia reticulata) (Buechel et al., 2018; Lucon-Xiccato & Bisazza, 2014; Vega-Trejo et al., 2020), three-spined sticklebacks (Gasterosteus aculeatus) (Bensky et al., 2017), and cichlids (Neolamprologus pulcher) (Fischer et al., 2021), and present a methodology for future studies to improve upon to investigate lionfish cognitive abilities. However, I found in the second experiment (**Chapter 4**), that lionfish were less successful in learning the association when a light source was used as stimuli, with only two of the eleven individuals learning the association. This may be due to the stimuli used in the second experiment (flashlights with differing spectrums vs. printed colors on laminated paper), or due to slight changes in methodology, such as having the lionfish individually housed vs. group housed. Future research on the basic cognitive abilities of lionfish can continue to improve upon my methodology and shed light on the most effective ways to test lionfish capabilities.

Additionally, in **Chapter 5** I show evidence of habituation learning in Mediterranean lionfish. Over the course of the study, 42 of the 61 individuals tested were encountered multiple times, and I found that these lionfish showed reduction in activity level and shorter burst distances over subsequent approaches. For this habituation to occur, lionfish would have needed to learn through repeated interactions what the consequences of interactions with researchers in our study would entail. For example, lionfish might have learned through repeated interactions with us that we are not a threat and therefore show shorter burst distance as there is less need to escape danger. Therefore, even though this population of lionfish overall showed limited long-term impact on behavior due to culling, they did show the ability to learn and change behavior based on repeated interactions during the course of the experiment through habituation.

Even with these insights into basic lionfish cognition, it is still unclear how lionfish's cognitive abilities may have an impact on their invasive success. I propose that future studies should look into comparing cognitive abilities from lionfish in invasive and native populations, as a start to uncovering how cognition may underlie invasive success. For example, understanding the link between invasive success and cognition can lead to insights into several processes, such as how invading and non-invading individuals differ cognitively from one another, what makes some species invasive and others not, and even the mechanisms through which these differences could have evolved. Indeed, a better understanding of the cognitive mechanisms that facilitate adaptation to novel environments can provide insight into how invaders can be managed to avoid destruction of native habitat.

Implications for Lionfish Management

One of the primary goals of research on invasive species is often to use new information to keep harmful invaders from spreading, and new invasions from occurring. Indeed, many previous studies on lionfish focus on their diet and feeding behaviors, as lionfish's most significant impact on invaded areas is caused by their high predation rates (del Río et al., 2022). As such, these studies have helped inform management efforts, as analysis of diet can help inform management coordinators of prey species at risk of overconsumption by lionfish, or track areas of high risk for impact by lionfish invasions (Albins, 2013; Eddy et al., 2016; Morris Jr. & Akins, 2009). Similarly, some of these studies can also reveal natural predators or deterrents to lionfish invasive success, such as the ongoing research on the effect the native Nassau grouper (Epinephelus striatus) has on lionfish behavior (Hackerott et al., 2013b; Raymond et al., 2015; N. S. Smith & Côté, 2021b).

Naturally, the next question in my research becomes: how do we put this new information on lionfish into action? Some research has suggested that insight into the sensory ecology of invasive species may be an underutilized area of research that could be used to improve current invasive species management strategies (Abrahams et al., 2017). For example, in both insects and vertebrate, knowledge of pheromones produced by invasive species has led management organizations to use these pheromones to lure invasive individuals into traps or otherwise disrupt their invasion attempts (Beroza & Knipling, 1972; Gaston et al., 1977; N. S. Johnson et al., 2009). Similarly, carp have been known to have highly sensitive hearing, so sound has been successfully tested as a method to control invasive carp species expansion in the Mississippi River (Elmer et al., 2021; Feely & Sorensen, 2023). Insights into lionfish vision, as I have explored in **Chapters 3 and 4**, and other sensory systems might therefore lead to developments in improved management strategies to deter lionfish expansion in invasive regions.

Similarly, knowledge of the behavioral flexibility of lionfish in local areas can have a direct impact on management strategies used in invasive locations. In the Caribbean, lionfish tend to display more wary behavior after repeated culling, resulting in the potential for decreased efficiency in using this method to reduce lionfish populations over time (Côté et al., 2014). In this case, other methods might have to be used in to control populations of lionfish that behaviorally respond to culling in order to maximize the success of reducing lionfish expansion and damage in invasive ranges. However, if some populations of invasive lionfish, such as those I studied in the Mediterranean, were found to be less responsive to repeated culling, this would ease pressure to change strategies and allow organizations to fully utilize culling as a removal method.

