UNIVERSITA' POLITECNICA DELLE MARCHE

DIPARTIMENTO DELLA SCIENZA DELLA VITA E DELL'AMBIENTE

CORSO DI LAUREA MAGISTRALE

IN

BIOLOGIA MARINA

Interazione tra impianti di acquacoltura e il tursiope *Tursiops truncatus* nel Golfo di Corinto, Grecia

Interaction between fish farms and the bottlenose dolphin *Tursiops truncatus* in the Gulf of Corinth, Greece

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Sessione primaverile

Anno accademico: 2020/2021

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Abstract

English version:

Due to the expansion of fish farm cages in coastal waters of the Mediterranean Sea, several populations of common bottlenose dolphins '*Tursiops truncatus*' come frequently in contact with these infrastructures. Some animals learned to take advantage of the new foraging opportunities provided by aquaculture facilities, where wild preys tend to be more concentrated and possibly easier to catch than in the surrounding environment. While this opportunistic behaviour is relatively well known, little information exists about the frequency and timing of feeding forays around coastal fish farms (hereafter, "farms"). As an attempt to bridge this gap, in the present thesis I investigated for the first time the occurrence and timing of dolphins visiting three aquaculture facilities (hereafter F1, F2 and F3) in the Gulf of Corinth (Greece) over three months (from the beginning of September to the end of November 2021) and I assessed the potential relationships between fish farm activities and dolphins' sightings. Data were collected from two land-based vantage points situated above the three seabass and seabream farms. Dolphins which have been observed during ca. 40% of the days investigated, showed significant variations in their presence among the three farm sites, favouring F2, likely due to both natural and anthropogenic factors. Dolphins' presence displayed significant daily variations, with higher occurrence during the afternoon than in the morning, and month-related changes (i.e. higher occurrence in October/November than in September). Multivariate models highlighted that, overall, sightings and number of dolphins were higher when farm activities occurred. This study emphasizes the potential of an inexpensive and non-invasive land-based research approach for dolphin

monitoring, which could be easily replicated across coastal areas and aquaculture facilities. This research is also important for a better understanding of the ecology of the bottlenose dolphins in the light of the progressive expansion of the aquaculture sectors in the Mediterranean Sea.

Versione italiana:

A causa dell'espansione degli allevamenti ittici nelle acque costiere del Mar Mediterraneo, diverse popolazioni di tursiopi "*Tursiops truncatus*" entrano frequentemente in contatto con queste infrastrutture. Alcuni animali hanno imparato a sfruttare le nuove opportunità di foraggiamento offerte dagli impianti di acquacoltura, dove le prede selvatiche tendono ad essere più concentrate e più facili da catturare rispetto all'ambiente circostante. Sebbene questo comportamento opportunistico sia relativamente ben noto, esistono poche informazioni sulla frequenza e sui tempi delle incursioni di alimentazione negli allevamenti ittici costieri (di seguito, "farms"). Nel tentativo di colmare questa lacuna, nella presente tesi ho studiato per la prima volta la presenza e la tempistica dei delfini che visitano tre impianti di acquacoltura (indicate come F1, F2 e F3) nel Golfo di Corinto (Grecia) nell'arco di tre mesi (da inizio settembre a fine novembre 2021) e ho valutato le potenziali relazioni tra attività legate all' allevamento ittico e presenza dei delfini. I dati sono stati raccolti da terra da due locazioni situati sopra i tre allevamenti di spigole e orate. I delfini che sono stati osservati durante ca. il 40% delle giornate, hanno mostrato variazioni significative della loro presenza tra i tre siti di allevamento, favorendo F2, probabilmente per fattori naturali e antropici che caratterizzano l'area. La presenza dei delfini ha mostrato variazioni giornaliere significative, con una frequenza maggiore durante il pomeriggio rispetto alla mattina e cambiamenti legati al mese (cioè una presenza maggiore in ottobre/novembre rispetto a

settembre). I modelli multivariati hanno evidenziato che, nel complesso, gli avvistamenti e il numero di delfini erano più frequenti ed elevati quando si verificavano le pratiche dell’acquacoltura. Questo studio mette in evidenza il potenziale di un approccio di ricerca non invasivo ed economico per il monitoraggio dei delfini, che potrebbe essere facilmente replicato in diverse aree costiere e negli impianti di acquacoltura. Questa ricerca è importante anche per una migliore comprensione dell'ecologia dei tursiopi alla luce della progressiva espansione dell'acquacoltura nel Mar Mediterraneo.

1.- Introduction

1.1 The state of aquaculture worldwide focusing on mariculture

The state of marine fishery resources has continued to decline (FAO, 2020). The proportion of fish stocks that are within biologically sustainable levels decreased from 90 % in 1974 to 65.8 % in 2017, where the Mediterranean and Black Sea had the highest percentage (62.5 %) of stocks fished at unsustainable levels (FAO, 2020). Therefore, a rising demand for fish protein, together with growing human population and globalization of trade and economic incentives, have resulted in a rapid worldwide expansion of aquaculture (Bostock et al., 2010), growing on average at 5.3 %per year in the period 2001-2018 (FAO, 2020).

Marine aquaculture has been one of the fastest growing food sectors in the world over the past 20 years (FAO, 2020; Naylor et al., 2021), with estimated sale value of US$106 billion (FAO, 2020).

Of the global fish production estimated to have reached about 179 million tonnes in 2018, 82 million tonnes came from aquaculture production reaching 46 %, up from 25.7 % in 2000. In 2018 aquaculture fish production was dominated by finfish accounting 54.3 million tonnes where 7.3 million tonnes came from marine and coastal aquaculture (FAO, 2020).

At the regional level, aquaculture accounted for 17.9 % of total fish production in Africa, 17 % in Europe followed by Americas and Oceania, with 15.7% and 12.7 % , respectively (FAO, 2020).

Clawson et al. (2022) counted and mapped the locations of mariculture farms worldwide resulting in 95443 facilities, where just 17% have known specific locations.

Marine aquaculture is carried out in different ecosystems. For some species whose production depend on the naturally occurring seed in the sea, the production cycle is entirely conducted in the marine environment.

Techniques are varied: feed-lotting consist in taking wild fish and fattening them in sea cages, while farming is based on producing fry and spat. Feeding mechanisms are equally diverse, ranging from no supplementary feeding (mostly for molluscs) to using vegetable products and whole fish (e.g. pilchards to fatten tuna), or fishmeal and pellets (most used), made from wild caught marine fish (Kemper et al., 2003). However, fed aquaculture (57 million tonnes) has outpaced non-fed aquaculture. (FAO, 2020).

In this regard, in 2018 about 88 % (156 million tonnes) of world fish production was utilized for direct human consumption and the remaining 12 % (22 million tonnes) was used for non-food purposes where 82% (or 18 million tonnes) was used to produce fishmeal and fish oil, as FAO in 2020 reported.

At the national, regional and global levels, aquaculture production by volume is dominated by a small number of "staple" species or species groups. Finfish farming is the most varied industry including 27 species and species groups, which accounted for over 90 % of total finfish production in 2018, of which the 20 most important species accounted for 83.6 % of total finfish production.

Coastal aquaculture plays an important role in livelihoods, employment and local economic development among coastal communities in many developing countries (FAO,2020).

1.2 Relevance of fish farm in the Mediterranean Sea

The aquaculture industry in the Mediterranean area has a long history and has grown tremendously since its inception, almost forty years ago

(UNEP/MAP/MED POL, 2004; Barazi-Yeroulanos, 2010), reaching over 500 sea-cage farms (Dempster, et al., 2008). Nowadays, the Mediterranean aquaculture industry consists of various segments, depending on the species produced (Barazi-Yeroulanos, 2010), applied technologies, production trends and the industry segments, but one aspect that is common to all countries is the tendency to move finfish farming from land-based to the sea (Massa, 2017). For instance, Trujillo et al. (2012) showed that the prevalent fish farming and its stationary cages can be seen off the coasts of 16 countries using satellite imagery available through Google Earth. Analysing 91% of the Mediterranean coastline, 248 tuna cages and 20,976 other fish cages within 10 km offshore have been counted, where the majority are in Greece (49%) and Turkey (31%) (Trujillo et al., 2012). In addition, about 225,736 tonnes of farmed finfish (not including tuna) were produced in the Mediterranean Sea in 2006.

In particular, Egypt, France, Spain, Italy, Turkey and Greece are the main producing countries in the Mediterranean area and dividing them into levels of development of the activity, Greece has the largest and most organized industry (Barazi-Yeroulanos, 2010) counting approximately 50 % of over 30 000 Mediterranean fish farms in 2006 (Trujillo et al., 2012).

Greek fish farming industry has expanded exponentially in more than 30 years, thanks to its advantageous morphology with many protected bays, its Mediterranean climate and subsidies from national government and the EU (Barazi-Yeroulanos, 2010). By the 1990s, Greece was already the leading producer (Barazi-Yeroulanos, 2010). Nowadays, Greece ranks first in the European Union and in Mediterranean countries and third in the world, accounting for about 40 % of the Mediterranean aquaculture production (Katranidis et al., 2003).

Greece exports 80 % of its aquaculture production, selling to 32 countries where the EU is the largest market (Barazi-Yeroulanos, 2010).

There are 189 Greek companies operating in the sector, but the industry has become highly concentrated over the past ten years with six companies controlling 60 % of national production and 16 companies controlling between 70–75 % of production, as highlighted by Barazi-Yeroulanos in 2010. The industry gives jobs to 12,000 people, crucially contributing to social and economic development of remote coastal areas communities (GFN, 2020).

The main species farmed in Greece are European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) (GFN, 2020), with an estimated yield of 145 000 tonnes in 2008, accounting for over 70 % of total aquaculture production in 2008 (Barazi-Yeroulanos, 2010) and over 80 % of the total production of these two species in 2010 (Massa, 2017).

1.3 Impacts of coastal marine fish farms

Interaction and compatibility of aquaculture with the environment and vice versa, is one of the main debated issues linked to aquaculture sustainability. The predicted future development and intensification of coastal and marine aquaculture is closely associated with a wide range of environmental issues which raise concerns about its long-term sustainability (IUCN 2007; Karakassis 2013; Price et al. 2015).

A considerable research effort has been invested over the last decades to study the environmental impacts of aquaculture (Gowen & Bradburry 1987, Wu 1995, Fernandes et al. 2001) and recently some projects have been developed to understand more about the potential effects on the marine environment due to mariculture. For instance, the European programs 'Medveg' and 'Meramed' were realized in the Mediterranean Sea (Medveg 2006; Meramed 2006) to provide information on the loss of dissolved and particulate effluents at fish farms, on the nutrient regeneration in fish farm sediments and on effects on population dynamics of seagrasses in fish farm surroundings (Marba` et al. 2006). Overall, the effects of mariculture can occur at various spatial and temporal scales (Karakassis 1998), depending on the nature of the waste released, the physical, hydrographic, and ecological characteristics of the site and the efficiency of the management of the farms (Machia, 2005). The interaction between the industry and the environment can be divided into biological, chemical and nutrients nature (Table 1.3.1).

Nature of interaction	**Origins**	**Impacts**
Biological	Escapees from cages	Interbreeding & competiton
	Diseases & Parasites	Transfer to wild population + low health conditions
	Fishmeal aggregation	Vulnerability to fishing
	Fishmeal and fish oil	Oversfishing
Chemical	Parasitides, disinfectants, antifouling	Affect no-targeted organisms, affect biodiversity
	Antibiotics, anaesthetics	Antibiotics resistance, affect biodiversity
Nutrients	Phospohorous, nitrogen, ammonium	Eutrophication, disrupt trophic balances, toxic to organisms, sedimen tation

Table 1.3.1. Interactions between aquaculture and the environment, their origins and impacts correlated (Porchas et al., 2014)

Regarding the biological nature of interaction, when farmed fishes accidentally escape from the cages, their interaction with wild species can "contaminate" wilderness exchanging gametes (Massa et al. 2017), since the farmed ones are biologically inferior in terms of competitiveness and productiveness compared to wild ones (Fleming et al. 1996). Biological interaction also includes the potential disease and parasites transmitted from the farmed to the wild population and the tremendous pressure on wild stock (mostly on small pelagic fishes) from feedings made of fishmeal and fish oil (Massa et al. 2017, Cao et al., 2015 as cited in Carballeira Braña et al. 2021). In these regards, feeding fish to fish, also called "Fishmeal trap", has received many criticisms as considered a very controversial aspect of marine aquaculture by many fishery ecologists (Shepherd, 2012).That is not to say that shellfish, seaweed and herbivorous fish aquaculture do not pose problems, but that their effects are likely to be more local in nature, rather than affecting the whole marine ecosystem (Kemper et al., 2003).

In addition, some of the main effects of marine aquaculture includes also chemical interactions relate to the discharge of different dangerous types of chemicals and metals from pesticides, fertilizers, disinfectants, antibiotics and oxidants (Naylor and Burke 2005) which affect biodiversity and ecosystem functioning (Massa et al., 2017).

But the environmental impacts come mainly from uneaten feed and faeces (Holmer et al., 2008). Direct sedimentation of organic particles to the seafloor (Borja et al. 2009 as cited in Tsikopoulou et al. 2018) and excess nutrients released from reared biomass can cause eutrophication, leading to progressive reduction of dissolved oxygen concentrations, which can impair biodiversity of the wild marine communities and ecosystem functioning (Karakassis et al. 2005 as cited in Tsikopoulou et al. 2018, Dell'Anno et al., 2008). This phenomenon is considered a major problem for different areas, such as Australia's estuaries and enclosed coastal waters (Zann 1995 as cited in Kemper et al., 2003). Some estimates indicate that in Mediterranean coastal areas, the release of nutrients from fish farming contributes for up to 7% and 10% of N and P total discharge, respectively (Pitta et al., 1999). Therefore, aquaculture installations can produce relevant shifts of the whole natural environment (Boyra et al., 2004; Machias et al., 2004).