Concluding Remarks on the Future of Invasive Ecology Research

In my thesis, I explore various factors of lionfish behavior and cognition that could impact the species' continued invasive success, as well as discuss their impact on lionfish management strategies and potential future research directions. I think my research is a start to unraveling the question of what makes lionfish so invasive, but also more generally, what makes a species a successful invader. As discussed in **Chapter 1**, the answer to this question is complicated and might be a question that is more species-dependent than previously expected. None the less, I argue that research into the general factors that impact invasive success, and namely the role cognition may play in this equation can lead to advancements in not just invasive ecology research, but also increase our general understanding of how environment factors shape these attributes, as invasive species provide natural experiments into the effect of environmental change on populations (Moran & Alexander, 2014; Sax et al., 2007). For example, having a better understanding of how cognitive abilities evolve during the invasion process can provide insight on how cognition has evolved in different environments and contexts. Cognition is a crucial factor in how animals adapt to novel environments, so as human interference in nature increases, learning how animals cognitively adjust to changing environments is necessary to understanding contemporary evolution. Therefore, through advancement in the field of invasion ecology, I think we will continue to advance our understanding of evolution in general.

References

Abrahams, M. V., Bassett, D. K., & Montgomery, J. C. (2017). Sensory biology as a risk factor for invasion success and native fish decline. Transactions of the American Fisheries Society, 146(6), 1238–1244. https://doi.org/10.1080/00028487.2017.1353545

Albins, M. A. (2013). Effects of invasive Pacific red lionfish Pterois volitans versus a native predator on Bahamian coral-reef fish communities. Biological Invasions, 15(1), 29–43. https://doi.org/10.1007/s10530-012-0266-1

Anton, A., Cure, K., Layman, C. A., Puntila, R., Simpson, M. S., & Bruno, J. F. (2016). Prey naiveté to invasive lionfish Pterois volitans on Caribbean coral reefs. Marine Ecology Progress Series, 544, 257–269. https://doi.org/10.3354/meps11553

Barbour, A. B., Allen, M. S., Frazer, T. K., & Sherman, K. D. (2011). Evaluating the potential efficacy of invasive lionfish (Pterois volitans) removals. PLoS ONE, 6(5), e19666. https://doi.org/10.1371/journal.pone.0019666

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(6782), 1–6. https://doi.org/10.1038/s41598-017-07326-1

Benkwitt, C. E. (2017). Predator effects on reef fish settlement depend on predator origin and recruit density. Ecology, 98(4), 896–902. https://doi.org/10.1002/ecy.1732

Bensky, M. K., Paitz, R., Pereira, L., & Bell, A. M. (2017). Testing the predictions of coping styles theory in threespined sticklebacks. Behavioural Processes, 136, 1–10. https://doi.org/10.1016/j.beproc.2016.12.011

Beroza, M., & Knipling, E. F. (1972). Gypsy Moth Control with the Sex Attractant Pheromone. Science, 177(4043), 19–27.

Buechel, S. D., Boussard, A., Kotrschal, A., van Der Bijl, W., & Kolm, N. (2018). Brain size affects performance in a reversal-learning test. Proceedings of the Royal Society B: Biological Sciences, 285(1871). https://doi.org/10.1098/rspb.2017.2031

Casas, F., Mougeot, F., Viñuela, J., & Bretagnolle, V. (2009). Effects of hunting on the behaviour and spatial distribution of farmland birds: importance of hunting-free refuges in agricultural areas. Animal Conservation, 12, 346–354. https://doi.org/10.1111/j.1469-1795.2009.00259.x

Côté, I. M., Darling, E. S., Malpica-Cruz, L., Smith, N. S., Green, S. J., Curtis-Quick, J., & Layman, C. (2014). What Doesn’t Kill You Makes You Wary? Effect of Repeated Culling on the Behaviour of an Invasive Predator. PLoS ONE, 9(4), e94248. https://doi.org/10.1371/journal.pone.0094248

Côté, I. M., Green, S. J., & Hixon, M. A. (2013). Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. Biological Conservation, 164, 50–61. https://doi.org/10.1016/j.biocon.2013.04.014

Côté, I. M., & Maljković, 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

D’Agostino, D., Jimenez, C., Reader, T., Hadjioannou, L., Heyworth, S., Aplikioti, M., Argyrou, M., & Feary, D. A. (2020). Behavioural traits and feeding ecology Mediterranean lionfish and naiveté native species lionfish predation. Marine Ecology Progress Series, 638, 123–135.

del Río, L., Navarro-Martínez, Z. M., Ruiz-Abierno, A., Chevalier-Monteagudo, P. P., Angulo-Valdes, J. A., & Rodriguez-Viera, L. (2022). Feeding ecology of invasive lionfish in the Punta Frances MPA, Cuba: insight into morphological features, diet and management. PeerJ, 10, e14250. https://doi.org/10.7717/peerj.14250

Deroy, E. M., Hussey, N. E., & Macisaac, H. J. (2020). Behaviourally-mediated learning ability in an invasive marine fish. Biological Invasions, 0123456789. https://doi.org/10.1007/s10530-020-02329-y

Dimitriadis, C., Galanidi, M., Zenetos, A., Corsini-Foka, M., Giovos, I., Karachle, P. K., Fournari-Konstantinidoy, 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 the thermal boundaries and future range expansion. Mediterranean Marine Science, 21, 62–69.

Eddy, C., Pitt, J., Morris, J. A., Smith, S., Goodbody-Gringley, G., & Bernal, D. (2016). Diet of invasive lionfish (Pterois volitans and P. miles) in Bermuda. Marine Ecology Progress Series, 558, 193–206. https://doi.org/10.3354/meps11838

Elmer, L. K., Madliger, C. L., Blumstein, D. T., Elvidge, C. K., Fernández-Juricic, E., Horodysky, A. Z., Johnson, N. S., McGuire, L. P., Swaisgood, R. R., & Cooke, S. J. (2021). Exploiting common senses: Sensory ecology meets wildlife conservation and management. Conservation Physiology, 9(1), coab002. https://doi.org/10.1093/conphys/coab002

Feely, J. R., & Sorensen, P. W. (2023). Effects of an ensonified bubble curtain and a cyclic sound on blocking 10 species of fishes including 4 invasive carps in a laboratory flume. Biological Invasions, 25(6), 1973–1989. https://doi.org/10.1007/s10530-023-03022-6

Ferreira, C. E. L., Luiz, O. J., Floeter, S. R., & Lucena, M. B. (2015). First Record of Invasive Lionfish (Pterois volitans) for the Brazilian Coast. PLoS ONE, 10(4), e0123002. https://doi.org/10.1371/journal.pone.0123002

Fischer, S., Balshine, S., Hadolt, M. C., & Schaedelin, F. C. (2021). Siblings matter : Family heterogeneity improves associative learning later in life. Ethology, 127, 897–907. https://doi.org/10.1111/eth.13196

Gaston, L. K., Kaae, R. S., Shorey, H. H., & Sellers, D. (1977). Controlling the Pink Bollworm by Disrupting Sex Pheromone Communication Between Adult Moths. Science, 196, 904–905. www.sciencemag.org

Guidetti, P., Vierucci, E., & Bussotti, S. (2008). Differences in escape response of fish in protected and fished Mediterranean rocky reefs. Journal of the Marine Biological Association of the United Kingdom, 88(3), 625–627. https://doi.org/10.1017/S0025315408000933

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(Supplement B), 214–222. https://doi.org/10.1111/j.1095-8649.2007.01575.x

Jiménez, C., Çiçek, B. ali, Jiménez, J. U., Hadjioannou, L., & Huseyinoglu, M. F. (2018). What is the roar about? Lionfish targeted removals in Costa Rica (Central America) and Cyprus (Mediterranean Sea). In M. F. Hüseyinoglu & B. Öztürk (Eds.), Lionfish Invasion and Its Management in the Mediterranean Sea (Issue 49, pp. 34–45).