The effects from mariculture are evidence both at small and large spatial scale. For instance, Cheshire et al. (1996) investigated the effects of tuna feedlotting at Port Lincoln, founding that the epibenthic communities were impacted up to 150 m from the cages and that there were significant infaunal communities within 20 m; both changes resulted from a large build-up of organic detritus. Many of the feedlots have since moved to more open water, where the effects on the immediate benthos would be less detrimental because of currents (Kemperetal et al.,2003). Another evidence have been revealed in the study conducted by Pargent-Martini (2006) where numerous changes were observed in the P. oceanica meadows even at a distance of 300 m as showed in Corsica and Malta or up to several hundred metres from the cages in Spain, contrary to what has been observed with other benthic populations, for which some authors notice no impact beyond 25–30 m from the cages (Karakassis et al. 2000, 2002; Machias et al. 2004). Therefore, the impact on P. oceanica meadows is perceptible over large distances.

Effects on small spatial scales (beneath or in close vicinity to the cages, normally not exceeding 25 to 30 m from the edge of fish cages) have been widely investigated, including effects on sediment chemistry (Hall et al. 1991, Holmer & Kristensen 1992, Karakassis et al. 1998, Holmer et al. 2002), on benthic communities (Brown et al. 1987, Weston 1990, Mazzola et al. 1999, 2000, Karakassis et al. 2000, La Rosa et al. 2001, Mirto et al. 2002), including seagrass meadows (*Posidonia oceanica*) (Holmer et al. 2008; Rountos et al. 2012) and maerl bed habitats (Sánchez-Jeréz 2011 as cited in Massa et al., 2017). All those small spatial scale impacts are mainly correlated to the organic enrichment of the sediments immediately beneath the sea cages as a direct result of the sedimentation of particulate waste products from the fish-farm (Hargrave et al., 1997; Karakassis et al., 1998; Holmer et al., 2008).

Focusing on the effects on *P. oceanica*, numerous changes are observed in the meadow especially in terms of density (number of shoots per m2) that showed a significant decrease in the vicinity of the cages and even at a distance of 300 m Pargent-Martini (2006). Moreover, the biomass of the epiphytes of P. oceanica leaves increases sharply close to fish farming facilities (Ruiz et al. 2001). Nevertheless, the maximum values are not observed at the closest station to the cages (where nutrient levels are highest), but at a distance between 20 and 40 m (Pergent et al. 1999; Dimech et al. 2000). This result could be clarified by the copper added to the fish food (estimated at between 450 and 500 g/year) (Pergent et al., 1999) and to the nets of the cages (antifouling operations) acting as an algicide in the proximity of the cages. Even the benthic macrofauna in the matte of P. oceanica shows impacts resulting in a greater biodiversity in a reference meadow compared with a meadow situated close to a fish farming facility (Pargent-Martini et al., 2006)

In addition, a study of the distribution of several species of macrofauna (such as echinoderms, decapods and molluscs) shows zonation in function of the distance from the cages (Dimech et al. 2000).

Furthermore, coastal aquaculture installations concentrate large numbers of a large variety of wild pelagic and demersal fish species of both ecological and economic importance by providing refuge from predators and food resources (Bjordal & Skar 1992; Castro, Santiago & Santana-Ortega 2002; Dempster et al. 2002; Boyra et al., 2004; Tuya et al., 2006; Fernandez-Jover et al., 2007; Valle et al., 2007; Fernandez-Jover et al. 2008). For these reasons, cages of coastal fish farms themselves act as fish aggregation devices (FADs) (Dempster and Tanquet, 2004). This phenomenon has been progressively documented during the last decade by scientists and fish farmers and local fishermen from several parts of the Mediterranean basin (Sanchez-Jerez et al., 2011). As an effect of this attraction, the increased fisheries landings for instance in the Greek coastal area with intensive fish farming, have been detected (Machias et al., 2006).

In several studies conducted in the Mediterranean, about 40 different species have been reported, which abundance and assemblage composition vary significantly across different geographical areas, farms and seasons (Sanchez-Jerez et al., 2011). Those aggregations include schools of bogue, Boops boops, in high abundance and biomass and because of this, bogue may serve as a model species for fisheries aquaculture interactions in the Mediterranean Sea (Arechava-Lopez, 2011).

In a study conducted in 2012, 65 % of cage aggregated fish were less than 11 cm (Bacher et al., 2012). It is therefore reasonable to assume that these small individuals are attracted not only by the available food, but also by the farm structures per se in search of shelter, as it has been suggested by other authors (Fernandez-Jover et al. 2009, Šegvi´c Bubi´c et al. 2011).

On the other hand, it is also reported as common to observe large shoals of bluefish (*Pomatomus saltatrix*) beneath fish cages, which not only feed on the small pelagic fish (e.g. *Sardinella aurita, Trachurus mediterraneus, Boops boops*) present in the surrounding area of the farm, but also break into cages and attack the cultured fish, particularly sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) (Sanchez-Jerez et al., 2008)). For this reason, the interaction of bluefish with sea-cage aquaculture is considered a large problem in some parts of the Mediterranean Sea (Sanchez-Jerez et al., 2008).

Small wild fish assemblages aggregating in the surrounding of the fish farms may also attract larger predatory species, due to the increased foraging opportunities (Sanchez-Jerez et al., 2011). For instance, larger species such as swordfish (*Xiphias gladius*), which is enlisted in the IUCN Red List of Threatened Species (category: least concern; see http://www.iucnredlist.org), Atlantic Bluefin Tuna (ABT; *Thunnus thynnus*) (Arechavala-Lopez et al., 2014b), Blue Shark (*Prionace glauca*), which is listed as "vulnerable" (Bacher et al., 2012), as well as some marine mammals, whose populations are deemed "critically endangered" in the Mediterranean, such as bottlenose dolphin (*Tursiops truncatus*) (criterionA3bcd;C1), and monk seal (*Monachus monachus*) (criterion A2abc; C2a(i); E) (e.g. Güc¸ lüsoy and Savas, 2003; Díaz-López and Bernal-Shirai, 2007; Piroddi et al., 2011) have also been observed in the vicinity of the farms.

Overall, serious impacts assessment from the industry proceedings has been lacking in past years and frameworks seeking sustainability need to be implemented (Bostock et al. 2009; Bohnes and Laurent, 2021 as cited in

Carballeira Brana et al 2021). In these terms, Holmer et al. (2008) and Wilson et al. (2009) agree to use Environmental Carrying Capacity (ECC) as an instrument to assess ecosystemic repercussions, where ECC represents the hypothetic load released by the farm that will be assimilated by the ecosystem (Carballeir Brana et al. 2021).

1.4 Sustainable management of aquaculture practices

The progressive expansion of aquaculture farms from land-based locations to the sea requires sounding management sustainable strategies (Papageorgiou et al. 2021). The development of aquaculture in the Mediterranean has, indeed, brought with it several environmental and socio-economic issues that influence the sustainability of the sector and could compromise its further development (Massa et al., 2017).

In fact, in addition to the impacts of aquaculture on the marine environment, there are interactions between different coastal stakeholders and the aquaculture sector that need to be considered. In this context, aquaculture struggles to be integrated within other activities and become accepted by coastal communities and thus is an important component of aquaculture development (Hishamunda et al., 2014). Therefore, assessing pollution sources that may affect a socio-ecological system have to be taken into consideration assuming an integrated management approach (FAO, 2006). This may lower down negative effects on the environment and also reduce conflicts (Dempster et al. 2008).

Papageorgiou et al. (2021) highlighted, sustainable aquaculture is a dynamic concept, integrating three main principles: economical profitability, environmental friendliness, and social equitability.

Different tools and plans have been proposed to assure sustainability with sectoral management strategies such as the ecosystem approach to aquaculture (Soto et al., 2008; FAO 2010) and multisectoral/cross-sectoral management strategies including ecosystem-based management (e.g. Levin et al., 2009; Curtin and Prelezzo 2010; Tallis et al., 2010;Cornier et al., 2018), integrated coastal zone management (Rochette and Bill´e 2012), marine spatial planning (e. g. Ehler et al., 2019; Stelzenmueller et al., 2021), and the Blue Growth Initiative (Ahmed and Thompson 2019; Venier et al., 2021).

Moreover, some advices have been provided by Gulnihal et al. in 2014, concerning feeding tips for fish farms operators both to enhance fish quality and reduce impacts, such as distributing the feeds efficiently to allow all the fish to feed, hence minimizing feed waste; avoiding overfeeding to prevent from effluent pollution and gill damages; adjusting the feeding in relation to the body weight percentage or give the correct amount of feed due to fish grows or selecting of the right feeding method for the species of interest.

Furthermore, one on the most important challenges for the sustainable development of the aquaculture sector on a broad scale is to reduce as much as possible the dependence on fishmeal and fish oil to feed carnivorous finfish species (Kemperetal 2003).

Therefore, feed efficiency needs to be improved by moving to feeds made of plant-based, polychaete-based or insect-based proteins (Boyd, 2015; Pahlow et al., 2015; Rhodes et al., 2016; Gómez et al., 2019; Llagostera et al., 2019 as cited in Carballeira Brana et al 2021). In fact, Beal et al. in 2018, estimated that the use of algae as substitute of fishmeal, would foster marine ecosystems conservation by lowering fishing pressure by 30 %.

Additionally, Pergent-Martini et al. (2006) highlighted the importance to choose the best positioning for mariculture facilitites to have less impacts on the surrounding marine environment. In this regard, especially in area with conservations interests there are some elements that need to be taken into account, for instance:

- Sites of Community Importance (SCIs – Habitat Directive). A suggested safe distance in function of environmental characteristics (currents, seabed typology, etc.) and the characteristics of the fish farming facility (number of cages, quantity of fish, etc.)
- Marine Protected Areas (existing or planned). A safe distance should be respected, in function of environmental characteristics and the characteristics of the fish farming facility.
- Protected Land Areas. For landscaping reasons, fish farming facilities should not have a negative visual impact from the land (distance, angles of view, size, etc.)
- Posidonia oceanica and C. nodosa meadows. A safe distance should be respected in function of environmental characteristics and the characteristics of the fish farming facility.
- Bathymetry. A depth of at least 30 m is required, which will generally position the fish farming facility away from the most sensitive populations and ensure better dilution of the effluents produced by the fish farm.
- Distance from the shore: at least 1000 m.
- River mouths. Care should be taken with these outlets for several reasons such as the introduction of fresh water, the interaction with currents and the introduction of pollutants

Likewise, to reduce environmental impacts especially on the benthic compartment, Correia et al., in 2020 argued that Integrated Multi-Trophic Aquaculture (IMTA) is the best option (Figure 1.4.1). This system combines the production of vertebrate and invertebrate species and macroalgaes, so nutrients, as organic and inorganic matters, are reused and turned into food, energy and fertilizers for other species such as mussels, clams, sea urchins, polychetes (as organic matter extractors e.g. faeces and uneaten feed) and macroalgaes (as inorganic nutrients extractors e.g. minerals or chemicals) (M.Correia et al., 2019). In this way circularity is achieved, cutting down losses and environmental damages. This switch of business model can be seen by fish farm managers as an opportunity to increment economic resilience via product diversification, efficient use of resources and increased public acceptability towards mariculture given the virtuous sustainable practices (Correia et al. 2020).

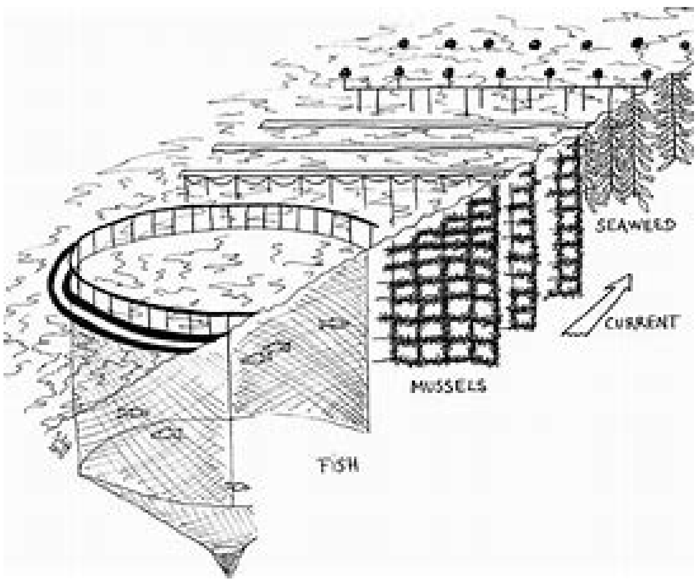

Figure 1.4.1: An integrated multitrophic aquaculture (IMTA) system

(WordEconomicForum, 2018)

1.5.1 Interaction between marine fish farms megafauna worldwide

As human populations have progressively increased marine space occupancy, different odontocete populations came into contact with humans' activities, facing with the challenges of adapting to artificially modified habitat. In the best scenario, they showed to become accustomed to human activities, for example by modifying feeding behaviour, taking advantage of foraging opportunities (Bearzi et al., 2019). This opportunistic behaviour occurs often in relation to fishing activities, which heads to forms of commensalism, mutualism, or depredation (Bonizzoni et al. 2014; Tixier et al. 2018). Historical reports, dated back to '70 and '80s, show cooperation between odontocetes and coastal fisheries, demonstrating that these adaptations occur since several centuries (Busnel 1973; Hall 1984).