Johnson, N. S., Yun, S.-S., Thompson, H. T., Brant, C. O., & Li, W. (2009). A synthesized pheromone induces upstream movement in female sea lamprey and summons them into traps. PNAS, 106(4), 1021–1026. www.pnas.org/cgi/content/full/

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, 138–147. https://doi.org/10.1016/j.marpolbul.2014.09.013

Kelber, A., Vorobyev, M., & Osorio, D. (2003). Animal colour vision - Behavioural tests and physiological concepts. Biological Reviews of the Cambridge Philosophical Society, 78(1), 81–118. https://doi.org/10.1017/S1464793102005985

Lucon-Xiccato, T., & Bisazza, A. (2014). Discrimination reversal learning reveals greater female Behavioural flexibility in guppies. Biology Letters, 10, 20140206. https://doi.org/10.1098/rsbl.2014.0206

Luiz, O. J., dos Santos, W. C. R., Marceniuk, A. P., Rocha, L. A., Floeter, S. R., Buck, C. E., de Klautau, A. G. C. M., & Ferreira, C. E. L. (2021). Multiple lionfish (Pterois spp.) new occurrences along the Brazilian coast confirm the invasion pathway into the Southwestern Atlantic. Biological Invasions, 23, 3013–3019. https://doi.org/10.1007/s10530-021-02575-8

Lythgoe, J. N., Muntz, W. R. A., Partridge, J. C., Shand, J., & Williams, D. M. B. (1994). The ecology of the visual pigments of snappers (Lutjanidae) on the Great Barrier Reef. Journal of Comparative Physiology A, 174(4), 461–467. https://doi.org/10.1007/BF00191712

Mccomb, D. M., Frank, T. M., Hueter, R. E., & Kajiura, S. M. (2010). Temporal Resolution and Spectral Sensitivity of the Visual System of Three Coastal Shark Species from Different Light Environments. Physiological and Biochemical Zoology, 83(2), 299–307. https://doi.org/10.1086/648394

Moran, E. V., & Alexander, J. M. (2014). Evolutionary responses to global change: Lessons from invasive species. Ecology Letters, 17(5), 637–649. https://doi.org/10.1111/ele.12262

Morris Jr., J. A., & Akins, J. L. (2009). Feeding ecology of invasive lionfish (Pterois volitans) in the Bahamian archipelago. Environmental Biology of Fishes, 86, 389–398. https://doi.org/10.1007/s10641-009-9538-8

Potier, S., Mitkus, M., & Kelber, A. (2018). High resolution of colour vision, but low contrast sensitivity in a diurnal raptor. Proceedings of the Royal Society B: Biological Sciences, 285, 20181036. https://doi.org/10.1098/rspb.2018.1036

Poursanidis, D. (2015). Ecological Niche Modeling of the invasive lionfish Pterois miles (Bennett, 1828) in the Mediterranean Sea. 11th Panhellenic Symposium on Oceanography and Fisheries, Mytilene, Levos Island, Greece, 621–624.

Poursanidis, D., Kalogirou, S., Azzurro, E., Parravicini, V., & Bariche, M. (2020). Habitat suitability , niche unfilling and the potential spread of Pterois miles in the Mediterranean Sea. Marine Pollution Bulletin, 154. https://doi.org/10.1016/j.marpolbul.2020.111054

Raymond, W. W., Albins, M. A., & Pusack, T. J. (2015). Competitive interactions for shelter between invasive Pacific red lionfish and native Nassau grouper. Environmental Biology of Fishes, 98(1), 57–65. https://doi.org/10.1007/s10641-014-0236-9

Rocha, L. A. (2003). Patterns of distribution and processes of speciation in Brazilian reef fishes. Journal of Biogeography, 30, 1161–1171.

Sax, D. F., Stachowicz, J. J., Brown, J. H., Bruno, J. F., Dawson, M. N., Gaines, S. D., Grosberg, R. K., Hastings, A., Holt, R. D., Mayfield, M. M., O'Connor, M. I., & Rice, W. R. (2007). Ecological and evolutionary insights from species invasions. Trends in Ecology and Evolution, 22(9), 465–471. https://doi.org/10.1016/j.tree.2007.06.009

Sbragaglia, V., Morroni, L., Bramanti, L., Weitzmann, B., Arlinghaus, R., & Azzurro, E. (2018). Spearfishing modulates flight initiation distance of fishes: the effects of protection, individual size, and bearing a speargun. ICES Journal of Marine Science, 75(5), 1779–1789. https://doi.org/10.1093/icesjms/fsy059