Marine cage fish farming attracts a variety of megafauna worldwide, including harbour seals (*Phoca vitulina*), grey seals (*Halichoerus grypus*), common bottlenose dolphins (*Tursiops truncatus*), cormorants (*Phalacrocorax carbo*), shags (Phalacrocorax aristotelis), grey herons (Ardea cinerea), gulls (*Larus spp.*), pelicans (*Pelecanus spp*.), grebes (*Podiceps spp*.), otters (*Lutra lutra*) and minks (*Mustela vison*) (EIFAC 1988; Ross 1988; Rueggeberg & Booth 1989; Pemberton & Shaughnessy 1993; Carss 1994; Morris 1996; Kemper et al. 2003; Dıaz Lopez et al. 2005; ; Dıaz Lopez & Shirai 2007; Dıaz Lopez 2012). In general, most of the literature has focused on pinnipeds that feed on finfish and some shellfish, but there is a lack of information on cetaceans and aquaculture (Wursig and Gailey, 2002; Kemper et al., 2003; Watson-Capps and Mann, 2005).

In all cases, aggregation at cage fish farms is mainly related to the availability of trophic resources, in terms of farmed fish, wasted feed and aggregated fauna (Díaz López, 2006; Ballester-Moltó et al., 2015; González-Silvera et al., 2015). These faunal groups may also interact among each other, generating an artificial ecosystem (Díaz López et al., 2008; Ballester-Moltó et al., 2015), The ecological relationships (predation, competition), interactions with human activity (husbandry, harvesting) and environmental conditions, determine the magnitude of the overall effects (Aguado-Gimenez et al., 2018).
Moreover, in the model presented by Diaz Lopez et al. (2008), the mixed trophic analysis (MTI) suggests that the aquaculture activities have a small positive impact on zooplanktivorous fish, cormorants, bottlenose dolphins and common grey mullets. On the other hand, finfish farm activities produce negative effects on cephalopods and zooplankton, due to its productions of positive effects on their predators (bottlenose dolphins, cormorants and zooplanktivorous fish).
A different interaction has been showed with mussel and oyster farms. For example, Würsig & Gailey (2002) pointed out that mussel farms are not attracting points to predators, such as cetaceans, due to great amounts of lines and buoys that would make it difficult to capture prey efficiently. In this way the loss of space cause by mussel farms seems to be the main negative impact for the animals. In New Zealand, mussel farming is now set up in areas where dusky, bottlenose and Hector's dolphins once used to feed, reproduce and rest (Würsig & Gailey, 2002). Ribeiro (2007) highlighted that the presence of oyster farming in Shark Bay, Australia, influenced movement patterns of Indian Ocean bottlenose dolphins, T. aduncus. Mothers and calves were excluded from areas after the setting up of oyster farms, however, dolphins returned after the farms were removed (Mann & Janik, 1999).

Nevertheless, the interactions between predators and marine farms often brings to negative effects both in terms of farming production and to wild fauna. As showed by Díaz López (2017), production loss was observed in different ways due to the predator species involved. Overall, the impact of marine predators on this industry was estimated as a loss of 2–10 % of their gross production owing to mammals, in particular seals (Nash et al. 2000). Moreover, direct predation from the cages was noticeable for marine birds and more difficult to observe with other marine fauna, such as bottlenose dolphins (as reported by Dìaz Lopez in 2017). Furthermore, the presence of mammalian and avian predators may also increase the stress levels of the farmed fishes and bring to death in some cases (Westers 1983; Price & Nickum 1995). Birds also transport bacterial pathogens in their gut and on their feet and are intermediate or definitive hosts to several cestodes, nematodes, trematodes and other parasites (Taylor 1992). Mariculturalists estimate a loss between 2 and 10 million of their gross production due to marine mammal predation (Nash et al. 2000) and 12% of aquaculture insurance claims worldwide are related to depredation and damage by pinnipeds (Kemper et al., 2003). In fact, many of the known interactions between marine mammals and finfish aquaculture involve pinnipeds and this is because research efforts have focused on the need to mitigate seal damages.

In contrast some studies, have noted no direct damages to the facilities caused by marine predators (Díaz López, 2006; Bearzi et al., 2009; Benmassaoud, 2017).

Conversely, several potential direct hazards to wild predators have been observed, such as risk of entanglement (Wursig & Gailey 2002; Diaz Lopez & Shirai 2007), habitat exclusion as a result of physical structures (Watson-Capps and Mann, 2005; Wu¨rsig and Gailey, 2002), alteration of natural behaviour

(Dıaz Lopez, 2009), acoustic devices (Dıaz Lopez & Marino 2011), and intentional killing of predators (Carss 1994).

The most serious, in terms of the reported number of instances and so possible threat to local populations, are entanglements of shortbeaked common dolphins (*Delphinus delphis*), common bottlenose dolphins *(T. truncatus*), Indo-Pacific bottlenose dolphins in anti-predator nets of finfish farms in Australia and another case is that of fatal entanglements of common bottlenose and short-beaked common dolphins in salmon farms in south eastern Tasmania, where anti-predator nets (typically having a mesh sizes greater than 6 cm) were involved in most cases (Kemper et al., 2003).

These hazards can cause significant problems where predator populations are limited or endangered. Moreover, some of these predator species are listed in Annex IV (a) in the European Habitats Directive (art. 12) (Office for Official Publications of the European Communities 2000).

1.6.1 Bottlenose dolphins and marine fish farms

The distribution of common bottlenose dolphins *Tursiops truncatus* (hereafter 'bottlenose dolphins') overlaps with aquaculture in several coastal areas worldwide (Würsig and Gailey, 2002; Watson-Capps and Mann, 2005).

Moreover, fish farms are known to attract a great variety of fauna, including key bottlenose dolphins' prey (Bearzi, Fortuna, & Reeves, 2009; Machias et al., 2006). Therefore, the concentration of these organisms attracts predators (such as dolphins) and the infrastructure itself may help in prey capture (Díaz López, 2006). In these terms, bottlenose dolphins are defined as an opportunistic forager, taking advantages of the concentrated prey and easily to catch (Bearzi et al., 2019).

Overall, the interaction between common bottlenose dolphins and marine finfish aquaculture seems to increase over the years (Diaz Lopez, 2017; Benmessaoud 2017) and has been observed in different part of the Mediterranean, such as in Cyprus waters (Bearzi, 2002), around Lampedusa Island (Pace et al. 2006), in the north of Sardinia (Díaz López, 2017) and in Greece (Bonizzoni et al. 2014; Piroddi et al., 2011)**.** However, data remain fragmented and disparate for very limited areas (Benmessaoud, 2017).

Productive waters around fish farms have then become important feeding spots for bottlenose dolphins, which seem to move from one farm to the next searching for prey (Bonizzoni et al., 2014). As Bearzi et al. (2009) observed, such behaviour is possibly a response to prey depletion.

In this regard, Diaz Lopez (2006) observed bottlenose dolphins hunting both schooling and solitary prey in the fish farm area, using individual and group feeding strategies developed only for this interaction. For instance, “encircling cage” was the most frequent technique seen in that study, where one or some dolphins swam around a floating fish farm cage, facilitating search and capture of prey. Other strategies observed were “feeding rush” (Shane, 1990) that consist in using the nets of the cages as barriers to feed on wild fishes; or “carousel swim” (Belkovich et al., 1991), a cooperative technique where dolphins surround wild fish schools, forcing them to swim in a ball very tight. The dolphins swam in circles around the fish, gradually tightening the school, also leaping laterally against the school of fish occasionally.

The strong interaction between bottlenose dolphins and fish farms was highlighted by Bonizzoni (2014), who reported bottlenose dolphin occurrence higher in areas within 5 km of fish farms and lower at distances up to 20 km. Moreover, other studies highlighted the potential of fish culturing in influencing bottlenose dolphin distribution (Piroddi et al., 2011; Diaz Lopez et al., 2005), so affecting the extent of home ranges (Eifler 1996). In general, preference for certain fish farms may be influenced by their spatial location, as well as by the fish species that aggregate around the cages**.** Furthermore, a seasonality in this occurrence have been showed in response to increase feeding opportunities in the surrounding area (Diaz Lopez et al., 2012). In fact, a peak of sightings was observed during periods with lower sea surface temperature (autumn and winter) (Diaz Lope, 2017), probably related to both changes in feeding opportunities and seasonal fluctuations in metabolic needs (Diaz Lope, 2017), offering an alternative food source for dolphins during periods with low prey abundance (Diaz Lopez et al., 2012).

Additionally, bottlenose dolphin presence in the fish farm seems to be related to harvesting operations, in accordance with observations of bottlenose dolphins predating on the fish that sometimes escape or are discarded during the harvesting procedure (Diaz Lopez, 2017).

Bottlenose dolphins in Mediterranean Sea has been classified as 'Vulnerable' under International Union for Conservation of Nature criteria (IUCN, 2012).

Therefore, understanding habitat selection by dolphins in the Mediterranean is necessary in terms of conservation management of a protected species and to mitigate any negative effect resulting from interactions with human activities (Bonizzoni, 2014).

1.7.1 Mitigation methods for fish farms and marine mammals' interaction

Marine mammals' interactions with aquaculture, particularly finfish farms, are inevitable. To minimise these, stringent requirements, including environmental impact assessments that predict the effects of habitat loss, nutrient concentration, monoculture and entanglement risk, should be put in place before permit applications are approved to develop new aquaculture initiatives or to expand existing operations. Recommendations on how to minimise or eliminate cetacean interactions with aquaculture have been addressed in few studies (e.g. Mate and Harvey 1987; Pemberton 1996; Reeves et al. 1996; Kemper and Gibbs 2001).

Overall, it is agreed that to minimise predators' damages to fish stock and gears is best done by modifying these, providing a physical barrier to these adaptable and persistent predators. For instance, human presence, top and underwater barrier nets, overhead lines were applied as anti-predator measures to protect the fish farm in Sardinia from 2004 and 2013 (Diaz Lopez & Shirai 2007; Diaz Lopez & Marino 2011). Moreover, the use of acoustic harassment devices was tested too.

In particular, acoustic devices (referred as AHDs or as Acoustic Deterrent Devices ADDs), have been a popular method of attempting to dissuade those predators from finfish farms (Kemper et al., 2003). These are sound generating devices that use a combination of intensity and frequency which is aversive to marine mammals and aims to keep them away from an area or a structure (Reeves et al. 1996). They are high-amplitude devices and should not be confused with 'pingers', which are of lower amplitude and are used to prevent bycatch of cetaceans in some fisheries. Acoustic devices cannot be expected to provide complete protection to farms. In fact, failure may result from improper maintenance or deployment of the equipment or because the sound is not particularly aversive to marine predators making them to get used to it (Kemper et al., 2003).

On the other hand, minimising entanglement of predators such as cetaceans and pinnipeds in finfish farms, has been the subject of few studies worldwide (Pemberton 1996; Kemper and Gibbs 1997, 2001). Many of the recommendations for instance adequate net tension reduces billowing; enclosing the anti-predator net at the bottom, stops dolphins and pinnipeds from being trapped between the cage and anti-predator nets. Furthermore, eliminating food wastage discourages other prey species and, therefore, again dolphins and pinnipeds from foraging around the nets. In addition, reducing the mesh size of the nets to less than 10 cm and repairing holes, reduces substantially the chance of marine mammal entanglement. In addition, pens that are not in use, and therefore often poorly maintained, are an entanglement threat. The simple remedy is to have all non-functioning nets removed from the water.

In conclusion, marine mammal interactions with aquaculture, particularly finfish farms, are unavoidable. To minimise these, stringent requirements and educational and awareness campaign should be put in pace.

Management actions in marine predators' hot spots habitat should be taken and should be contingent upon gaining financial backing, environmental audit and aquaculture management approvals.

2. Aim and objectives

In the present study I analysed the occurrence and timing of bottlenose dolphins visiting aquaculture facilities in the Gulf of Corinth (Mediterranean Sea) over three months and I investigated the potential relationships between bottlenose dolphin occurrence and farm activities.

In particular, the specific objectives of this research are:

1. assessing the frequencies of dolphins' occurrence close to the fish farms and potential changes in their presence in relation to the different farms investigated;
2. investigating daily changes (morning vs. afternoon) and month-related variations of dolphins' presence close to the fish farms;
3. assessing the variations in the presence of dolphins in relation with the presence/ absence of fish farm activities;
4. Investigating the relationships between dolphins' group size and temporal (e.g. morning-afternoon and months) and anthropogenic variables (i.e. fish farm types and activities).

3. Materials and Methods

3.1 Study area

The Gulf of Corinth (GOC), between the Peloponnese and mainland Greece, is a semi enclosed basin of 2400 km2 marked by broad bays separating the Peloponnese from mainland Greece. The Gulf is approximately 128-km long and more to 35-km wide. It is separated to the west from the outer Gulf of Patras and the Ionian Sea by the 1.9-km-wide Rion-Antirion strait and it is bounded to the east by the narrow Corinth Canal (25-m wide). The western sector of the Gulf leading to open Ionian Sea waters is relatively shallow, with a maximum depth of 65 m under the Rion-Antirion bridge. The central sector of the Gulf includes a large basin with depths of 500–900 m.

The Gulf waters are generally oligotrophic and quite transparent (Bearzi, Bonizzoni, Agazzi, Gonzalvo, & Currey, 2011) and the freshwater inputs are variable but with a low trend, generally.

Input of contaminants comes mainly from city sewage, industrial discards and agriculture runoff (Botsou and Hatzianestis, 2012).For instance, a factory processing bauxite for aluminium production has been in service since 1966,located close to the city of Antikyra.

The National Strategy and Action Plan for the conservation of cetaceans in Greece, 2010–2015 (Notarbartolo di Sciara & Bearzi, 2010, as cited in Bearzi et al., 2016) identified the Gulf of Corinth as an area of special conservation importance. In 2007, the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea, and Contiguous Atlantic Area (ACCOBAMS), approved by Greece, had cataloged the Gulf of Corinth as an area of special importance for common dolphins and other cetaceans, asking for the design of a marine protected area (resolution 3.22; ACCOBAMS, 2007). In the same period, Greenpeace proposed the creation of a marine reserve (Greenpeace, 2007, as cited in Bearzi et al., 2016). Therefore, parts of the Gulf of Corinth have been included in a proposed network of marine protected areas (Giakoumi et al., 2012; Issaris et al., 2012; Stelzenmüller et al., 2013; Vassilopoulou et al., 2012 as cited in Bearzi et al., 2016).