Schofield, P. J. (2010). Update on geographic spread of invasive lionfishes (Pterois volitans [Linnaeus, 1758] and P. miles [Bennett, 1828]) in the Western North Atlantic Ocean, Caribbean Sea and Gulf of Mexico. Aquatic Invasions, 5(Supplement 1), S117–S122. https://doi.org/10.3391/ai.2010.5.S1.024

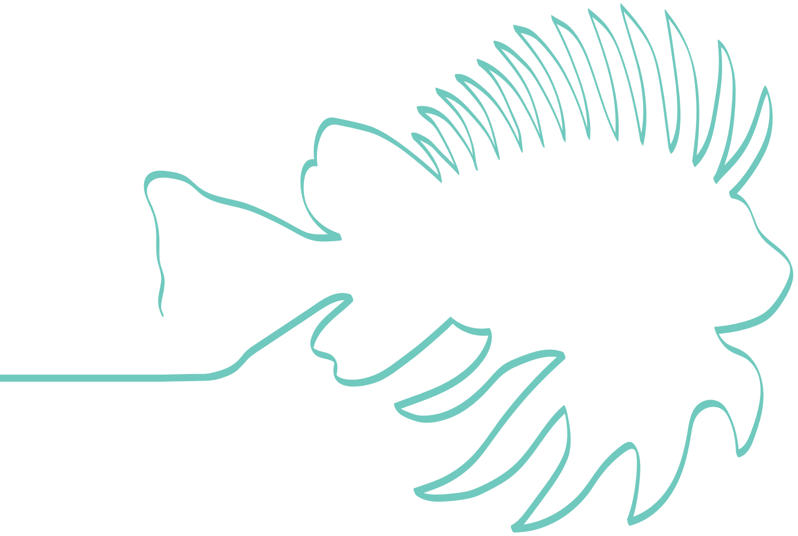
Siebeck, U. E., & Marshall, N. J. (2000). Transmission of ocular media in labrid fishes. Philosophical Transactions of the Royal Society B: Biological Sciences, 355(1401), 1257–1261. https://doi.org/10.1098/rstb.2000.0679

Smith, N. S., & Côté, I. M. (2021). Biotic resistance on coral reefs? Direct and indirect effects of native predators and competitors on invasive lionfish. Coral Reefs, 40, 1127–1136. https://doi.org/10.1007/s00338-021-02117-7

Szabo, B., Damas-moreira, I., & Whiting, M. J. (2020). Can Cognitive Ability Give Invasive Species the Means to Succeed ? A Review of the Evidence. Frontiers in Ecology and Evolution, 8(187), 1–13. https://doi.org/10.3389/fevo.2020.00187

Vega-Trejo, R., Boussard, A., Wallander, L., Estival, E., Buechel, S. D., Kotrschal, A., & Kolm, N. (2020). Artificial selection for schooling behaviour and its effects on associative learning abilities. Journal of Experimental Biology, 223, jeb235093. https://doi.org/10.1242/jeb.235093

Summary



Worldwide, the number of invasive species has been on the rise due to human-induced global change. Many theories have been proposed as to what makes a good invader, including how the impact of different life history characteristics, sensory system adaptations, behaviors, and even more recently, cognition may play a role in the invasive success of a species. However, it still remains enigmatic what general traits are most crucial to invasive success across species. Instead, research into invasive ecology has shown more success when studying the specific traits of a single invasive species and how those traits then impact its invasive success. This also has the added benefit of allowing management organizations to use this specific knowledge about invading species to reduce or prevent further introductions of that species in areas that are vulnerable to invasion.

The Indo-Pacific lionfish (Pterois spp.) is a recent invader spreading through the Caribbean and Mediterranean Seas, and is commonly regarded as one of the most successful marine invaders. While lionfish's life history and physical characteristics in relation to invasion events are well documented, the behavior and cognition of this species, and how these traits contribute to their invasive success, is vastly understudied. In my thesis, I investigate how lionfish behavior may impact their invasive success, ranging from species-level evolutionary adaptations, to personality changes individuals can make to adjust better to novel environments.

First, in **Chapter 2**, I use a citizen science survey to track the spread of the lionfish invasion in the Mediterranean. In previous decades, heavy focus has been paid to the invasion of lionfish in the Caribbean, but more recently lionfish have also begun to spread into the Mediterranean Sea via the Suez Canal. Therefore, in this chapter I aim to update the record of lionfish sightings in their newest invasive range using an under-utilized expert resource: diving centers. From our survey, we found reports that lionfish are rapidly expanding their invasive range in the Mediterranean, both north and west, even past the predicted limits to their invasion range. Lionfish therefore seem to be able to invade a wider range of environments than previously thought, though it still remains unclear how.

In **Chapter 3**, I begin to investigate a potentially overlooked factor to lionfish's invasive success – their visual capabilities. Color vision is widespread among animals, and is often tightly linked to a species' light environment. For example, diurnal species tend to possess increased spectral sensitivity when compared to nocturnal species, who tend to have better low-light vision. However, crepuscular species live in a unique environment where light levels rapidly change during peak foraging and hunting periods, and it remains enigmatic what visual capabilities these species possess: are they more like diurnal or nocturnal species, or a combination of both? Lionfish have generally been thought to be crepuscular and nocturnal hunters, and therefore unlikely to possess color vision. However, lionfish live in coral reefs where light is abundant, and have even sometimes been found to hunt diurnally in invasive ranges, making it is unclear whether or not lionfish possess color vision. Using genetic and behavioral analyses, we show that lionfish possess color vision, a trait that could potentially increase their hunting efficiency in a range of different environments.

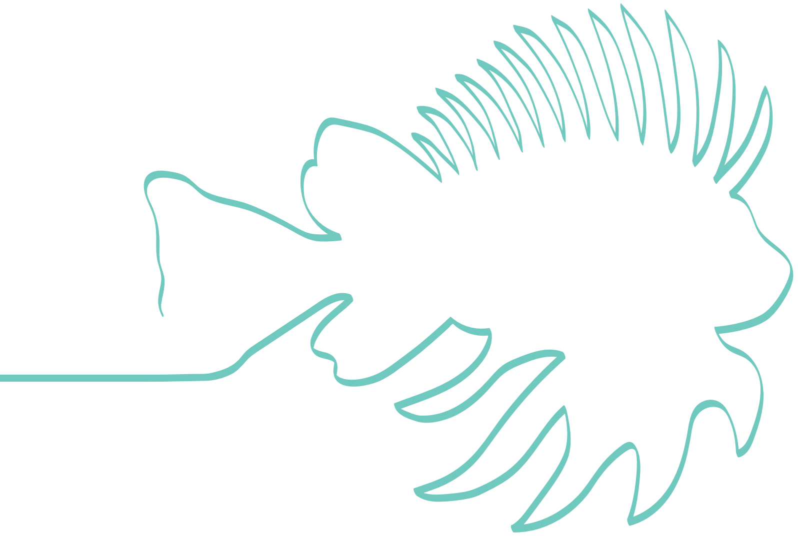
Then, in **Chapter 4**, I continue my investigation of lionfish vision and explore whether lionfish are able to detect UV vision. Many coral reef fish species use the reflection of UV light to communicate with conspecifics, as most aquatic predators are UV-blind, creating a "secret" communication system for prey fish to signal to others while minimizing their risk of predation. Exploitation of this system by a predator that can detect UV light, therefore, would likely help facilitate prey detection and increase predator efficiency. Recently, indirect evidence from another study, as well as results from our genetic analysis of opsin expression conducted in **Chapter 3** suggests that lionfish might be able to see into the UV spectrum. Building off of our investigation of lionfish color vision, we improved upon our original associative learning apparatus and found the first behavioral evidence that lionfish can indeed detect UV light. This may have implications for lionfish invasive success, as increased hunting efficiency through increased spectral sensitivity in the UV spectrum could increase hunting efficiency and, in turn, invasive success.

Next, in **Chapter 5**, I explore how lionfish adapt to local environments within invasive ranges, specifically how lionfish differ in boldness based on the culling frequency of their local environment. Previous work on lionfish in the Caribbean have found that lionfish are less bold after repeated culling, however, how resilient to culling lionfish are in their newest invasive range, the Mediterranean, remains enigmatic. To explore this, we compared the response of lionfish to an approaching free diver holding a metal pole in protected and non-protected within an area of Cyprus that is a highly invaded by lionfish. We also assessed activity, hiding patterns, and site fidelity of these fish to determine any differences in general behavior between the protected

and non-protected areas. Overall, we found limited effects of culling on the traits measured, indicating surprising resilience in Mediterranean lionfish. I predict that this response may be an example of lionfish's behavioral flexibility, as lionfish populations locally adapt to the different invasion environments between the Caribbean and Mediterranean.