Focusing on the site considered in this study, it is in the northern part of the Gulf, important for fishery and aquaculture production.

3.2 Aquaculture in the study area

The northern shore of the Gulf gives shelter to 17 fish farms that produce mainly European sea bass, *Dicentrarchus labrax*, and gilthead seabream, *Sparus aurata*. In particular, the marine farm considered in this study (Galaxidi Marine Farm S.A) is operating since 1987, and it is one of the first aquaculture farming companies in the country, where 98% of the production is exported in many European countries such as Spain, Italy, Germany, the Czech Republic, France, Switzerland and Austria.

It is characterized by two hatcheries and six-unit farms, where cages are placed from 40 up to 150m water depths, counting about 331 cages and covering a sea surface area of 357.000sq.m.

The farm has an entire vertical production, from egg stage to whole fish, where the main species farmed are: Sea Bass (Dicentrarchus labrax); Sea Bream (Sparus aurata) and Meagre (Argyrosomus regius). Moreover, Greater amberjack (Seriola dumerili) is at an experimental stage of production.

Since 2008, organic sea bream and sea bass have been raising, certified according to NATURLAND Standards for Organic Aquaculture.

3.3 Bottlenose dolphins in the study area

Previous studies reported the occurrence of bottlenose dolphins in the Gulf varying among years and preferring habitat situated in the northern/central coastal sector of the Gulf with waters shallower than 300 m. For the years 2012–2015 an average of 39 animals were reorted, with a no significant interannual variability. Some of the dolphins observed in the GOC can cross the strait toward the offshore waters of the Ionian Sea. Bearzi et al. in 2011 showed that some individuals previous identified in the Gulf were sighted in other areas of western Greece, up to 265 km apart.

Bottlenose dolphins in the GOC (Figure 3.3.1) appear to be strongly attracted to fish farms on the northern coast where the aquaculture facilities are concentrated, taking advantages of a higher concentration of prey possibly in response to prey depletion (Bearzi et al., 2008) and low prey availability away from fish farms.

Bottlenose dolphins in the Mediterranean are classified as Vulnerable due to declines as a result of many threats such as overfishing of their prey, mortality in fishing gear, health effects caused by pollution and culling (Bearzi et al., 2008).

Figure 3.3.1 Image of a bbottlenose dolphin close to fish farm in Greece

3.4 Data collection

Land base observations were conducted in the Gulf of Corinth between September and November 2021 (Figure 3.4.1). In particular, data were collected from two land-based points (38.3568096, 22.3842530 and 38.3440817, 22.3843043) which allowed to observe three seabass and seabream farms: F1 (organic); F2: (oldest) and F3 (biggest and exposed to the open sea).

In land base work, all the visual area was observed to search for groups of bottlenose dolphins during the entire time of observations using a binocular and naked eye. Overall, the priorities in this data collection's methods were: scanning, collecting farms' activities, presence/absence of bottlenose dolphins and other megafauna, dolphins' group size and composition and their distance from the cages when spotted. In particular, the data where organized in two diverse sheets referred as 'Survey' and 'Sighting'.

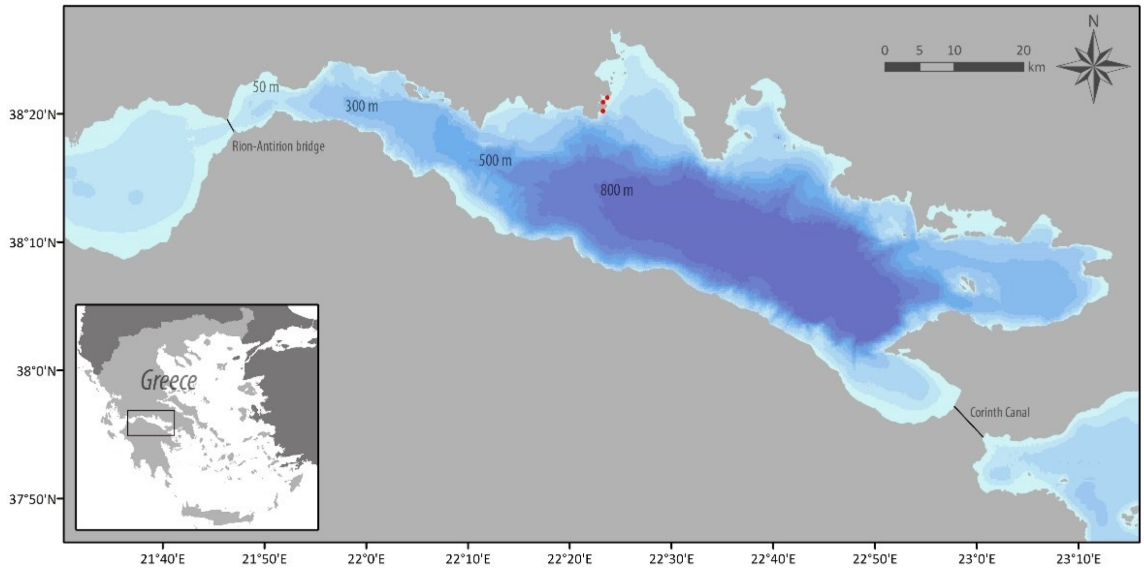

Figure 3.4.1. Study area with details of the three fish farms

3.5 Survey

During the survey, environmental data and anthropogenic activities were collected every five minutes starting with the start time, which was the time of the first survey. Specifically, the data collected during the survey included: the date, the start time, the sea state, the visibility (low, average, high, very high); rain or fog; the farm activities, the presence/absence of dolphins and the presence of other megafauna such as turtles, tuna or seals. The observations were considered satisfactory when sea condition where less than 3 on the Douglas sea force scale and the visibility was high or very high and not reduced by fog, rain or other adverse circumstances.

3.6 Sightings

The sightings collected data every five minutes when dolphins were spotted. The data collected during the sightings were: the distance from the cages (between cages, <50, 50-100, 100-200, 200-500 or >500m), the occurring farm activities (e.g., during practices of feeding, harvesting, maintenance and biomass recovery) and size and composition of the group (referred as adults or calves). In this study both new-borns and immature individuals were included in the category “calves”, where new-born is <1,5 m with fetal folds, swimming with the mother and touching her abdomen (“infant position”) and immature dolphins is two-thirds or less the adult's length of 2,5 m (Diaz Lopez and Methion, 2018). If dolphins were spotted, observation sessions continued until the animals moved away from the farm area and out of sight.

3.7 Data analysis

All data collected were then transcribed manually, reviewed and entered into a database.

Data were analysed with Excel (Version Office 2203) and Python in the development environment called Jupiter (version 6.3.0).

To calculate the relative frequency of occurrence of bottlenose dolphins, referred to as the encounter ratio (ER) (Díaz López , 2007) was analysed as follow:

$$ER = Ne / h$$

Where *Ne* is the number of encounters (number of sightings) and *h* is the total number of hours searching for dolphins.

To assess the: difference between dolphins' presence/absence distributions among the farms; the difference between dolphins' presence/absence distributions among the different months (September, October and November); the difference between dolphins' presence/absence distributions among different time of the day (Morning-Afternoon) and the difference between dolphins' presence/absence with or without farms' activities in all farms and in the diverse farms, Chi-Square tests were applied. The Chi-Square is a statistical test that verifies if two multinomial distributions are the same, this test is often called the chi-squared test for homogeneity. The p-value is used to determine whether the null hypothesis should be accepted or rejected, where null hypothesis means that two multinomial distributions are the same (Valli 2001, 72). Commonly, 0.05 (or 5%) of the level of risk is used in scientific research. This means that the result is 95% valid for the entire population, but at the same time, the probability of error is five percent. (Heikkilä 2005, 212-232). Therefore, small P-values reject the null hypothesis and conclude that the variables distributions are not the same.

The formula for Chi- Square test is:

$$X^2 = \Sigma \, (O - E)^2 \, \mathbf{E}$$

Where:

- Σ means to sum up
- O = each Observed value
- E = each Expected value

To assess the relation between dolphins' presence (in terms of presence/absence) or group size, and others independent variables (such as moment of the day, month, distance from the cages, different farm type and farm's activities), linear and generalized linear models have been applied.

Linear models assume that a response variable Y can be written as a linear function of predictor variables X1,……,Xp plus an error term:

$$y_i = a + \sum_{j=1}^{p} b_j x_{ij} + \epsilon_i,\ i = 1, ..., n$$

Where epsilon_i is the error in the i-th value of Y.

Linear models can be written in matrix form as:

$$y = X\beta + E$$

Where:

- Y:n-dimensional vector of observed response variable,
- X: matrix whose first column has all 1's and other columns are observed predictor variables,
- E: n-dimensional vector of errors,
- Beta: vector of parameters to estimate.

In a linear model there are the fallowing assumptions to take in consideration:

- Constant variance (homoscedasticity). This means that the variance of the errors does not depend on the values of the predictor variables.

- Independence of errors. This assumes that the errors of the response variables are uncorrelated with each other.
- Lack of perfect multicollinearity in the predictors.

Generalized linear model is an extension of classical linear model. The model can be written as:

$$\mathbb{E}(Y_i) = \mu_i = \sum_{j=1}^{p} x_{ij}\beta_j; \quad i = 1, ..., n$$

Where Xij is the value of the j-th covariate for observation i. In matrix notation we may write:

$$\mu = X\beta$$

Where X is the model matrix, Beta is the vector of parameters and Mi is a column vector nx1.

In the generalized linear model, that is a generalization of linear model the assumptions are:

1. The distribution of Yi belongs to an exponential family and are independent random variables;
2. $\eta_i = \sum_{j=1}^{p} x_{ij}\beta_j;$
3. $g(\mu_i) = \eta_i,$ where *g* is called link function

First, I applied linear models and then a Negative Binomial model has been created as considered performing better to analyse count data. The negative Binomial consider t a discrete distribution on the nonnegative integers y with density, that can be written as:

$$f(y) = \binom{y+k-1}{y} p^y (1-p)^k$$

The multicollinearity has been then calculated to satisfy the linear models' s assumptions. In statistics, multicollinearity (also collinearity) is a phenomenon in which one predictor variable in a multiple regression model can be linearly predicted from the others with a substantial degree of accuracy. Multicollinearity affects calculations regarding individual predictors, and it refers to a situation in which more than two explanatory variables in a multiple regression model are highly linearly related. The indicators that multicollinearity may be present in a model include the following: the Pearson correlation coefficient and the variance inflation factor (VIF).

In statistics correlation refers to the degree to which a pair of variables are linearly related. Pearson (LSM) is a measure of linear correlation between two sets of data. It is the ratio between the covariance of two variables and the product of their standard deviations where the result always has a value between −1 and 1.

$$\rho_{X,Y} = \frac{\mathrm{cov}(X,Y)}{\sigma_X \sigma_Y}$$

where: ·

- cov is the variance
- σx is the standard deviation of X
- σY is the standard deviation of Y

In statistics, the variance inflation factor (VIF) is the ratio (quotient) of the variance of estimating some parameter in a model that includes multiple other terms (parameters) by the variance of a model constructed using only one term. It quantifies the severity of multicollinearity in an ordinary least squares regression analysis.

$$VIF = \frac{1}{1 - R_i^2}$$

Where R2 is the coefficient of the regression with Xi on the left side and all the other predictor variables on the right-hand side.

4. Results

4.1. Frequencies of dolphins' occurrence in the farms and differences among the investigated farms

Out of 38 days of survey effort (232.66 h), 2400 observations each one of five minutes (total 200 h) have been selected as satisfactory (considering the sea state less than 3 on the Douglas scale and the visibility very high or high and not reduced by fog, rain or other adverse circumstances).

Dolphins were observed in 15 days (39.47%), totalling 20 sightings, with up to 3 sightings per day.

In particular, 2 sightings of dolphins have been recorded in F1, 10 in F2 and 8 in F3 with ca. 2 %, 15 % and 9% frequency of presence, respectively. In Figure 4.1.1 is reported the total number of observations and the number of sightings of dolphins in the three farms (F1, F2 and F3).

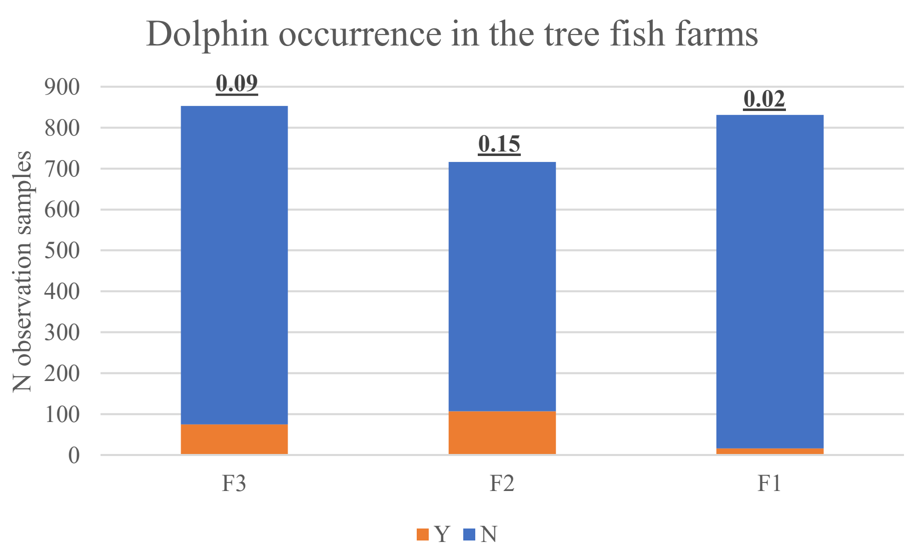

Figure 4.1.1. Presence/absence of dolphins recorded in Farm1, Farm2 and Farm3, where Y: observations' samples in presence of dolphins, N: observations' samples in absence of dolphins. The values 0.09; 0.15 and 0.02, labelled on each column, referred to the relative frequency of dolphins' presence.