Finally, in **Chapter 6**, I discuss the implications of my research as it relates to my thesis topic: What makes lionfish such successful invaders? In my thesis, I explore a variety of behavior traits and cognitive abilities that could contribute to lionfish's invasive success, including their visual capabilities (**Chapters 3** and **4**), behavioral flexibility (**Chapter 5**), and learning ability (**Chapters 3-5**). I argue my research is a start to unraveling the question of what makes lionfish so invasive, but also more generally, what makes a species a successful invader.

Acknowledgements

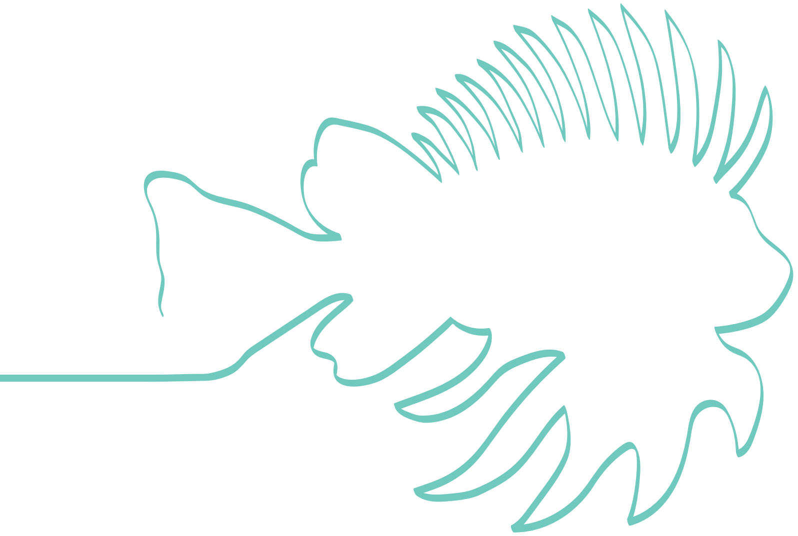


Acknowledgements

First and foremost, I would like to thank my supervisors **Alexander Kotrschal** and **Marc Naguib** for their supervision and guidance throughout my PhD. I would also like to thank my lab members **Hannah De Waele** and **Catarina Vila Pouca** for their help and support during all the lab experiments. I'd also like to thank all the MSc and BSc students that helped in any of the lionfish or cichlid projects over the past four years, as none of this would be possible without their work

Lastly, I'd like to thank my partner **Niels Mencke** and my parents **Vince and Maggie Phillips** for all their support throughout the years and their belief in my ability to actually get it all done.

Curriculum Vitae & List of Scientific Publications



Curriculum Vitae

Elizabeth Phillips was born on August 29th, 1997 in Illinois, USA. She completed her Bachelor's degree at the University of Illinois at Urbana-Champaign (USA) in the spring of 2019 with a major in Psychology and minor in Anthropology. During her bachelor's, she worked in multiple research labs, such as the Rhodes Neuroscience Lab and the Evolution & Biomechanics Lab. Elizabeth worked on projects spanning a wide range of fields, from parental care behavior in fish, to the biomechanics of running in humans. Her honors thesis investigated the step-fathering behavior of non-breeding clownfish (Amphiprion ocellaris) in the absence of the breeding pair.

After graduating, she worked as a research technician in the Boughman lab at Michigan State University (USA). Here, she helped on projects investigating sensory evolution in the three-spined stickleback (Gasterosteus aculeatus), as well as conducted an independent side project exploring the effect of a behavior-altering parasite (Schistocephalus solidus) on stickleback vision.

In January 2020, Elizabeth joined the Behavioural Ecology group at Wageningen University as a PhD student under the supervision of Alexander Kotrschal and Marc Naguib. Her research broadly focused on behavior and cognition of fish, including work on the invasive lionfish (Pterois spp.) in the lab and in the field, as well as lab-based work on social competence in the Princess of Burundi cichlid (Neolampologus pulcher).

List of Scientific Publications

Journal Publications

Phillips EW*, Bottacini D*, Schoonhoven A, Kamstra Y, de Waele H, Jimenez C, Hadjioannou L, Kotrschal A (2024) Limited effects of culling on the behaviour of invasive lionfish (Pterois miles) in the Mediterranean. Journal of Fish Biology. (*equal contribution)

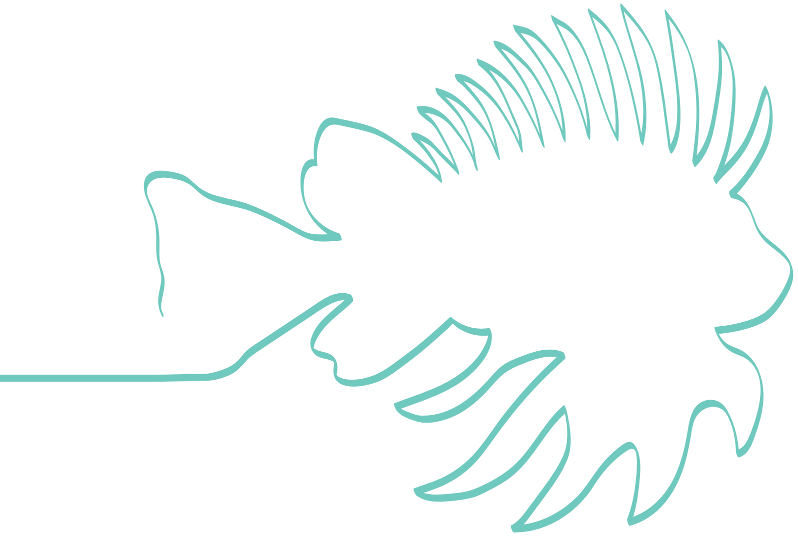
Phillips EW, Kotrschal A (2021) Where are they now? Tracking the Mediterranean lionfish invasion via local dive centers. Journal of Environmental Management, 298, 11354.

Phillips E, DeAngelis R, Gogola JV, Rhodes JS (2020) Spontaneous alloparental care of unrelated offspring by non-breeding Amphiprion ocellaris in absence of the biological parents. Scientific Reports, 10, 4610.

In Preparation

Phillips EW, Ramirez MAV, Douwes H, Bloch N, Kotrschal A (In prep) Color Vision in Lionfish: Gene Expression and Behavioral Evidence for Color Vision in Lionfish.

WIAS Training & Supervision Plan (TSP)



WIAS Training & Supervision Plan (TSP)

Activity	Year	Credits*
The Basic Package		**1.7**
WIAS Introduction Day	2020	
WGS Scientific Integrity Course	2020	
WGS Ethics in Animal Sciences Course	2022	
Disciplinary Competences		**13.4**
Writing a WIAS Research Proposal	2020	
Laboratory Animal Science: Design and Ethics in Animal Experimentation	2021	
RMarkdown	2021	
LAS Species Specific Course on Fish	2022	
Bayesian Statistics	2023	
Big Data in the Life Sciences	2023	
Applied Statistics	2023	
Professional Competences		**5.4**
Searching & Organizing Literature for PhD Candidates	2020	
Starting to Supervise BSc & MSc Thesis Students	2020	
Research Data Management	2020	
Reviewing a Scientific Manuscript	2021	
WAC 2022 Organizing Committee	2021-22	
PhD Workshop Carousel	2022	
Adobe Illustrator - Scientific Artwork & Infographics	2023	
The Final Touch	2023	

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

Activity	Year	Credits*
Presentation Skills		**4.0**
Oral Presentations		
WIAS Annual Conference	2021	
International Society for Behavioural Ecology	2022	
Association for the Study of Animal Behaviour	2023	
Poster Presentations		
WIAS Annual Conference	2020	
Netherlands Society for Behavioural Biology	2021	
Teaching Competences		**6.0**
Teaching Assistant	2020-21	
Life History Evolution		
Practical Supervisor	2020-22	
Behavioural Ecology		
Animal Behaviour		
Thesis Supervisor	2020-23	
Four MSC Students		
Five BSc Students		
Education & Training Total Credits		**30.5**

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