The relative frequency of dolphins' occurrence was then calculated by using the encounter ratio (ER) (Díaz López ,2007), with a value equal to 0.10 for all of the farms, with a majority observed in F2 (E.R. 0.17) as showed in Table 4.1.1.

Table 4.1.1. Encounter ratio (ER) of dolphins assessed for all farms (global) and for the three different farms (F1, F2 and F3).

	F1	**F2**	**F3**	**GLOBAL**
N SIGHT.	2	10	8	20
HOURS	69.25	73.75	71.08	214.08
ER.	0.03	0.17	0.11	0.10

To assess the presence of statistically significant differences between dolphins' presence/absence among the diverse farms, a chi-square test was applied. The test showed significant differences between the three farms (CHI-2: 69.78; $p<0.05$ and $p<0.005$: d.f. 2), with a preference of presence for F2, as highlighted also by the ER value.

4.2. Changes of dolphins' presence during the study period (September-November 2021)

Of 2400 total records between September and November 2021, 968 (80.67 h) have been collected in September, 966 (80.50 h) in October and 466 (38.83 h) in November.

The chi-square test applied to assess the differences in the overall dolphin occurrence (considering all the three farms) among months highlighted significant differences between September, October and November (CHI-2= 31.61; $p<0.05$; d.f. 2), with values higher in October and November than in September (Figure 4.2.1) as also showed by the higher ER value (0.14 and 0.10 in October and November vs. 0.06 in September).

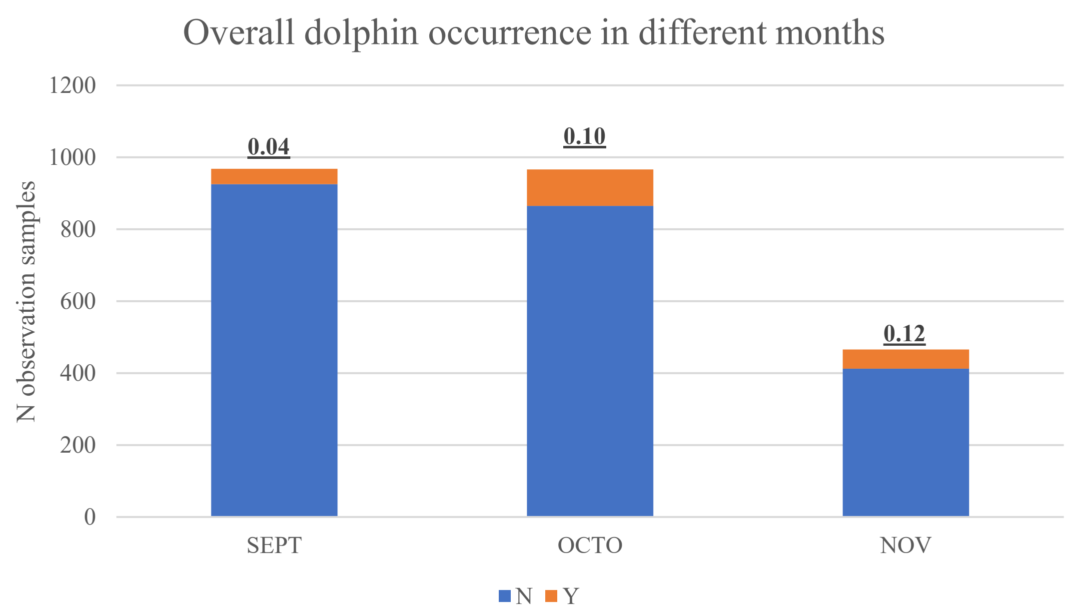

Figure 4.2.1 Overall dolphin occurrence in the different months. The values 0.04; 0.10 and 0.12, labelled on each column, referred to the relative frequency of dolphins' presence.

The chi-square test was also applied to assess differences of dolphin occurrence among time periods in the three farms (Figure 4.2.2). The test provided evidence of significant variations in all the fish farms (CHI-2= 31.60, $p<0.05$; d.f. 2; CHI-2= 24.45, $p<0.05$: d.f. 2; CHI-2= 53.07, $p<0.05$: d.f. 2 for F1, F2 and F3, respectively). The ER calculated for the three distinct months in F1, F2 and F3 revealed a higher presence of dolphins in October (0.21) and November (0.23) for F2 (versus 0.09 in September) and in October for F3 (0.20 versus 0.07 and 0.00 in September and November) and a slight higher presence in September (0.04) and November (0.07) for F1 when compared to October (0.00) (Table4.2.1).

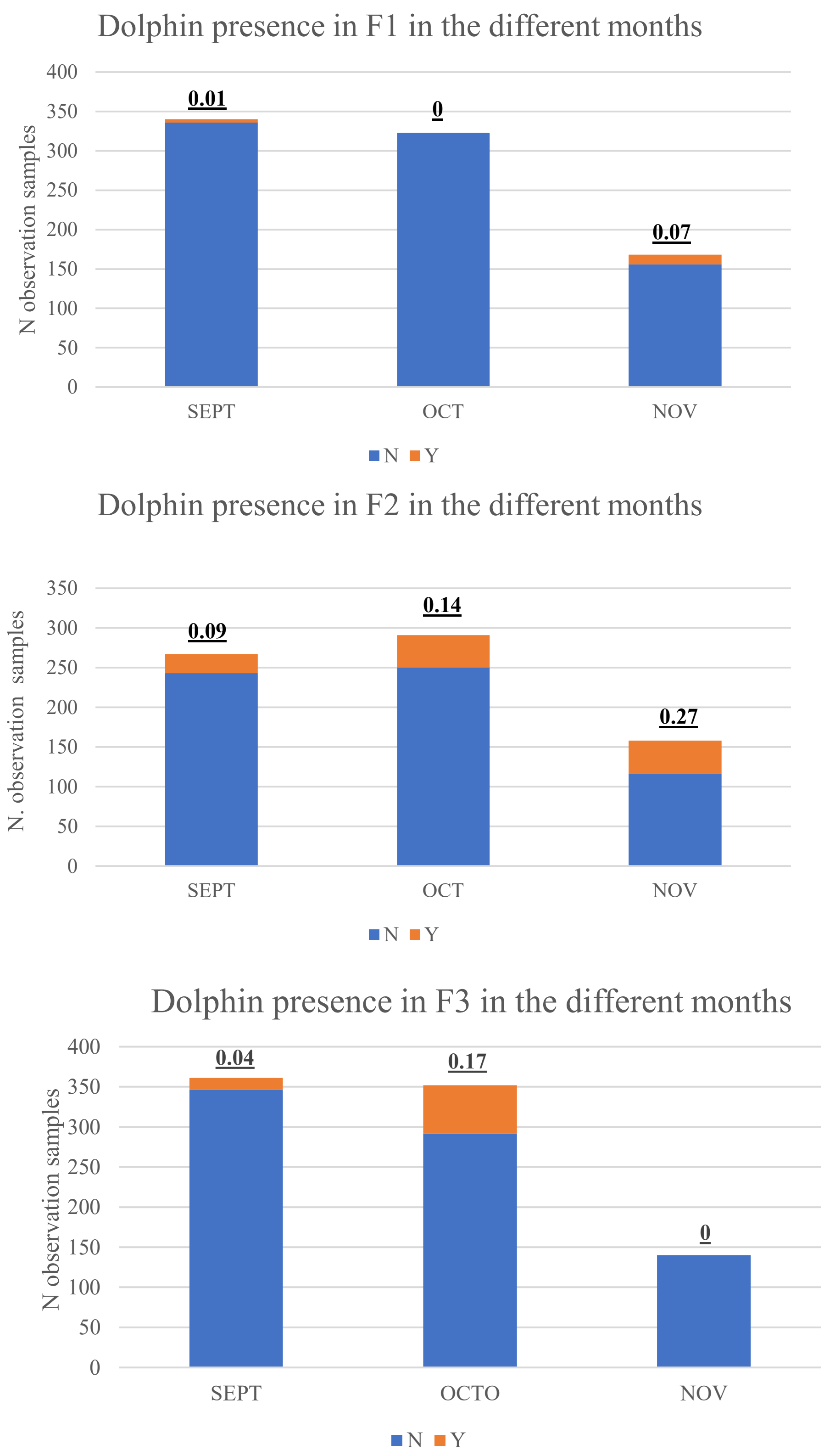

Figure 4.2.2 Dolphin occurrence assessed in the three farms (F1, F2 and F3) in the different months. The values 0.01;0.00;0.07 in F1, 0.09;0.14;0.27 in F2 and 0.04;0.17;0 in F3, labelled on each column, referred to the relative frequency of dolphins' presence for September, October and November, respectively.

Table 4.2.1 Encounter ratio estimated for the three farms in September, October and November 2021. Reported is also the overall encounter ratio (global) considering the farms altogether.

	ENCOUNTER RATIO			
	F1	**F2**	**F3**	**GLOBAL**
September	0.04	0.09	0.07	0.06
October	0.00	0.21	0.20	0.14
November	0.07	0.23	0.00	0.10

4.3 Dolphin occurrence in relation to the moment of the day (morning vs. afternoon)

Of 2400 total records between 7:0 and 19:00, 198 were characterised by the presence of dolphins.

Since it was not possible to apply the chi square test with frequencies < 5, we grouped each time slots in morning (referred to 7:00 - 12:59) and afternoon (referred to 13:00 - 19:00). The test highlighted significant differences in the dolphin occurrence between morning and afternoon (CHI-2: 22.96; $p<0.05$: d.f. 1; Figure 4.3.1), with higher occurrence in the afternoon than in the morning as showed by the ER value (Table 4.3.1).

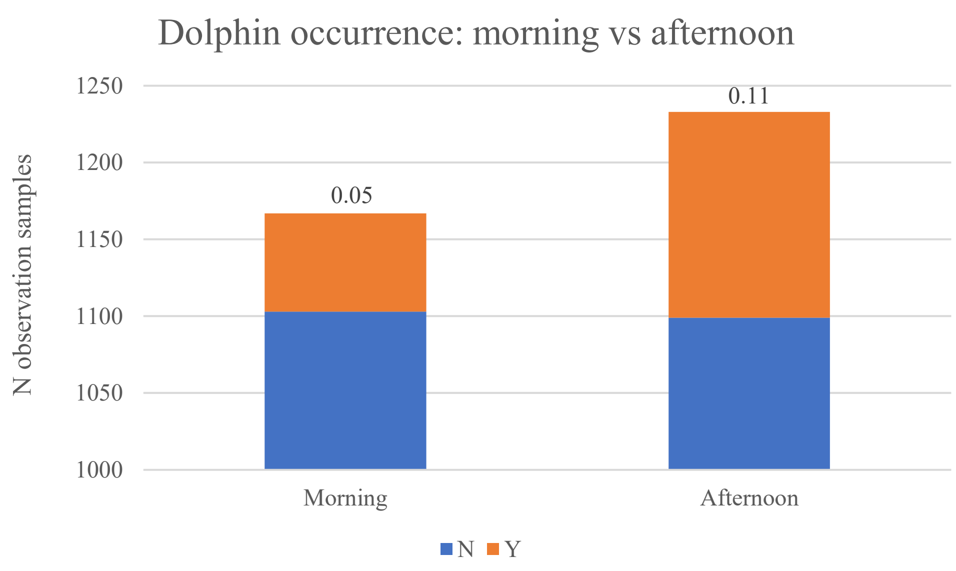

Figure 4.3.1 Overall dolphin occurrence in the morning and in the afternoon in the investigated areas. The values 0.05 and 0.11, labelled on each column, referred to the relative frequency of dolphins' presence in the morning and in the afternoon, respectively.

The chi square test provided also evidence of significant variations in relation to the moment of the day in F2 and F3 (CHI-2= 4.61; $p<0.05$: d.f. 1 and CHI-2= 45.96; $p<0.05$: d.f. 1 respectively), while no significant differences were observed in F1 (CHI-2= 0.48; $p>0.05$: d.f. 1; Figure 4.3.2). This was also confirmed by the ER value which was lower in in the morning than in the afternoon for F2 and F3 (Table 4.3.1).

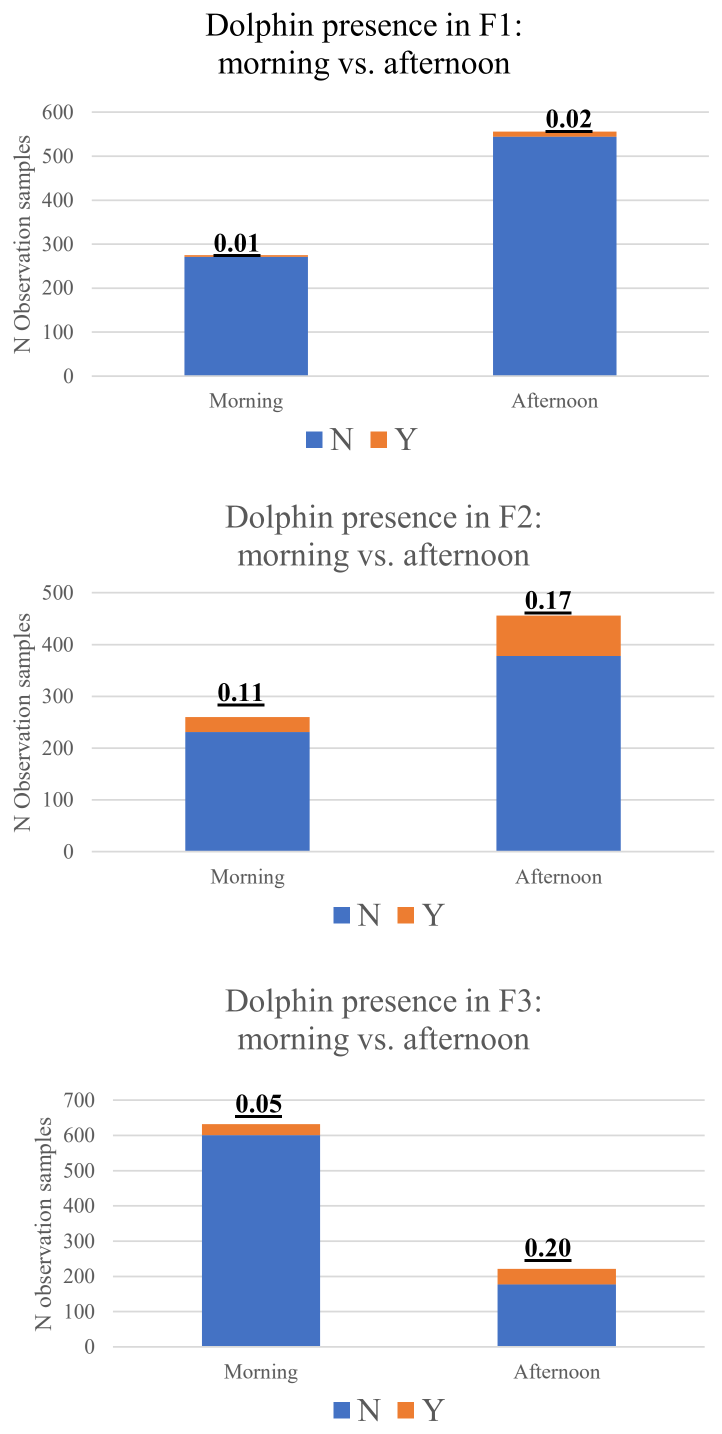

Figure 4.3.2 Dolphin occurrence assessed in the morning and in the afternoon in the three farm sites (F1, F2 and F3). The values 0.01, 0.02 in F1, 0.11, 0.17 in F2 and 0.05, 0.20 in F3 ,labelled on the columns, referred to the relative frequency of dolphins' presence in the morning and in the afternoon, respectively.

Table 4.3.1 Encounter ratio estimated for the three farms in the morning and the afternoon of the investigated time interval (September-November 2021). Reported is also the overall encounter ratio (global) considering the farms altogether.

ENCOUNTER RATIO				
	F1	**F2**	**F3**	**GLOBAL**
Morning	0.04	0.09	0.07	0.06
Afternoon	0.021	0.21	0.22	0.13

4.4 Presence of dolphins during fish farm activities

Of 1753 records registered with farms' activities and 647 with none, the presence of dolphins were observed in 153 observations each one of five minutes during farms' activities, while in 45 with no activities. The chi-square test showed no significant differences in the presence of animals when farm activities occurred (CHI-2=1.96; p>0.05: d.f. 1; Figure 4.4.1), as confirmed also by similar ER values with farm activities and not (0.10 vs 0.11, respectively).

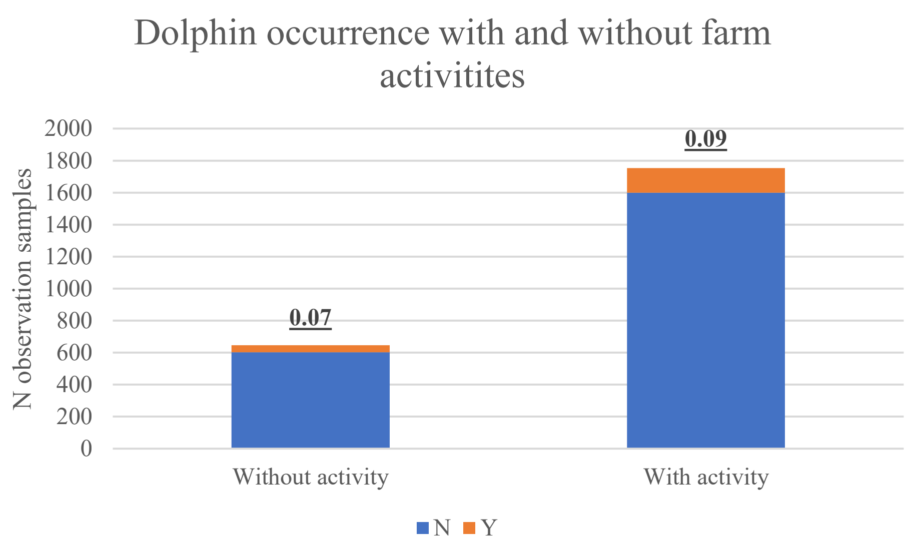

Figure 4.4.1 Overall dolphin occurrence with and without farm activities in the investigated areas. The values 0.07 and 0.09, labelled on each column, referred to the relative frequency of dolphins' presence with and without farms' operations, respectively.

As far as the three different farms, the chi square test highlighted the lack of significance difference in F3 (CHI-2=1.54; $p>0.05$: d.f. 1) and the presence of significant variation in F1 (CHI-2=8.19; $p<0.05$: d.f. 1) and F2 (CHI-2=22.0; $p<0.05$: d.f. 1; Figure 4.4.2). The ER value was higher when no activities occurred in F2 (0.34 vs 0.16), whereas was higher with farm operations in F1 and F3 (Table 4.4.1).

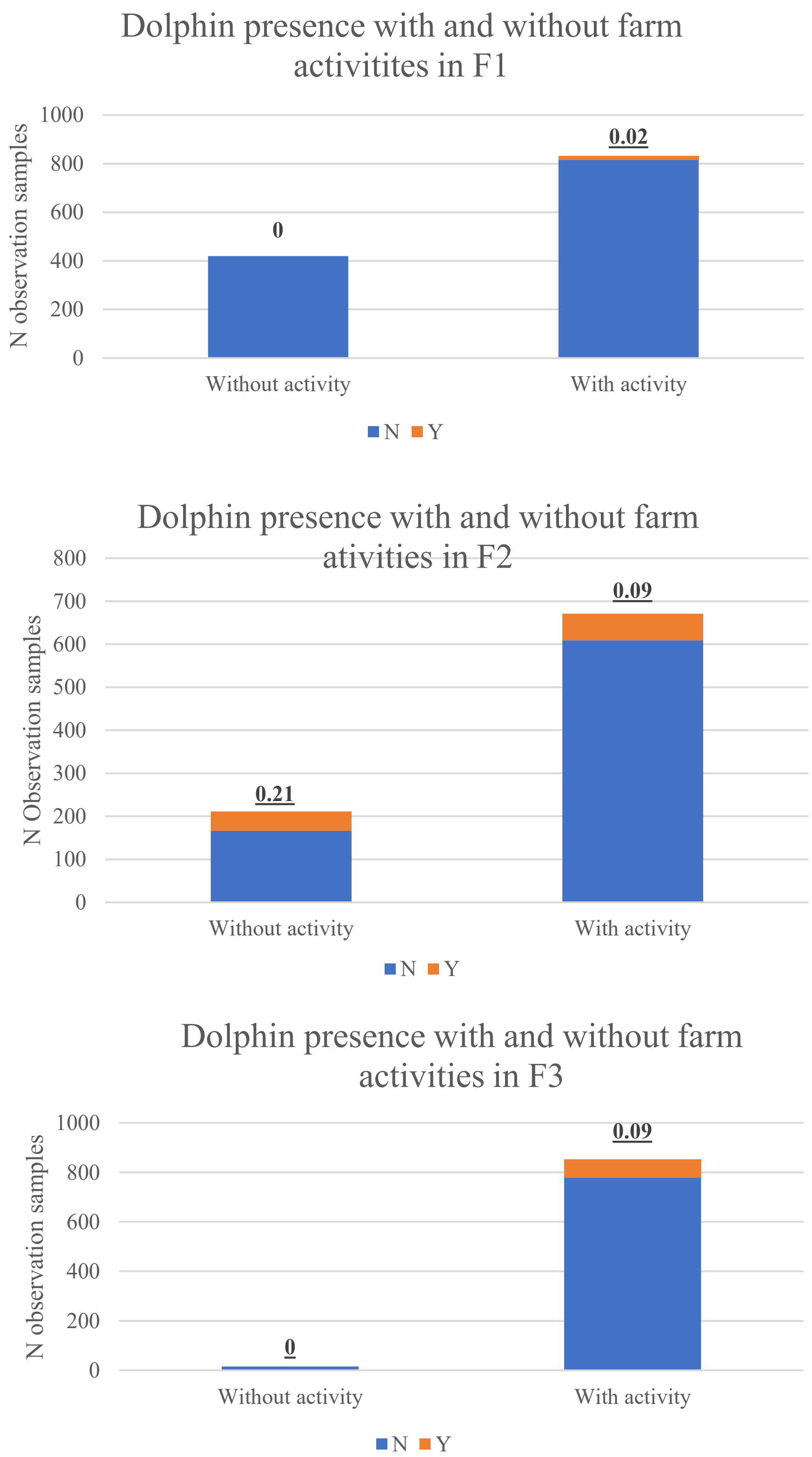

Figure 4.3.2 Dolphin occurrence in relation to the presence or not of farm activities in the three sites (F1, F2 and F3). The values 0, 0.02 in F1, 0.21; 0.9 in F2 and 0; 0.09 in F3, labelled on each column, referred to the relative frequency of dolphins' presence with and without farms' operations.

Table 4.4.1 Encounter ratio estimated for the three farms in relation to the presence or not of farm activities. Reported is also the overall encounter ratio (global) considering the farms altogether.

	ENCOUNTER RATIO			
	F1	**F2**	**F3**	**GLOBAL**
No activities	0.00	0.34	0.00	0.11
With activities	0.03	0.17	0.11	0.10

4.5 Dolphins' group size and composition

Based on 198 observations, the number of dolphins' groups was on average 3.7±1.2 individuals (range 1–5) and quite similar in the different fish farms (Figure 4.5.1): 3.25±0.43 individuals (range: 3-4) in F1, 3.66±1.00 individuals (range: 2-5) in F2 and 4.57±1.13 individuals (range 1-5). In all of the observations samples (198), the composition of the dolphins' group was represented by adults animals.

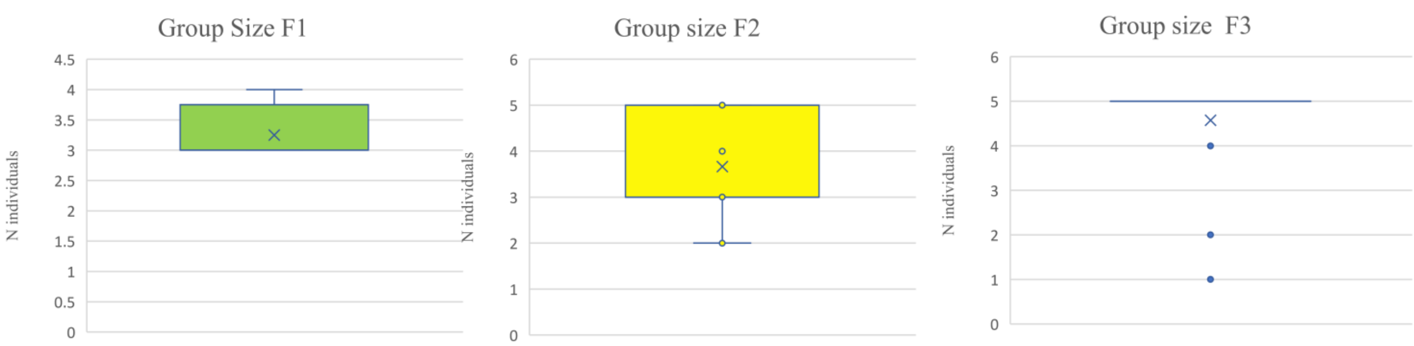

Figure 4.5.1 Box plot of the number of dolphins encountered in the three different farms during the entire study period.

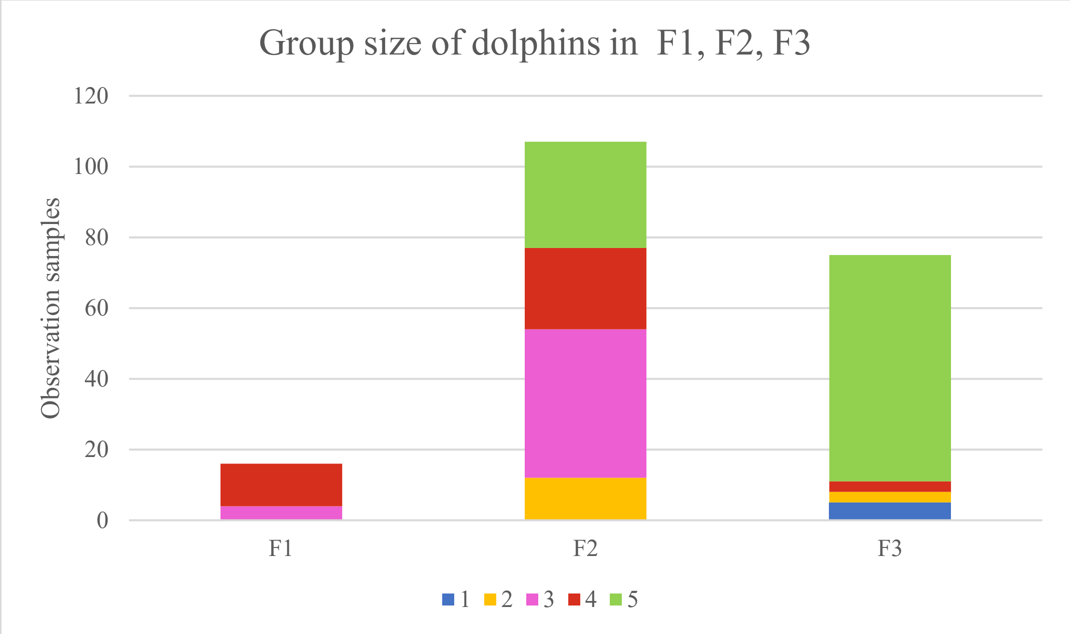

Figure 4.5.2. Histogram showing the number of observations of dolphins' occurrence in the three farms, where each colour represents the group size.

4.6 Relationships between dolphins' group size and temporal (e.g. morning-afternoon and months) and anthropogenic variables (i.e. farms type and farms' activities)

By the chi-square tests, we have evaluated the difference in dolphin occurrence considering only one parameter for each test (e.g. farm typology, month, morning/afternoon, farm activity). To estimate the relative relevance of the temporal and anthropogenic variables on dolphin occurrence, we used a multivariate regression approach, considering the number of individuals in each sighting and not only the presence/absence of dolphins.

Firstly, the so call "zero level" was fixed for the categorical variables:

- F1 = farm zero level,
- September = moth zero level,

- Morning = daytime zero level,
- No farm activities = activity zero level,
- No dolphins' presence = the zero level for the various categories representing the distances (m) from the cages when dolphins were spotted (between cages, <50; 50-100; 100-200; 200-500; >500).

To reduce collinearity that affect the accuracy of coefficient estimates in the regression a Pearson correlation coefficient was calculated and then the results visualized in a Heat Map (Figure 4.6.1), this coefficient analyses the degree of correlation between each couple of variables. The highest value of the correlation occurred between Morning-Afternoon and Farms' Activities (-0.58), but this value (as absolute value), being below 0.7, revealed the lack of collinearity between all the features considered.

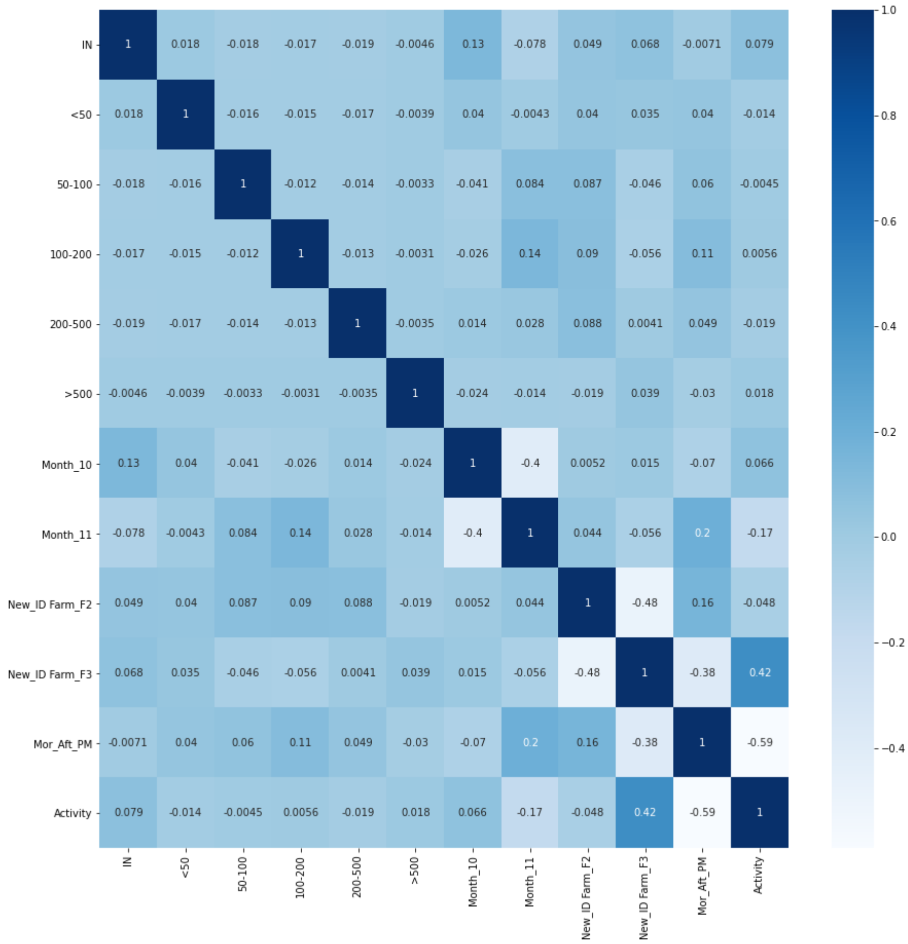

Figure 4.6.1 Heat map of the Pearson correlation coefficient between each couple of variables considered.

To develop a reliable multivariate regression model we need to reduce also the multicollinearity, for this reason we calculated the VIFs (Variance inflation factors) of each independent variable which resulted always <0.5 , so that all predictor variables were accepted. For the multiple linear regression model we considered the number of dolphins in each sighting as a response variable and all the other features of the dataset as covariates. We rejected this model for different reasons: i) the lack of statistical significance for F2 and F3 (p= 0.098 and p=0.059 respectively), ii) similar coefficients among the distance categories (3.85; 3.47; 3.55; 3.97 and 3.47 for ‘between cages’, <50, 50-100, 100-200, 200-500, >500, respectively), iii) negative values for November (coeff=-0.06) and F2 (coeff=-0.02, opposite from what expected; Table 4.6.1).

Table 4.6.1 Output of the multivariate linear regression model.

OLS Regression Results

Dep. Variable:	TOT_Group_size	R-squared:	0.921
Model:	OLS	Adj. R-squared:	0.920
Method:	Least Squares	F-statistic:	2313.
Date:	Thu, 05 May 2022	Prob (F-statistic):	0.00
Time:	16:46:24	Log-Likelihood:	-678.14
No. Observations:	2400	AIC:	1382.
Df Residuals:	2387	BIC:	1457.
Df Model:	12		
Covariance Type:	nonrobust		

	coef	std err	t	P>|t|	[0.025	0.975]
const	-0.0827	0.023	-3.631	0.000	-0.127	-0.038
IN	4.2752	0.043	98.890	0.000	4.190	4.360
<50	3.8566	0.049	78.245	0.000	3.760	3.953
50-100	3.4758	0.059	59.157	0.000	3.361	3.591
100-200	3.5599	0.063	56.883	0.000	3.437	3.683
200-500	3.9724	0.055	71.820	0.000	3.864	4.081
>500	3.4792	0.228	15.259	0.000	3.032	3.926
Month_10	0.0401	0.015	2.716	0.007	0.011	0.069
Month_11	-0.0685	0.019	-3.650	0.000	-0.105	-0.032
New_ID Farm_F2	-0.0283	0.017	-1.657	0.098	-0.062	0.005
New_ID Farm_F3	0.0340	0.018	1.890	0.059	-0.001	0.069
Mor_Aft_PM	0.0595	0.017	3.520	0.000	0.026	0.093
Activity	0.0695	0.020	3.546	0.000	0.031	0.108

On the basis of such results, a Negative Binomial model was created since it might fit better with count data. However, also this model was rejected due to the lack of statistical significance of October, November and Morning-Afternoon (p=0.06; 0.09; 0.08 respectively), and similarity among the distances' coefficients (coeff= 7.32; 7.35; 7.30; 7.29; 7.37; 7.27 for between cages', <50, 50-100, 100-200, 200-500, >500, respectively; Table 4.6.2).

Table 4.6.2 Output of the generalized multivariate linear model.

```
                 Generalized Linear Model Regression Results
==============================================================================
Dep. Variable:          TOT_Group_size   No. Observations:                 2400
Model:                             GLM   Df Residuals:                     2387
Model Family:         NegativeBinomial   Df Model:                           12
Link Function:                     log   Scale:                          1.0000
Method:                           IRLS   Log-Likelihood:                -525.12
Date:                 Thu, 05 May 2022   Deviance:                       77.812
Time:                         16:46:24   Pearson chi2:                 3.41e+03
No. Iterations:                     15
Covariance Type:             nonrobust
==================================================================================
                     coef    std err          z      P>|z|      [0.025      0.975]
----------------------------------------------------------------------------------
const             -6.5932      0.542    -12.168      0.000      -7.655      -5.531
IN                 7.3270      0.410     17.855      0.000       6.523       8.131
<50                7.3502      0.407     18.081      0.000       6.553       8.147
50-100             7.3025      0.447     16.323      0.000       6.426       8.179
100-200            7.2937      0.470     15.504      0.000       6.372       8.216
200-500            7.3705      0.439     16.776      0.000       6.509       8.232
>500               7.2714      0.911      7.983      0.000       5.486       9.057
Month_10           0.1356      0.272      0.499      0.618      -0.397       0.669
Month_11          -0.0194      0.324     -0.060      0.952      -0.654       0.615
New_ID Farm_F2     0.3027      0.327      0.926      0.355      -0.338       0.944
New_ID Farm_F3     0.2484      0.353      0.704      0.482      -0.443       0.940
Mor_Aft_PM         0.0456      0.242      0.189      0.850      -0.428       0.519
Activity           0.3261      0.251      1.301      0.193      -0.165       0.818
==================================================================================
```

To remove the similarity among the distances' coefficients in the previous models, the six features related to the distances of dolphins from the cages were eliminated and substituted with a single flag ('Presence'), which is 1 when there is a dolphin sighting and 0 otherwise.

This modification was included in a new linear model which was rejected again due to negative value for November and F2 (coeff= -0.09 and -0.03, respectively), not coherent with the data (Table 4.6.3). This bias is probably due to the overfitting effect of the variable 'Presence' which explains alone a large portion of the variance.

Table 4.6.3 Output of the modified multivariate linear model

OLS Regression Results

Dep. Variable:	TOT_Group_size	R-squared:	0.921
Model:	OLS	Adj. R-squared:	0.920
Method:	Least Squares	F-statistic:	3965.
Date:	Thu, 05 May 2022	Prob (F-statistic):	0.00
Time:	16:46:24	Log-Likelihood:	-680.61
No. Observations:	2400	AIC:	1377.
Df Residuals:	2392	BIC:	1423.
Df Model:	7		
Covariance Type:	nonrobust		

	coef	std err	t	P>|t|	[0.025	0.975]
const	-0.0927	0.023	-4.087	0.000	-0.137	-0.048
Presence	3.9735	0.025	160.773	0.000	3.925	4.022
Month_10	0.0574	0.015	3.903	0.000	0.029	0.086
Month_11	-0.0997	0.019	-5.359	0.000	-0.136	-0.063
New_ID Farm_F2	-0.0378	0.017	-2.212	0.027	-0.071	-0.004
New_ID Farm_F3	0.0538	0.018	3.004	0.003	0.019	0.089
Mor_Aft_PM	0.0633	0.017	3.757	0.000	0.030	0.096
Activity	0.0692	0.019	3.550	0.000	0.031	0.107

Even a Generalized Linear Model (Negative Binomial) was tested, but refused too, for high p values of different covariates and a negative coefficient for November (-0.06; Table 4.6.4) also related to the overfitting effect revealed in the previous simulation.

Table 4.6.4 Output of the Generalized Linear Model (Negative Binomial)

```
                 Generalized Linear Model Regression Results
==============================================================================
Dep. Variable:          TOT_Group_size   No. Observations:                 2400
Model:                             GLM   Df Residuals:                     2392
Model Family:         NegativeBinomial   Df Model:                            7
Link Function:                     log   Scale:                          1.0000
Method:                           IRLS   Log-Likelihood:                -512.37
Date:                 Thu, 05 May 2022   Deviance:                       52.307
Time:                         16:46:24   Pearson chi2:                 6.02e+03
No. Iterations:                      9
Covariance Type:             nonrobust
==================================================================================
                     coef    std err          z      P>|z|      [0.025      0.975]
----------------------------------------------------------------------------------
const             -7.0746      0.683    -10.360      0.000      -8.413      -5.736
Presence           7.8775      0.590     13.357      0.000       6.722       9.033
Month_10           0.1279      0.257      0.498      0.618      -0.375       0.631
Month_11          -0.0640      0.316     -0.203      0.839      -0.683       0.555
New_ID Farm_F2     0.2629      0.328      0.802      0.423      -0.380       0.906
New_ID Farm_F3     0.2513      0.351      0.716      0.474      -0.437       0.939
Mor_Aft_PM         0.0638      0.234      0.273      0.785      -0.394       0.522
Activity           0.3054      0.239      1.279      0.201      -0.163       0.773
==================================================================================
```

For all these reasons, the feature ‘Presence’ was eliminated from the model and a new linear model (LM) and a generalised linear model (GLM) with the remaining covariates was developed and used. The output of these models (Tables 4.6.5-4.6.6) were consistent with the observed dataset, with the exception for the high p value in November in the LM (p=0.06).

Tables 4.6.5 Output of the linear model after removing the feature “Presence”

OLS Regression Results

Dep. Variable:	TOT_Group_size	R-squared:	0.063
Model:	OLS	Adj. R-squared:	0.061
Method:	Least Squares	F-statistic:	26.95
Date:	Thu, 05 May 2022	Prob (F-statistic):	3.09e-31
Time:	16:46:25	Log-Likelihood:	-3642.9
No. Observations:	2400	AIC:	7300.
Df Residuals:	2393	BIC:	7340.
Df Model:	6		
Covariance Type:	nonrobust		

	coef	std err	t	P>|t|	[0.025	0.975]
const	-0.4697	0.077	-6.063	0.000	-0.622	-0.318
Month_10	0.2837	0.050	5.640	0.000	0.185	0.382
Month_11	0.1197	0.064	1.878	0.061	-0.005	0.245
New_ID Farm_F2	0.4386	0.058	7.587	0.000	0.325	0.552
New_ID Farm_F3	0.3751	0.061	6.141	0.000	0.255	0.495
Mor_Aft_PM	0.4018	0.057	7.001	0.000	0.289	0.514
Activity	0.2607	0.067	3.900	0.000	0.130	0.392

Tables 4.6.6 Output of the generalized linear model after removing the feature “Presence”

Generalized Linear Model Regression Results

Dep. Variable:	TOT_Group_size	No. Observations:	2400
Model:	GLM	Df Residuals:	2393
Model Family:	NegativeBinomial	Df Model:	6
Link Function:	log	Scale:	1.0000
Method:	IRLS	Log-Likelihood:	-1547.4
Date:	Thu, 05 May 2022	Deviance:	2122.3
Time:	16:46:25	Pearson chi2:	7.51e+03
No. Iterations:	11		
Covariance Type:	nonrobust		

	coef	std err	z	P>|z|	[0.025	0.975]
const	-4.2646	0.202	-21.148	0.000	-4.660	-3.869
Month_10	0.7896	0.104	7.570	0.000	0.585	0.994
Month_11	0.5483	0.129	4.245	0.000	0.295	0.801
New_ID Farm_F2	2.0847	0.160	13.045	0.000	1.772	2.398
New_ID Farm_F3	1.7629	0.170	10.341	0.000	1.429	2.097
Mor_Aft_PM	0.8947	0.101	8.861	0.000	0.697	1.093
Activity	0.7099	0.132	5.394	0.000	0.452	0.968

The outputs of these models indicate that the number of dolphins in each sighting is positively related with fish farm activity and is higher in the afternoon and in fish farm F2.

A STEPAIC backward has been performed, eliminating one variable at each step, to identify the predictor variables less relevant in explaining the variance of the response. In particular, the STEPAIC has been applied on the linear model, because faster and easier to develop with the Python program, but results are still valid for the GLM. On the basis of the STEPAIC backward analysis, we found an increase of the AIC values, indicating that the LM and GLM with all the covariates considered, expect for the variable "Presence", are the best models.

Also, the use of univariate model highlighted a significant positive correlation between the number of dolphins and the farm activities (coefficient=0.14; P=0.05). Moreover, univariate linear model was also used to identify potential correlation between the number of dolphins and the investigated months, since the p-value of the LM in November 2021 was not significant (P=0.06), in contrast with the GLM. The univariate linear model indicates a significant positive relationship both in October and November (coeff=0.29 and $p<0.01$ in October and coeff=0.17, $p<0.01$ in November, respectively).

5. Discussion and conclusions

This study has investigated for the first time the occurrence, timing and seasonality of dolphins visiting aquaculture facilities in the Gulf of Corinth, Greece and provided insights on the potential relationships between farm activities and dolphins' sightings. This study is relevant for a better understanding of the ecology of the bottlenose dolphins in the light of the progressive expansion of the aquaculture sectors in the Mediterranean Sea.

5.1 Frequencies of dolphins' occurrence in the farms and differences among the investigated farms

In the current study dolphins have been observed during ca. 40% of the days investigated, with the highest frequency of occurrence close to the fish farm F2 (14.9%) and lowest close to F1 (1.9%). Statistical analysis based on chi square test (CHI-2: 69.78; $p<0.05$ and $p<0.005$: d.f. 2) and encounter ratio value which was higher for F2 (ER 0.14) followed by F3 (ER 0.11) and F1 (ER 0.03) further support the preference of dolphins for the area surrounding the fish farm F2.

Finally, these results were confirmed by multivariate linear (LM) and generalised linear (GLM) models which highlight dolphins' preference for F2 and F3 farms based on their positive and statistically significant coefficients. Moreover, dolphins apparently prefer F2 compared to F3 as the coefficients in F2 in both models are greater than F3.

Such differences of dolphin preference can be explained by the different characteristics of the farming systems and by different environmental conditions (e.g., bathymetry, current circulation, food availability) that can play all together an important role in influencing the distribution patterns of dolphins (Machia, 2005). To this regards, Piroddi and colleagues (2011) highlighted that dolphins' preference for certain fish farms may be due to their spatial location, as well as by the fish species that aggregate around the cages. In the present study, F2 is the oldest installation (1987) and it is located in a small bay characterised by weak current regimes. Such characteristics may promote a high trophic enrichment due to farming activities which, in turn, can attract a high number of dolphins' prey (Dempster et al., 2002; Machias et al., 2005) resulting in a high occurrence of dolphins (also confirmed by some personal declarations from local workers). On the other hand, F1 may be less attractive to dolphins due to its farming system based on certified NATURLAND standard for organic aquaculture, while F3 is the biggest, containing only adult reared fishes and exposed to strong currents favouring the dispersion of the farm' s food, faeces or in general particulate organic matter.

5.2 Changes of dolphins' presence during the study period (September-November 2021)

The analysis of dolphin presence during the entire study periods (from the beginning of September to the end of November 2021), highlighted the presence of significant differences between the three investigated months (CHI-2= 31.61; $p<0.05$; d.f. 2), with a higher relative frequency of occurrence in November compared with the other months. The values of encounter ratio further confirm such findings, since they increase from September to October/November 2021 (0.06, 0.14 and 0.10 in September, October and November, respectively).

Both the LM and GLM models provide support that the number of dolphins' sightings is higher in October and November than in September, but with some differences. The LM assigns a coefficient for dolphin presence in October double the one in November, but this latter coefficient is not statistically significant. The

GLM assign to October and November similar coefficients. This can be due to the fact although the relative frequency of occurrence is higher in November, the number of observations is lower than in October.

Despite our survey is restricted to only three months, it can be argued that dolphins' interaction with aquaculture facilities may strongly vary on a relatively short time scale with a peak of occurrence in late autumn. The highest relative frequency of occurrence of dolphins close to the different fish farms in November 2021 may due to the fact that during this period the animals spend most of their time foraging and for accumulating fat before the winter months (Diaz Lopez 2012; Andres et al., 2021). Moreover, fish farms provide an alternative food source during periods with low prey abundance (i.e. winter), because hunting at fish farms usually requires less effort and so becoming a more appealing option than hunting wild fish over broad scales during these periods (Diaz Lopez , 2017). In particular, the density and biomass of wild fish has been observed to decrease in the Ionian Sea in winter season (Madurell, Cartes & Labropoulou, 2004), so the fish farm area may offer valuable foraging grounds for bottlenose dolphins. To this regard, fish- survey campaigns, conducted by Bräger et al. (2016), during bottlenose dolphin surface foraging events in the waters of the Gulf of Ambracia, provided evidence that dolphins primarily feed on two epipelagic zooplanktivorous fish species of the family *Clupeidae* including the European pilchard (*Sardina pilchardus*) and the round sardinella (*Sardinella aurita*). Specific interviews of the fish farm personnel carried out in the present study highlighted that the fish species most regularly encountered around fish farms belong to the family *Carangidae* (such as the horse mackerel or Safridia or Sparidae, *Boops boops*).

The higher relative frequency of occurrence of dolphins in November is also consistent when the different fish farms are considered separately, with the exception of F3 where no sightings have been observed. This is possibly related to the fact that F3 is located offshore and so more subjected to strong currents and waves often occurring in late autumn, as documented in November during this study. In such harsh environmental conditions, dolphins could prefer sheltered bays, as the area where F2 is located and in which high occurrence has been reported by the present results and declared also by personal testimony from local farm's workers.

5.3 Dolphin occurrence in relation to the moment of the day (morning vs. afternoon)

The occurrence of dolphins close to the different fish farms changed not only on monthly basis but also on a daily basis. Indeed, statistical analysis revealed significant changes of dolphin presence between morning and afternoon. Such differences hold true also when the fish farms were considered separately, with the exception of the fish farm F1, where no significant differences were observed (CHI-2= 0.48; $p<0.05$: d.f. 1). The outputs of the LM and GLM models indicate that the number of dolphins is higher in the afternoon than in the morning.

Overall, these results do not agree with findings reported by Diaz Lopez (2017) who did not find any significant diurnal variation of dolphin occurrence or by Diaz Lopez (2012) who reported dolphin occurrence related to the moment of the day, with a minimum during the evening hours. A possible explanation of the daily variations (morning vs. afternoon) of dolphin occurrence close to the fish farm I observed, is that the abundance of preys (wild fish around the fish farm cages) is not the same during the day, as suggested by Diaz Lopez (2012).

5.4 Presence of dolphins during fish farm activities

In the present study we also investigated the potential effect of fish farm activities on the occurrence of dolphins. Statistical analysis showed overall the lack of significant differences, in the presence of animals when farm activities occur (CHI-2=1.96; p>0.05: d.f. 1). However, when fish farms were considered separately significant differences were observed for F1 (CHI-2=8.19; p<0.05: d.f. 1) and F2 (CHI-2=22.0; p<0.05: d.f. 1), but not for F3. The ER value was higher when no activities occurred in F2 (0.34 vs 0.16), whereas it was higher with farm operations in F1 and F3. The ER value estimated for F1 should be viewed with caution since only 2 sightings and 16 records of dolphin presence were available. Previous findings reported an increase of the frequency of occurrence of dolphins caused by the loss of fish during fish farm harvesting operations (Diaz Lopez, 2012). In the present study, no damages to the farm facilities have been directly observed and reported by the workers at the farms, so that other factors can be involved in the higher occurrence of dolphins in F3. The higher occurrence of bottlenose dolphins in F2 without farming operations may be associated with a higher availability of wild prey attracted from the facilities. At the same time, it is worth to note that F2 is located close to the land-based hatchery-centre, where all the farms' boats leave and pass close to F2 to reach other farms' units. Therefore, the noise produced by farming operations in F2 may amplify the noise produced from boat traffic thus determining unfavourable conditions for dolphin occurrence.

To further investigate the potential effect of farm activities on the occurrence and number of individuals of dolphins we used a multivariate liner model (LM) and a generalised linear model (GLM). Such models reveal that, overall, sightings and number of dolphins are higher when farm activities are present.

This result seems in contrast with the result of the chi-square test which revealed the lack of significant differences between the presence or not of fish farm operations and with the E.R. value in F2 that is higher when no activities occurred. However, such discrepancies depend on the fact that chi-square test is a univariate test where only the occurrence of dolphins (presence/absence) has been considered whereas the multivariate regressions consider the effect of all the covariates and as response variables and not only the occurrence but also the number of the dolphins.

5.5. Dolphins' group size and composition

Dolphins' groups resulted in average value of 3.7±1.3 individuals (range 1–5). The mean number of dolphin group size is slightly lower than the one observed in the North-eastern of Sardinia (4.35±0.37 individuals; Díaz López, 2006b; 2008; 2012). A study conducted in the whole Gulf of Corinth reported an average number of bottlenose dolphins of 8±44.5 individuals (range 1–28) (Bearzi et al., 2016). The relatively low number of dolphin group size encountered in the present study close to the fish farm areas indicates that some individuals are attracted to a certain extent to the fish cages. This is in line to what reported by Diaz Lopez (2012) who suggested individual preferences for aquaculture facilities. In general, the group sizes tend to be higher in presence of calves or dangers (Benmessaoud, 2017). In particular, the influence of calves in group size have been reported for several areas, as the North-western coastal of Sardinia, Italy (Díaz López, 2012).

In the present study, an average of 3.25±0.43 individuals (range 3-4) was found in F1, 3.66±1.00 individuals (range 2-5) in F2 and 4.57±1.13 individuals (range 1-5) in F3. The higher group size in F3 may be related to the huge number of

cages and several farm's activities occurring simultaneously, which can be "perceived" by the animals as a danger presence.
The composition of the group founded in this study, agree with the hypothesis that the group size tends to be lower when no calves are present (Benmessaoud, 2017). In fact, dolphins' units were represented by adult animals in all the sightings. This could be also related to the period of the year, as new-borns are mostly observed in summer (Diaz Lopez, 2012).

During the last years, marine aquaculture has generated a worldwide interest because of the overexploitation of wild stocks combined with a growing demand for fish and seafood products worldwide (FAO, 2007). The expansion of marine aquaculture industries has so caused growing uncertainties regarding their environmental impact. This study provides new findings on the interaction between a marine top predator (such as bottlenose dolphin) and fish farms. In a relatively short time scale (three months), it is showed a relative high frequency of occurrence of dolphins in fish farm areas, likely related to the high abundance of wild prey availability around the cages, as well as the preference for certain aquaculture facilities, dictated by some characteristics associated both to the natural environmental characteristics and the farming-system.
Moreover, this study highlights the potential of an inexpensive and non-invasive land-based research approach for dolphin monitoring, which could be easily replicated in different costal marine ecosystems characterised by the presence of fish farms (possibly in conjunction with other approaches and taking advantage of i.e. digital photography, unoccupied aerial vehicles and underwater acoustic recordings).

A better understanding of the interaction between dolphins and fish farms are fundamental for both aquaculture management and biodiversity protection and conservation. With the recently launched initiative by the Greek Ministry of Environment, Energy and Climate Change in the context of the project 'LIFE IP 4Natura - Integrated Actions for the Conservation and Management of Natura 2000 sites, Species, Habitats and Ecosystems in Greece' (LIFE16 IPE/GR/000002), some restrictions to the expansion of fish farms should be proposed with the implementation of Natura 2000 network site management plans. These are important points to improve the conservation status of target habitats and species included in the Birds and Habitats Directives in Greece, such as bottlenose dolphins. In particular, the Gulf of Corinth has been already listed as an area of special importance for common dolphins and other cetaceans in 2007 (resolution 3.22; ACCOBAMS, 2007, as cited in Bearzi et al., 2016).

Therefore, future studies on cetaceans should be based on long-term research, allowing to document long-terms effects due to ecosystem changes caused by human activities. It is also fundamental to acquire information about cetaceans' habits (i.e. habitat use and movement patterns), at different spatial-temporal scales for planning the sustainable use of marine space, including the installations of fish farms. Finally, it is necessary to protect bottlenose dolphins classified as 'Vulnerable' species by IUCN (2012), by mitigating any negative effect resulting from human activities, such as the aquaculture sector in the Mediterranean Sea.

6. References

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